Effects of phosphorus on the growth and nitrogen fixation rates of *Lyngbya majuscula*: implications for management in Moreton Bay, Queensland

Ibrahim Elmetri[1,2,*], Peter R. F. Bell[1]

[1]**Division of Chemical Engineering, The University of Queensland, Brisbane, Queensland 4072, Australia**
[2]***Present address:* Institute of Technology and Engineering, Massey University, Turitea, New Zealand**

ABSTRACT: Significant acetylene reduction and therefore N_2 fixation was observed for *Lyngbya majuscula* only during dark periods, which suggests that oxygenic photosynthesis and N_2 fixation are incompatible processes for this species. Results from a series of batch and continuous-flow-culture reactor studies showed that the specific growth rate and N_2 fixation rate of *L. majuscula* increased with phosphate (P-PO$_4$) concentration up to a maximum value and thereafter remained constant. The P-PO$_4$ concentrations corresponding to the maximum N_2 fixation and maximum growth rates were ~0.27 and ~0.18 μM respectively and these values are denoted as the saturation values for N_2 fixation and growth respectively. Regular monitoring studies in Moreton Bay, Queensland, show that concentrations of P-PO$_4$ generally exceed these saturation values over a large portion of the Bay and therefore, the growth of the bloom-forming *L. majuscula* is potentially maximised throughout much of the Bay by the elevated P-PO$_4$ concentrations. Results from other studies suggest that the elevated P-PO$_4$ concentrations in the Bay can be largely attributed to discharges from waste-water treatment plants (WWTPs), and thus it is proposed that the control of the growth of *L. majuscula* in Moreton Bay will require a significant reduction in the P load from the WWTP discharges. If the current strategy of N load reduction for these discharges is maintained in the absence of substantial P load reduction, it is hypothesised that the growth of *L. majuscula* and other diazotrophs in Moreton Bay will increase in the future.

KEY WORDS: *Lyngbya majuscula* · Nitrogen fixation · Phosphorus · Continuous culture · Growth kinetics · Moreton Bay

INTRODUCTION

Extensive blooms of the cyanobacterium (blue-green alga) *Lyngbya majuscula* have recently been recorded in the Deception Bay region of northern Moreton Bay Queensland (see Fig. 1) and in other regions along the coast of Queensland (Dennison et al. 1999). The blooms in Deception Bay have had a major impact on the ecology, with associated fish and sea grass kills being recorded. Directly associated human health problems, namely respiratory problems and skin irritations have led to closure of beaches in Moreton Bay. A very extensive bloom of another cyanobacterium, *Trichodesmium*, occurred in Moreton Bay in November

2002; this also led to the closure of beaches. Such blooms of cyanobacteria appear to be on the increase in other regions along the coast of Queensland (Bell et al. 1999) and are affecting many coastal regions around the world (Zimmerman & Montgomery 1984, Lapointe & Clark 1992, Porter & Meier 1992, Shannon et al. 1992, Lapointe et al. 1993, Sellner 1997, Nagle & Paul 1998, Kuffner & Paul 2001). The N_2 fixing ability of these taxa suggests that nutrients other than nitrogen, e.g. phosphorus (P) and/or iron (Fe), are contributing to the occurrence of these blooms. P and Fe are required by all algae for the synthesis of various cell constituents, but N_2-fixing cyanobacteria (diazotrophs) require additional amounts of these nutrients
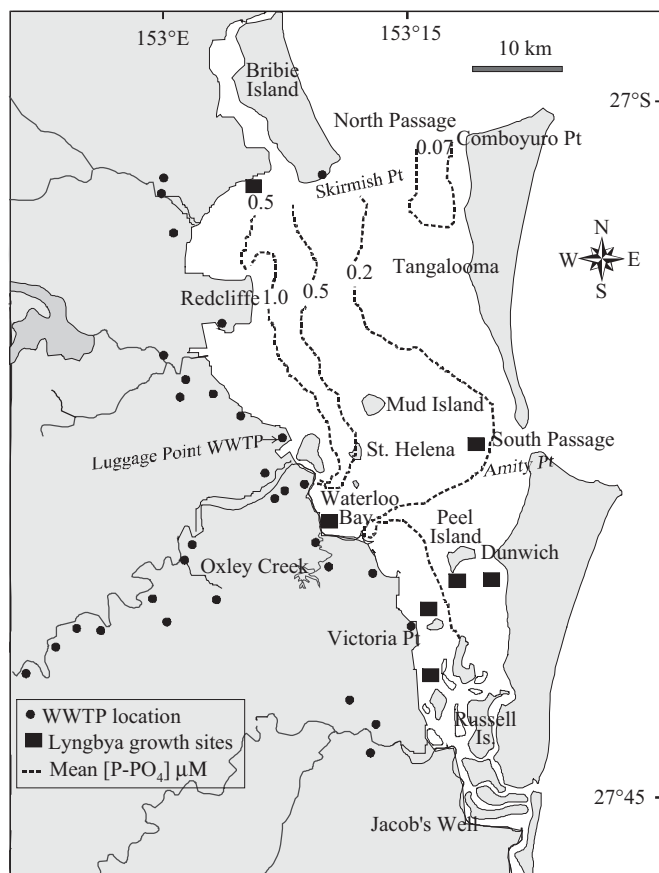
Fig. 1. The Moreton Bay region of SE Queensland, Australia show-ing locations of waste-water treatment plants (WWTPs), sites of significant *Lyngbya majuscula* growth (LMS 2003) and contours of mean P-PO$_4$ concentrations (EHMP 2001)

for synthesis and activation of nitrogenase (Howarth et al. 1988, Paerl 1990). The 'new' N fixed by the dia-zotrophs provides an important source of N for other alga taxa; it has been suggested that oceanic produc-tivity depends, to a significant extent, on this 'new' N and therefore, the productivity in the oceanic regions as a whole could well be P-limited (Tyrrell 1999).

The genus *Lyngbya* is a widespread and often a dominant component of marine and freshwater micro-bial mats. Previous studies have shown that various marine and freshwater species of *Lyngbya*, including *L. majuscula*, fix N$_2$ (Jones 1990, Paerl et al. 1991, Phlips et al. 1992, Dennison & Abal 1999). Dennison et al. (1999) hypothesised that Fe was primarily responsi-ble for *L. majuscula* blooms in Deception Bay. This hypothesis was based on a set of batch bioassays, which showed that Fe-EDTA stimulated the N$_2$ fixation of *L. majuscula*. However, the same study showed that the addition of EDTA alone also stimulated the N$_2$ fix-ation rate of *L. majuscula*, which suggests that it was

the bioavailability of some trace component, not neces-sarily Fe, that was limiting the N$_2$ fixation in their sam-ples. Dennison et al. (1999) recognise that P loading is often an important factor in controlling the growth of cyanobacteria in coastal ecosystems but suggested it was not an important factor in the Deception Bay region because the concentrations of P-PO$_4$ were relatively low. However, it is noted that no studies to date have determined the relationship between the growth of *L. majuscula* and the concentrations of P-PO$_4$. The present study uses a series of batch and semi-continuous studies to investigate the importance of P-PO$_4$ in promoting the N$_2$ fixation rate and growth rate of *L. majuscula*, and shows that the concentrations of P-PO$_4$ throughout most of Moreton Bay are actually relatively high when compared with the P-require-ments of *L. majuscula*.

It is generally accepted that the growth rate of algae is a function of intracellular P concentration and that this internal concentration is a function of the external P concentration. Therefore, an exact relationship between external P concentrations and growth rate during P-limited growth conditions is only deter-minable during steady-state growth situations, i.e. where the external- and hence the internal-cellular P concentrations remain constant (Fuhs 1969, Droop 1973, Goldman 1977). Such steady-state conditions cannot be achieved in batch cultures during nutrient-limited growth situations because the internal and external P concentrations are varying, and therefore chemostat type studies have been employed in some past studies on phytoplankton growth (Fuhs 1969, Goldman 1977). Practically though, such procedures are not useful for studying all algal taxa. For example, some taxa will not grow under continuous light condi-tions (e.g. *Trichodesmium* spp.) and others (e.g. macroalgae) will not readily mix and exit with the flow of medium. Both these aspects are relevant to the growth of *Lyngbya majuscula*. Firstly, it grows in the light but, as is discussed below, it fixes N$_2$ mainly in the dark, and secondly, it grows as long continuous fila-ments that cannot easily mix and exit with the medium. Thus, continuous flow culture methods had to be devised that allowed the biomass to be retained and yet maintained a relatively constant external P-PO$_4$ concentration throughout each experiment.

MATERIALS AND METHODS

All chemicals used in the study were analytical grade reagents. All glassware and pump tubing were acid-washed and thoroughly rinsed with Milli-Q water prior to use. *Lyngbya majuscula* Harvey ex Gomont was collected from a shallow region at the northern

end of Deception Bay in February 2000 (Fig. 1) and cultured in a nitrogen-free artificial seawater medium based on the Aquil medium of Morel et al. (1979) containing 3 µM P-PO$_4$. For the purpose of this study, the culture was adapted and grown in an enriched seawater medium (ESW) (Table 1), because the salt solution used to make the Aquil medium was found to contain significant levels of soluble reactive phosphorus (i.e. P-PO$_4$ of 0.4 µM). The seawater was collected from the northern section of the outer Great Barrier Reef near to Agincourt Reef, in a deep channel away from the influence of the coral reefs; the seawater was pre-filtered through Millipore GF filters, filter-sterilised through 0.2 µm sterile-filter and stored in 20 l acid-washed containers. This seawater was analysed for P-PO$_4$, ammonia and nitrate on a Lachat autoanalyser; all were found to be below the detection limits (0.03 µM P and 0.07 µM N). All media were filter-sterilised using 0.2 µm sterile-filters and were stored at 4°C in a refrigerator. All media were again filter-sterilized prior to use. Examination of the cultures under 1000× magnification using normal light and epifluorescence microscopy (Hass 1982) demonstrated that the stock cultures were unialgal and that minimal, if any, bacterial growth occurred on the surface of the filaments or in the medium.

Growth experiments. The growth studies were investigated using batch and continuous cultures in 35 ml glass Petri dishes (10 cm diameter) fitted with glass tops. Filaments were selected from the stock culture and were cut to 3 mm lengths just prior to the start of each experiment. The growth conditions were maintained at an average laboratory temperature of 25°C and cultures were exposed to a 12 h light:12 h dark light cycle. Light irradiance (PAR) of 55 µmol quanta m^{-2} s^{-1} was provided from a bank of cool fluorescent tubes above the culture vessels. Biomass was determined daily by measuring the length of the filaments (mm) in the glass culture dishes under a stereomicro-

scope. It is noted that the filaments are denser than the medium, and hence they sank and stayed on the bottom of the chamber even during the continuous flow studies. The measurements were conducted at the same time each day to provide accurate daily growth rate calculations. On occasion, some objectiveness was required in the measurement in order to take into account bending and tangles of filaments. Forceps were used to gently grip and straighten the filaments to facilitate the measurements.

In each batch experiment, one 3 mm filament of *Lyngbya majuscula* was placed in a culture dish with 25 ml of the various ESW media. The ESW media were made up with a wide range of P-PO$_4$ additions (0, 0.08, 0.16, 0.33, 0.66, 1, 4 µM). Cultures were grown in triplicate for each treatment for up to 16 d and biomass was measured daily.

The continuous-flow culture (CFC) system consisted of a pump, tubing and the same type of culture dish used for the batch studies (35 ml glass Petri dish with glass cover). Two small holes were drilled, 1 on each side of the glass cover, to provide for the inlet and outlet tubing (3 mm OD; 0.9 mm ID). Medium was pumped to and from each CFC reactor at the same flow-rate using multiple-head peristaltic pumps. The flow rates (F, ml h^{-1}) were measured and calibrated using a 5 ml measuring cylinder and a timer prior to the start of each experiment, and were monitored daily by measuring the outflow volume collected. The volume of liquid in each CFC reactor (V, ml) was maintained constant at 25 ml; this was aided by placing the exit flow tube at a fixed height above the bottom of the CFC reactor. An initial series of experiments was carried out with a residence time ($\tau = V/F$) of ~2 h, i.e. the reactor volume was changed every 2 h which is equivalent to a dilution rate ($D = 24 \times F/V$) of ~12 d^{-1}. It was noted that with the initial experiments the exit P-PO$_4$ concentration tended to drop by about 0.03 µM after about 4 d growth, and therefore the flow rate was increased by about 10% every 4 to 5 d to counteract this. Mixing was aided by placing the inflow tube above the solution surface and the energy of the waves generated from the falling drops supplied significant mixing in the vessel. The ESW outflow (exit) samples were collected daily, filtered (0.2 µm filter pore size) and analysed for P-PO$_4$ using the method of Parsons et al. (1984).

The CFC experiments were initiated by filling each culture vessel with 25 ml of the various media containing the different P-PO$_4$ concentrations. Four 3 mm filaments of *Lyngbya majuscula* were placed in each CFC reactor. Flow of medium was started immediately and maintained on a continuous basis over the entire culture period (~2 wk). In order to achieve a wide range of exit P-PO$_4$ concentrations, 8 inlet P-PO$_4$ concentrations

Table 1. Enriched seawater medium (ESW)

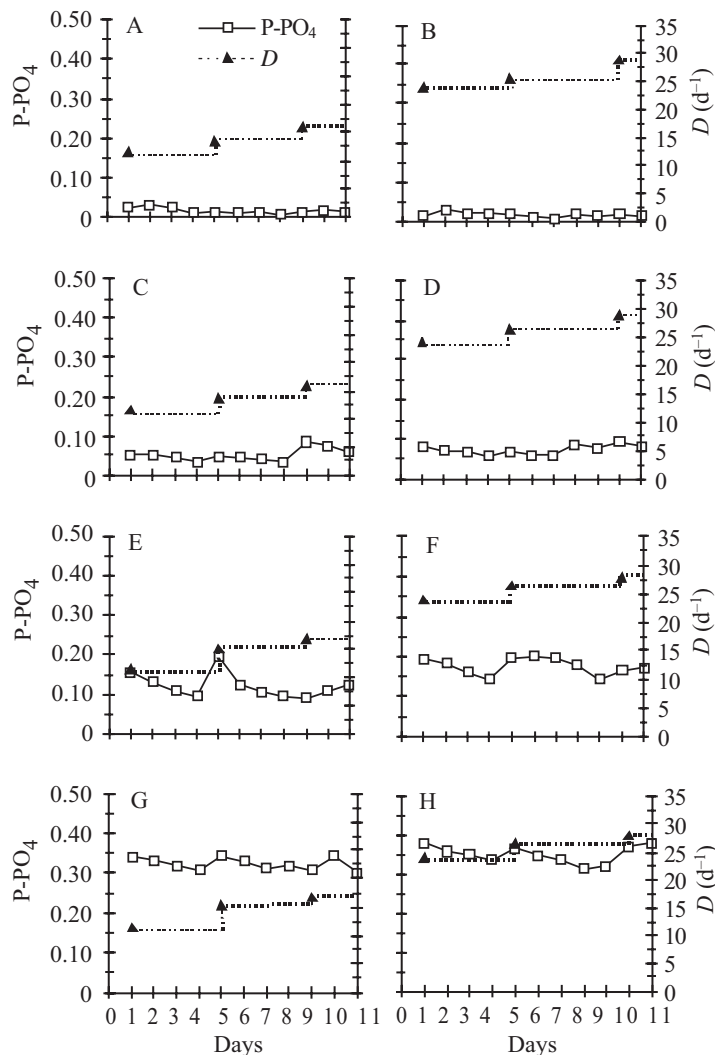| Nutrient	Chemical used	Concentration (M)
P-PO$_4$	KH$_2$PO$_4$	3.00×10^{-6}
Trace metal	Fe-EDTA	2.50×10^{-7}
	Na$_2$H$_2 \cdot$EDTA$\cdot$2H$_2$O	$4.5 \ \times 10^{-6}$
	CuSO$_4 \cdot$5H$_2$O	9.97×10^{-10}
	(NH$_4$)$_6$Mo$_7$O$_{24} \cdot$4H$_2$O	1.50×10^{-9}
	CoCl$_2 \cdot$6H$_2$O	2.50×10^{-9}
	MnCl$_2 \cdot$4H$_2$O	2.30×10^{-8}
	ZnSO$_4 \cdot$7H$_2$O	4.00×10^{-9}
Vitamins	B$_{12}$	3.62×10^{-7}
	Biotin	2.05×10^{-6}
	Thiamine HCl	2.88×10^{-4}

Fig. 2. Time course of CFC exit P-PO$_4$ concentrations (µM). (A–H) represent the different CFC reactors with high and low dilution rates, D (d^{-1})

N$_2$ fixation. Preliminary studies were conducted on the stock cultures to determine the effects of the light/dark cycle on the N$_2$ fixation. Four actively growing samples were transferred to 15 ml wide mouth serum bottles for determination of N$_2$ fixation rates using the acetylene reduction method (Capone 1993). The filaments had been growing for 5 d on a 12 h light:12 h dark cycle. Samples were selected following an initial 3 h exposure to light. Each bottle was sealed with a long-skirted red-silicone rubber serum stopper. Acetylene gas, obtained from a cylinder, was injected into each bottle using a gas-tight syringe to give an initial gas phase concentration of at least 15%. A 0.1 ml gas phase sample was extracted from each bottle at zero time using a gas tight syringe and was analysed immediately for ethylene concentration using a Photovac 10s plus a portable gas chromatograph fitted with a photo ionisation detector. The sample bottles were incubated in the laboratory at 25 ± 1.5°C for periods of 9 h in the light (45 µmol quanta m^{-2} s^{-1}) followed by 12 h in the dark. Gas samples (0.1 ml) were taken periodically throughout the light/dark incubation period with a gas tight syringe and analysed for ethylene. The gas phases of several control blanks, i.e. bottles containing filtered medium with added acetylene but with no added *Lyngbya majuscula*, were also analysed at zero time and after the incubation period in order to determine the background ethylene concentration in the absence of any acetylene consumption by *L. majuscula.* The bottle contents were gently removed at the end of each experiment and the length (mm) of the filaments was estimated under a stereotype microscope fitted with a scale. A molar conversion factor of 4:1 was assumed in converting the ethylene production rates to N$_2$ fixation rates (Capone 1993).

The effect of various P-PO$_4$ concentrations on the N$_2$ fixation rate was also investigated. The *Lyngbya majuscula* filaments used in this experimental work were removed from the stock cultures, cut into 10 mm lengths under a stereomicroscope and transferred to ESW containing no added P (P-PO$_4$ < 0.03 µM) and incubated (25 ± 1.5°C; 12 h dark:12 h light; 55 µmol quanta m^{-2} s^{-1}) for 3 d in order to starve the cells from the stored phosphorus. The phosphorus-deficient filaments were then transferred to 100 ml flasks containing 50 ml of medium containing various amounts of added P-PO$_4$: 0.1, 0.2, 0.33, 0.67, 1.0 µM. The cultures were grown for 2 d in order to establish a pseudo-steady state equilibrium between the external P concentration and the cell P concentration. A 'zero'-control sample was also prepared by incubating filaments

in the range of 0.02 to 0.4 µM were used in conjunction with a series of low-flow ($D \approx 12$ d^{-1}) and high-flow ($D \sim 30$ d^{-1}) experiments. To demonstrate there was no loss of P-PO$_4$ caused by other factors, a control CFC reactor containing no *L. majuscula* was run in parallel.

Each CFC experiment provided 1 value for the mean specific growth rate (μ) at 1 mean ambient concentration of P-PO$_4$. In carrying out these calculations, the results for the first 5 d of each experiment were ignored in order to avoid any adaptation/nutrient storage effects. Thus, mean specific growth rates were determined during the 5 d growth period from Days 6 to 11 for each reactor. The success of the reactor set-up and operating procedures are well demonstrated by the achievement of essentially constant reactor exit P-PO$_4$ concentrations for significant periods for all experiments (Fig. 2).

for 2 d in ESW with no added P-PO$_4$. After the incubation period, the filaments plus medium were transferred to 15 ml wide mouth serum bottles for determination of dark-induced N$_2$ fixation rates using the acetylene reduction method as described above. All experiments were done in duplicate. Light-induced N$_2$ fixation was not investigated in this set of experiments because, as discussed below, the initial experiments had shown this to be minimal when compared with the dark-induced N$_2$ fixation. Samples for P-PO$_4$ analysis were collected from all cultures, filtered (0.2 µm pore size) and analysed using the method described in Parsons et al. (1984).

RESULTS AND DISCUSSION

N$_2$ fixation studies

Only minimal acetylene reduction, and consequently N$_2$ fixation, was observed during the light periods, which suggests that oxygenic photosynthesis and N$_2$ fixation are incompatible processes for this species. These results confirm those of Lundgren et al. (2003), who found that natural populations of *Lyngbya majuscula* only fixed nitrogen during the dark phase of a diel cycle but do not agree with those of Jones (1990) and Dennison et al. (1999), who found that field-collected samples of *L. majuscula* exhibited nitrogenase activity only during the light phase. Paerl et al. (1991) found that *L. aestuarii*, which if one were to adopt the classification of Drouet (1968) is the same species as *L. majuscula*, exhibited nitrogenase activity in both the light and dark but the fixation in the dark was much greater than that exhibited in the light. The temporal separation of photosynthetic (light) and N$_2$ fixation (dark) processes as suggested by our results has previously been observed for other non-heterocystous diazotrophs, e.g. *Gloeothece* sp. and *Oscillatoria* sp. (Stal & Krumbein 1985). Stal & Krumbein (1985) also showed that when the dark cycle is reduced to 4 h, significant N$_2$ fixation by *Oscillatoria* spp., which normally only occurs in the dark, also occurs in the light. Also, Phlips et al. (1992) showed that aerobic N$_2$ fixation by *L. wollei* varies significantly with light intensity and with the duration of light:dark cycles. Thus, it is possible that the results of Jones (1990) and Dennison et al. (1999) for their field-collected samples are a reflection of the changed light/dark cycle conditions between the field and the laboratory. It is also possible that the field samples might have been contaminated with other bacteria/cyanobacteria although Jones (1990) suggests this is unlikely for his samples.

The dark-stimulated acetylene reduction rate, and consequently N$_2$ fixation rate, increased linearly with

P-PO$_4$ concentration up to 0.27 µM and then showed no further increase up to 0.85 µM (Fig. 3). These findings are in general agreement with the work of Stewart & Alexander (1971), which showed that the acetylene reduction rate for 3 freshwater diazotrophs was proportional to the P-PO$_4$ concentration up to approximately 0.6 µM with no further increase beyond 1.6 µM. Mague et al. (1974) also showed that increased P-PO$_4$ concentrations stimulated acetylene reduction in samples containing the N$_2$ fixing *Richelia intracellularis*, and Carpenter & Price (1977) and Sañudo-Wilhelmy et al. (2001) found that higher cellular N$_2$ fixation rates for *Trichodesmium* correlated with elevated P-PO$_4$ concentrations. In physiological terms, N$_2$ fixation is a very energetically demanding process, requiring between 12 and 16 molecules of ATP for the reduction of 1 molecule of N$_2$ (Postgate 1982) and thus, the strong dependence of N$_2$ fixation on the concentration of P-PO$_4$ is not surprising.

Batch growth studies

Results from the batch studies (Fig. 4) show the final biomass achieved is dependent on the initial P-PO$_4$ concentration, i.e. the system is P-limited. The results also show that the initial growth rates for all treatments including the 'zero'-control medium were essentially the same over the first 3 to 4 d, suggesting that a 3 to 4 d lag period is required for this diazotroph to adapt to the new environment. It is noted that the initial growth during this period would be largely driven by the initial cellular P content, which would be essentially the same for all cultures. The results for the higher concentration media ($\geq$0.33 µM) are quite similar for the first 10 d of growth, while there is a definite drop-off in growth during that period for the cultures in media with lower initial concentrations of P-PO$_4$. As noted
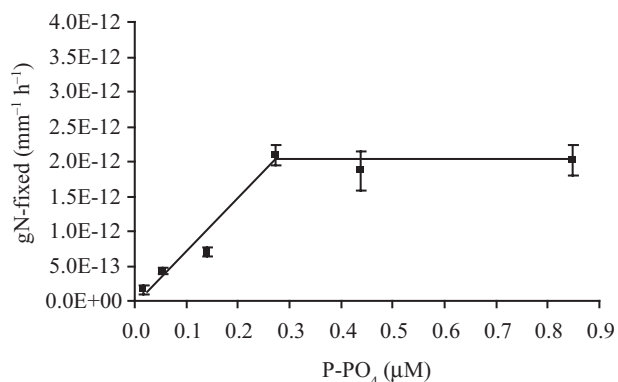

Fig. 3. *Lyngbya majuscula*. Mean nitrogen fixation rates for different P-PO$_4$ concentrations. Error bars denote SD
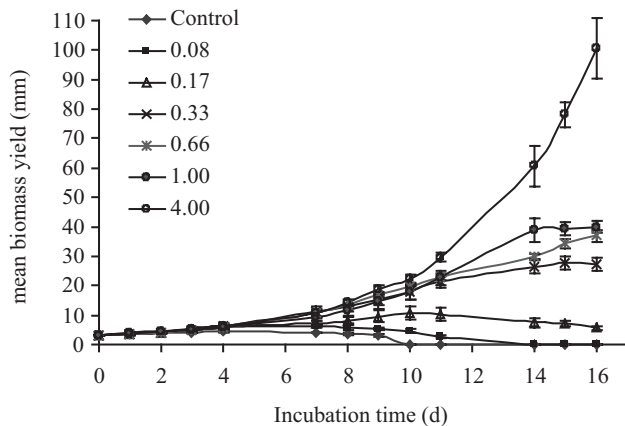
Fig. 4. *Lyngbya majuscula*. Effect of P-PO$_4$ concentrations on growth in batch culture studies. Legend shows P-PO$_4$ concentration in µM. Error bars denote SD

above, the concentration of P-PO$_4$ would be varying with time in these batch cultures, and therefore it is not possible to determine the direct relationship between external P concentrations and growth rate from such experiments. Overall, such batch studies can provide useful information on the nutrient uptake kinetics from pulsed inputs and the status of nutrient limitation, but provide little useful information on the effects of low levels of limiting nutrients on growth rates.

Continuous flow culture studies

The results from the continuous growth studies (Fig. 5) show that the growth rate increases with P-PO$_4$ concentration up to about 0.18 µM, beyond which a plateau is reached at ~0.33 d^{-1}. This result is similar to that obtained for the relationship between N$_2$ fixation
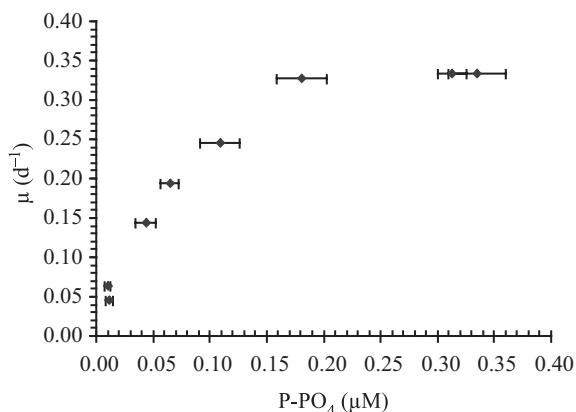

Fig. 5. *Lyngbya majuscula*. Effect of P-PO$_4$ concentration on specific growth rate, µ (d^{-1}) in continuous flow culture (CFC) studies. Error bars denote SD

rate and P-PO$_4$ concentration suggesting that the N$_2$ fixation rate could play an important role in controlling the growth rate. Various formulations have been used in the past for describing the relationship between phytoplankton productivity or growth rate and the concentration of P-PO$_4$ in a P-limited system. Many workers have assumed a Monod type relationship (Tyrrell 1999) but the formation of the plateau suggests such a relationship would not be appropriate for our data. Early modelling studies on phosphorus-limited growth of phytoplankton in the marine environment (Steele 1958, Riley 1963) assumed that below a limiting or saturation concentration (C_s = 0.4 µM), there was a linear relationship between the P-PO$_4$ concentration and growth rate:

$$\mu \;=\; \frac{C}{C_s}\mu_{max} \qquad C \leq C_s \qquad\qquad (1)$$

$$\mu \;=\; \mu_{max} \qquad C > C_s \qquad\qquad (2)$$

where µ = specific growth rate d^{-1}, μ_{max} = maximum specific growth rate (varies with light intensity and temperature) (d^{-1}) and C = P-PO$_4$ concentration (µM). Our data could be fitted to such a saturation type kinetics model ($C_s \approx 0.18$ µM; $\mu_{max} \approx 0.33$ d^{-1}) but the fit would not be good for values $\leq C_s$; our data suggest that a curvilinear linear relationship would be more appropriate in the lower P-PO$_4$ concentration range 0.0 to 0.18 µM.

Other experimental studies on P-limited growth support the concept of a saturation type model. The results of Thomas & Dodson (1968) obtained from batch studies on P-limited growth of the diatom *Chaetoceros* exhibit a reasonable fit to a linear saturation type model with $C_s \approx 0.22$ µM. Thomas & Dodson (1968) attempted to fit the hyperbolic Monod relation to their results but experienced some difficulty in doing so. Also, Fuhs et al. (1969) conclude that such a linear saturation model or a sharply bent asymptotic curve would be applicable to results that they obtained for P-limited growth of phytoplankton in their continuous-flow culture experiments, but their experimental procedures did not allow them to quantify such a relationship.

Implications for management of *Lyngbya majuscula* blooms in Moreton Bay

The discharge of effluents from waste-water treatment plants (WWTPs) is the principal external source of P to Moreton Bay (Fig. 6) (Eyre & McKee 2002). Many of the WWTPs discharge directly to the Bay, while others discharge to the estuarine sections (Fig. 1). In order to quantify the effects of the discharges on the water quality within the Bay, it is nec-

essary to have information on the water quality outside the Bay, but this is not available. There is a limited amount of data for the eastern extremities of the Bay near to the entrance to Moreton Bay, and these data suggest an 'incoming-background' concentration for P-PO$_4$ (C_b) of ~0.07 µM (EHMP 2001). It is noted that the Bay waters would influence these waters, and therefore the true 'incoming-background' concentration could be somewhat less than this value; further studies are required to establish a more reliable estimate of C_b. Recent water quality data from within the Bay (Fig. 1) show that the concentration of P-PO$_4$ is much greater than C_b over most of Moreton Bay. Modelling studies on the effects of the WWTP discharges on the Bay show that the elevated concentrations (i.e. $\geq C_b$) of P-PO$_4$ within the Bay can be attributed largely, if not solely, to the WWTP discharges (McEwan et al. 1998, Bell et al. 2003). The results in Fig. 5 show that the growth rate of *Lyngbya majuscula* is extremely sensitive to small increases in concentration of P-PO$_4$ above C_b. Thus, the results suggest that, provided it can be verified that C_b is significantly less than C_s, the P-PO$_4$ discharges from the WWTPs are producing an accelerated growth regime for *L. majuscula* throughout most, if not all, of Moreton Bay. Indeed, it is noted that P-PO$_4$ concentrations generally exceed C_s throughout most of the Bay, and consequently there is potential for *L. majuscula* to grow at its maximum rate throughout most of the Bay. Dennison et al. (1999), in dismissing the importance of P-PO$_4$ in the formation of *Lyngbya* blooms in Moreton Bay, had suggested that P-PO$_4$ concentrations were low throughout the *Lyngbya*-bloom region, whereas in fact they ranged from 0.2 to 0.4 µM and averaged 0.28 ± 0.02 µM throughout the bloom period (Watkinson 2000), i.e. they were greater than C_s and therefore would have provided for a maximal growth environment. We

hypothesise that elevated P-PO$_4$ concentrations in the Bay are a primary contributor to the formation of blooms of *L. majuscula* within Moreton Bay and that, if it can be established that the WWTP discharges are the primary cause of the elevated P-PO$_4$ concentrations, then a reduction in the extent of these blooms will require a substantial reduction in the loads of P-PO$_4$ from the WWTPs.

Results from some other studies had suggested that algal productivity in Moreton Bay is limited by the availability of N, and based on these results, an extensive program on the reduction of N loads from WWTPs was recommended and is now being implemented (Dennison & Abal 1999, Abal et al. 2001). It is important to note that the present emphasis of reducing only N loads from the WWTPs will reduce not only the concentrations of N in the Bay but will also reduce the N:P ratio. This is important because it is now generally accepted that the conditions long recognised as promoting the growth of diazotrophs in freshwater systems, namely low N concentrations and a low N:P ratio, will also promote growth of diazotrophs in coastal waters (Niemi 1979, Sellner 1997). It is suggested that the control of bloom-forming diazotrophs such as *Lyngbya majuscula* in Moreton Bay will require consideration of both aspects, namely N concentrations and the N:P ratio. It is hypothesised that that the lower N concentrations and N:P ratios that will occur in many, if not most, regions of the Bay as a result of the N-only removal strategy, will lead to an increase in the growth of *L. majuscula* and other diazotrophs in Moreton Bay.

Conclusions

Significant acetylene reduction, and consequently N$_2$ fixation, was observed for *Lyngbya majuscula* during dark periods and only a minor amount occurred during light periods, which suggests that oxygenic photosynthesis and N$_2$ fixation are incompatible processes for this species. The specific growth rate and N$_2$ fixation rate of *L. majuscula* increased with P-PO$_4$ concentration up to a maximum value and thereafter remained constant. The P-PO$_4$ concentration corresponding to the maximum N$_2$ fixation rate was ~0.27 µM and that corresponding to maximum growth rate was ~0.18 µM. These values are denoted as the saturation values for N$_2$ fixation and growth respectively. The concentrations of P-PO$_4$ throughout Moreton Bay generally exceed these saturation values, and hence the growth of the bloom-forming *L. majuscula* is potentially maximised throughout much of the Bay by the elevated P-PO$_4$ concentrations. Other studies suggest that the elevated P-PO$_4$ concentrations in the Bay can be largely attributed to discharges from WWTPs.
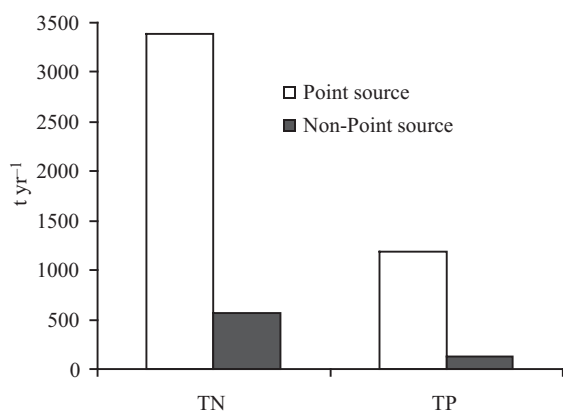

Fig. 6. Comparison of point source (i.e. WWTP discharges) and non-point source (i.e. run-off) loads of TN and TP to Moreton Bay (after Eyre & McKee 2002)

Thus, it is proposed that the control of the growth of *L. majuscula* in Moreton Bay will require a significant reduction in the P load from the WWTP discharges. If the current strategy of N load reduction for these discharges to Moreton Bay is maintained in the absence of substantial P load reduction, it is hypothesised that the growth of *L. majuscula* and other diazotrophs in Moreton Bay will increase in the future.

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