

ECOPHYSIOLOGY OF THE MARINE
CYANOBACTERIUM, LYNGBYA
MAJUSCULA (OSCILLATORIACEA).

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THESIS SUBMITTED TO THE DEPARTMENT OF BOTANY,
UNIVERSITY OF QUEENSLAND,
FOR THE PARTIAL FULFILMENT OF BACHELOR OF SCIENCE (HONS).

15TH MAY, 2000.

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This thesis is being submitted in the form of a scientific paper for the purpose of publishing in the Experimental Journal of Marine Biology and Ecology. A table of contents are attached for the purpose of this thesis.

I declare that this thesis does not contain any material that has been submitted by me previously for any degree or diploma to a university, and to the best of my knowledge, it does not contain any material published or written by another person, except where due reference is made in the text.

Andrew Watkinson

May 2000

ACKNOWLEDGMENTS

Thank-you to my supervisors, Bill and Judy, for all the help you have given over the past couple of years. The advice and trust you both put into my research will prove invaluable in the years to come. Thank- you also to my parents who have supported me throughout my life and given me the freedom to chose my own directions, even if some of them were pretty naive.

To my partner in crime, Ian, who always kept the pressure on me by having everything done 6 weeks early. We have travelled a long road together, and I think its time for a well- earned rest. Thank-you to my extended field crew, in particular Matty and Rory, who both willingly rose in the early hours of the morning at a moments notice to brave the *Lynghya* waters of Deception Bay. Thanks also to Greg Savage for all his help and information in relations to the bloom.

Thanks also to Simon, Dieter and Catherine for all the editing help. I must also thank Chris and Ben, for taking time away from their precious coffee drinking, to help me with mapping and datalogger problems, and the rest of the Marbot crew for being such a great bunch of people.

Thanks also to my best friend Becky, who has supported me through a lot and is always there for me.

Thank-you to Dan Wruck and Co. at Queensland Health for not only analysing all my nutrients, but taking an interest in my research to ensure quality results. Thanks also to the School of land and water at UQ for elemental analysis.

Science is facts; just as a house is made of stone, so is science made of facts; but a pile of stones is not a house, and a collection of facts is not necessarily science.

Jules Henri Poincaré (1854-1912)
French mathematician.

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ABSTRACT

A bloom of the marine cyanobacterium *Lyngbya majuscula* in Moreton Bay, Australia (27°05' S, 153°08' E) has been reoccurring for several years. This bloom was studied in detail over the period Jan-Mar 2000. *In situ* data loggers and field sampling characterised various environmental parameters before and during the *L. majuscula* bloom which was mapped repeatedly. The effect that the *L. majuscula* had on various seagrasses in the bloom region was assessed with repeated biomass sampling. Various ecophysiological experiments were conducted on *L. majuscula* collected in the field and transported to the laboratory. In these experiments, short term (2 h) ^{14}C incorporation rates and longer term (7 d) pulse amplitude modulated (PAM) fluorometry assessments of photosynthetic capacity were measured. The bloom initiated in January 2000 following December rainfall events and water temperatures in excess of 24°C and high light conditions, expanded rapidly ($100\text{ m}^{-2}\text{ min}^{-1}$) to a maximum extent of 8 km^{-2} and an average biomass of $210\text{ g}_{\text{dw}}^{-1}\text{ m}^{-2}$ in late February, followed by a rapid decline. Seagrass biomass declined in areas of dense *L. majuscula* accumulation, especially *Syringodium isoetifolium* biomass. Dissolved and total nutrient concentrations did not significantly ($p > 0.05$) differ preceding or during the bloom. However, water samples from the creeks discharging into the study region indicated elevated concentrations of total iron ($2.7 - 80.6\text{ }\mu\text{M}$) and dissolved organic carbon ($2.5-24.7\text{ mg L}^{-1}$), associated with low pH values (3.8-6.7). ^{14}C incorporation rates by *L. majuscula* were significantly ($p < 0.05$) elevated by additions of iron ($5\text{ }\mu\text{M Fe}$), an organic chelator, ethylenediaminetetra-acetic acid ($5\text{ }\mu\text{M EDTA}$) and phosphorus ($5\text{ }\mu\text{M PO}_4^{-3}$). Photosynthetic capacity measured with PAM fluorometry was also stimulated by various nutrient additions, but not significantly ($p > 0.05$). These results suggest that the *L. majuscula* bloom could have been stimulated by availability of bioavailable iron, perhaps complexed by dissolved organic carbon. The rapid bloom expansion observed could then be sustained by additional inputs of nutrients and iron through sediment efflux, stimulated by decomposing *L. majuscula* mats.

INTRODUCTION

Cyanobacterial blooms in Australia's freshwater supplies have been well documented in Australia since early 1878, when a report was published in *Nature*, on stock deaths resulting from water contamination in Lake Alexandrina, South Australia (Francis, 1878). While much of the early research and observations of cyanobacterial abundance was restricted to freshwater environments, the ecology and proliferation of marine cyanobacteria, especially toxic forms, is becoming increasingly important. Of particular concern is the increasing frequency of nuisance cyanobacterial blooms in urbanised coastal areas, such as *Lyngbya majuscula* occurrences in Southeast Queensland (Dennison *et al.*, 1999; Duffy & O'Neil, submitted).

Lyngbya majuscula (Oscillatoriaceae) is a filamentous, non-heterocystous marine cyanobacterium inhabiting tropical and sub-tropical estuarine and coastal waters (Jones, 1990; Shannon *et al.*, 1992). It is composed of thin, unbranched filaments, often olive- grey in colour (Cribb, 1996). *L. majuscula* is often found forming benthic mats loosely attached to a substrate form, such as seagrass, macroalgae or rock outcrops (Diaz *et al.*, 1990; Dennison *et al.*, 1999). Additionally, mats can rise to the surface through the accumulation of gas bubbles (Benz *et al.*, 1979; Diaz *et al.*, 1990; Dennison *et al.*, 1999). Seasonal blooms of *L. majuscula* have been reported in the Deception Bay/ Pumicestone passage areas of Moreton Bay, Queensland, for nearly a decade (Dennison & Abal, 1999). There is anecdotal evidence that the blooms have increased in severity and extent since first reports were made in the early 1990s.

Nitrogen has been identified as the major limiting nutrient for productivity in Moreton Bay (Horrocks *et al.*, 1995; O'Donohue & Dennison, 1997; Udy & Dennison, 1997). Therefore, it is not surprising to find the proliferation of a nitrogen-fixing organism under potentially N-limiting conditions. Substantial rates of nitrogen fixation ($0.09\text{--}1.24 \mu\text{M g}^{-1} \text{h}^{-1}$) have been recorded for *L. majuscula* in Deception Bay (Duffy & O'Neil, submitted). Estimates of nitrogen input through nitrogen fixation have been calculated at $1.14 \text{ mg N m}^{-2} \text{h}^{-1}$ during the peak periods of blooms (Duffy & O'Neil, submitted). Once fixed as cyanobacterial biomass, this nitrogen has the potential to be released to the environment through direct release, or trophic interactions.

Phosphorus often becomes a limiting nutrient controlling cyanobacterial growth through high metabolic demands (ATP) and a tendency to precipitate from solution (Cosgrove, 1977). Growth of cyanobacteria is often directly related to phosphorus loading since nitrogen deficiencies can be overcome through nitrogen fixation (Paerl *et al.*, 1987), which is often limited by phosphorus availability (Paerl *et al.*, 1991)

Micronutrients, such as iron (Paerl *et al.*, 1994) and molybdenum (Howarth & Cole, 1985; Paerl *et al.*, 1987), can be potentially limiting factors for N₂ fixation and consequently, cyanobacterial growth. Iron has been identified as an important limiting nutrient in plankton productivity (Tranter & Newell, 1963; Martin *et al.*, 1990), and in particular, cyanobacterial productivity (Paerl *et al.*, 1994). Iron is a major component of ferredoxin, one of the primary constituents of Photosystem I (Syrett, 1981), which provides energy for N₂ fixation. The nitrogenase enzyme, required for N₂ fixation, contains iron in both of its subunits (Postgate, 1987).

Preliminary studies suggest that iron may be a key nutrient stimulating blooms in Deception Bay (Duffy & O'Neil, submitted). Concentrations of total Fe measured in the vicinity of the *Lyngbya majuscula* blooms in Moreton Bay were very high (Dennison & Abal, 1999). It is hypothesised that these high concentrations may be due to leachate from hydric soils and other soil disturbances in the area. Previous studies have shown *L. majuscula* to be stimulated by iron additions in culture (Gross & Martin, 1996) and blooms have been reported in mullet aquaculture facilities in Egypt fed with the effluent from an iron crusher factory (Sadek *et al.*, 1986).

Blooms of *Lyngbya majuscula* have had serious detrimental ecosystem impacts to affected areas including: i) localised seagrass loss, ii) poor crab and fish harvests (reported by fishermen) in bloom years resulting from decline in biota, iii) increase in bacterial biomass with bloom decomposition, and iv) significant localised input of bioavailable nitrogen through nitrogen fixation and release of organic and inorganic nitrogen through decay (Duffy & O'Neil, submitted). In addition, the local economy suffers through affected commercial and recreational fisheries, declining recreational use of the region due to health concerns, and the removal of large beach wracks of decaying *L. majuscula* by local government for health and aesthetic reasons.

Lyngbya majuscula has also been shown to contain a suite of toxic secondary metabolites. These secondary metabolites have been shown to have adverse effects on humans, with reports of severe contact dermatitis, eye irritation and asthma-like symptoms (Dennison & Abal, 1999). Blooms of *L. majuscula* have been implicated as the causative agent in fish kills (Sadek *et al.*, 1986) and off-flavour in fish (Brown & Boyd, 1982).

The role of abiotic factors (ie. light, water temperature, salinity and rainfall) and ambient water quality parameters (ie. dissolved inorganic nutrients and trace metal concentrations) have been shown to be critical for the formation of cyanobacterial blooms in both marine (Sellner, 1992; Sellner, 1997) and freshwater environments (Codd *et al.*, 1994). The aim of the present study was to investigate the role of various environmental parameters in the stimulation of *Lyngbya majuscula* blooms in Deception Bay. This information may ultimately aid in devising a management strategy in order to potentially mitigate future *L. majuscula* blooms.

METHODS

STUDY SITE

This study was conducted in northern Deception Bay, at the southern end of Pumicestone Passage (27°05' S, 153°08' E). Deception Bay forms part of a larger embayment, Moreton Bay, adjacent to the city of Brisbane in southeast Queensland, Australia (Figure 1). Moreton Bay is influenced by a strong east-west gradient in water quality through elevated terrigenous inputs on the western shores (Neil, 1998), and increased oceanic flushing on the eastern shores via both the North and South Passages (Dennison & Abal, 1999). Deception Bay is no exception to this, influenced by terrigenous input, via the Caboolture River and Pumicestone Passage, and oceanic flushing via North Passage. The study area was largely restricted to the shallow banks adjacent to Pebble and Godwin Beaches (figure 2), based on previous reports of *Lyngbya majuscula* blooms in the region (Dennison *et al.*, 1999; Duffy & O'Neil, submitted).

Three major sites were chosen within the study area to monitor ambient environmental parameters (Figure 1). Site 1 was located in a deep channel adjacent to the seagrass beds, at a water depth of 5.6 m above Lowest Astronomical Tide (L.A.T.) (27°05.777' S, 153°09.297' E). Site 2 was located in shallow mixed species seagrass meadows adjacent to Godwin Beach consisting of the seagrasses *Zostera capricorni*, *Halophila spinulosa*, *Halophila ovalis* and *Halodule univernis*, at an approximate depth of 2.5 m above L.A.T (27°05.755' S, 153°06.976' E). Site 3 was located in shallow monospecific *Z. capricorni* beds adjacent to Pebble Beach (27°05.096' S, 153°08.693' E) at a depth of 2.5 m above L.A.T. An additional 7 sites were sampled to examine water quality in local creeks and waterways, following a rainfall event in November 1999.

FIELD OBSERVATIONS AND MEASUREMENTS

ABIOTIC MONITORING

Water column salinity and temperature were monitored in the bloom area throughout the study period using a surface data logger (392 Data Recorder) fitted with a salinity/temperature sensor. Measurements were taken every 15 min and averaged on a daily basis. Surface light was monitored adjacent to the bloom area on Bribie Island (27°05.623' S, 153°09.565' E). A cosine corrected 2π photosynthetically active radiation quantum light sensor was used to take measurements every 15 min and daily total light calculated ($\text{mol quanta m}^{-2} \text{ d}^{-1}$).

The Bureau of Meteorology Climate Centre supplied rainfall and wind data for the study period. Total daily rainfall was used from a weather station approximately 20 km from the study region. Wind speed and direction was recorded at midday, approximately 30 km from the study area.

Secchi depth (Z_{SD}) was measured at site 1 over the sampling period for all sampling times with the exception of sampling done on the 20th March 2000. Light attenuation (K_d) in the water can be calculated from secchi depth using the equation described in Kirk (1994). Although the available secchi depths, which were measured during field trips planned and undertaken in good weather conditions, could generate bias, these secchi depths are likely to be representative of light penetration during *Lyngbya majuscula* bloom initiation due to the calm weather conditions recorded in this period. The generated K_d ranged from 0.59 to 1.3 m^{-1} , with a mean of 0.78 m^{-1} .

WATER COLUMN NUTRIENTS

Water quality sampling was conducted over 7 trips between September 1999 to April 2000, including the periods both preceding and during the bloom. For total nutrient analysis, site water was collected into pre-rinsed 100 ml polycarbonate bottles. Water was also filtered through 0.45 μm Gelman Poretics glass fibre filters into pre-rinsed 100ml polycarbonate bottles for dissolved nutrient analysis. Samples were frozen on dry ice at the sites and subsequently analysed for dissolved and total nutrients on an automated LACHAT 8000QC flow injection system (Hosomi & Sudo, 1986; Clesceri *et al.*, 1998).

For total water column iron analysis, samples (250 mL) of site water were collected and fixed with 5mL of 70% nitric acid (v/v). Iron content was determined using inductively coupled plasma atomic emission spectrometry following a 50x dilution (AS3641.2, 1999). Water (60 mL) from the bloom monitoring sites and four of the runoff monitoring sites was filtered through 0.45µm Gelman Poretics glass fibre filters (GF/F) and frozen in pre-rinsed 100ml polycarbonate bottles for dissolved organic carbon analysis (catalytic combustion to CO₂ using a Shimadzu TOC-5000).

ELEMENTAL ANALYSIS

Samples of shoot, root and rhizome of the seagrass *Zostera capricorni* were collected for elemental analysis. Approximately 20 seagrass shoots with attached rhizome and roots were removed from sediments, washed and frozen on site. Subsequently the samples were separated into leaf and root/ rhizome material and washed in deionised water to remove any sediment. When present, samples of *Lyngbya majuscula* were also collected and frozen on site. Samples were sorted to remove non-*Lyngbya* material and rinsed in deionised water to remove sediments. Nine sediment cores were also taken to a depth of five cm, using 60 mL syringe corers, homogenised into a sample jar and frozen on site. All samples were then dried at 60°C, and homogenised into a fine powder using a stainless steel ball-bearing grinder (Reutch). A subsample was added to 15mL HNO₃/HClO₄ and digested through stepwise heat digestion to 180°C. Digested samples were diluted with triple-deionised water and elemental analysis of both the seagrass and the sediment was performed using inductively coupled plasma atomic emission spectrometry (Environmental Monitoring Systems Laboratory, 1996).

Samples of *Lyngbya majuscula* were collected for tissue carbon, nitrogen, phosphorus and iron content. Samples were rinsed in deionised water, dried and homogenised into a fine powder using a stainless steel ball-bearing grinder (Reutch). Tissue iron and phosphorus content was determined using inductively coupled plasma atomic emission spectrometry (Environmental Monitoring Systems Laboratory, 1996) as described above. Tissue carbon and nitrogen content and $\delta^{15}\text{N}$ signature was determined on a continuous flow isotope ratio mass-spectrometer (CF-IRMS, Tracer Mass, Europa scientific, Crewe, UK). The ratio of ¹⁵N

to ^{14}N in dried plant tissue, the relative amount of ^{15}N , or δ (delta) ^{15}N was determined using the following equation:

$$\delta^{15}\text{N} = [(atom \% sample - atom \% standard)/atom \% standard] * 1000$$

(Equation 1)

BLOOM EXTENT AND BIOMASS

The progression of the *Lyngbya majuscula* bloom was investigated by mapping aerial extent and biomass with bloom duration. After the initiation of the bloom, a ground truthing survey was conducted approximately every 2 weeks to map the spread of the bloom. Surveys were undertaken with the aid of local commercial fisherman and their observations of bloom extent. Each survey was aimed at identifying the presence or absence of *L. majuscula* across the study site through the use of viewing buckets, or by snorkelling and manta-towing areas in question. From these surveys, maps of the bloom area were developed in relation to local landmarks and structures, such as oyster leases, navigation beacons and topography using a global positioning system (GPS) fitted with a Garmin GBR21 differential. ArcView GIS (version 3.1.) was then implemented to generate the final maps of *L. majuscula* extent. In all, 5 maps were generated over the duration of the bloom, which lasted approximately 3 months.

Seven sites were sampled for biomass of *Lyngbya majuscula* as well as associated seagrass throughout the bloom. These sites included the 3 water quality sites and an additional four sites within the bloom area (Figure 1). At each site, 3 replicate cores were randomly sampled to a depth of 10 cm with a steel corer (diameter 15 cm). Cores were rinsed in a mesh bag to remove associated sediments and frozen until sorted. Biomass cores were sorted into seagrass leaf material, seagrass root/ rhizome material and *L. majuscula* biomass. Samples were dried (60°C) and weighed ($\pm 0.001\text{g}$), and biomass was calculated as $\text{g}_{\text{dw}} \text{m}^{-2}$. The change in seagrass biomass was calculated through % decline of seagrass biomass, leaf and root/ rhizome, over the period of the bloom as:

$$\% \text{ decline} = 100 - [(B_{\text{F}} - B_{\text{I}}) * 100]$$

(Equation 2)

where B_{F} is final biomass of leaf or root/ rhizome material and B_{I} is the respective initial biomass at that site with the onset of the bloom.

LABORATORY EXPERIMENTS AND MANIPULATIONS

FLUOROMETRY

PAM (Pulse Amplitude Modulation) fluorescence (Schreiber & Bilger, 1987) was used to determine photosynthetic response of *Lyngbya majuscula* to a multitude of experimental conditions. Two measures of fluorometry based photosynthetic response were incorporated for these experiments, photochemical efficiency and rapid light curves.

Photochemical efficiency (Fv/Fm ratio) provides a measure of the potential maximum Photosystem II quantum yield (Schreiber *et al.*, 1994) and was measured by emitting a saturating pulse of light to a dark acclimated photosystem where electron transport between the two photosystems is greatly reduced. From the resulting light pulse, fluorescence will be emitted at a maximum, and the photochemical efficiency or yield was calculated as:

$$(F_v/F_m) = (F_m - F_o) / F_m \quad (\text{Equation 3})$$

where Fm is maximum fluorescence and Fo is minimal fluorescence. The resulting ratio can provide a useful tool for examining the physiological status of the plant. In these experiments, photochemical efficiency was measured after a period of 15 minutes dark acclimation.

Rapid light curves (RLC) are another non-intrusive fluorometry technique for measuring photosynthesis. *Lyngbya majuscula* was exposed to nine saturating light pulses (>2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), where each pulse is preceded by a period of actinic light, and the intensity of actinic light increases stepwise. The intensity of intermittent actinic light ranged from approximately 20 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ to between 1200 and 1900 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. At each saturating pulse, the photosynthetic yield (Fv/Fm) was calculated as described in equation 3. From the photosynthetic yield, the electron transport rate (ETR) was calculated as:

$$\text{ETR} = F_v/F_m \times \text{PAR} \times 0.5 \times 0.84 \quad (\text{Equation 4})$$

where PAR is the actinic irradiance in $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, 0.5 is the multiplication factor to account for both photosystems and 0.84 is the species specific fraction of incident quanta absorbed by the cyanobacteria (White & Critchley, 1999).

From experimental rapid light curves, the maximum electron transport rate (ETR_{MAX}) was generated and used for comparison and analysis of data. The maximum electron transport rate (ETR_{MAX}) of a rapid light curve was described as the maximum rate after the light limiting phase, and was calculated from light curves using hyperbolic tangent modelling as described in Chalker (1981). Using this modelling, the associated saturating irradiance (I_k) was also calculated.

^{14}C INCORPORATION

Productivity of *Lyngbya majuscula* was measured using the ^{14}C incorporation technique (Parsons *et al.*, 1984). Approximately 0.5 cm^3 of biomass was added to 25 mL polycarbonate vials containing 20 mL of filtered seawater ($0.45 \mu\text{m}$ Gelman Poretic GF/F). A dark control and a filtered seawater control were included to account for any dark fixation and background counts. Aqueous ^{14}C sodium bicarbonate ($4 \mu\text{Ci}$) was added to samples, which were then incubated for 1-2 h at between $500\text{--}700 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ in a flow-through water bath at approximately $25 \pm 2 \text{ }^\circ\text{C}$.

After incubation, samples were filtered onto pre-weighed $8 \mu\text{m}$ polycarbonate filters using a vacuum filtration system. Samples were subsequently rinsed with filtered seawater and 10 % HCl to remove any residual unincorporated ^{14}C bicarbonate. A final rinse with 6 % isotonic ammonium formate was conducted to remove any salt for subsequent accurate dry weight determinations. Samples were dried (60°C) and weighed (± 0.001). The samples were placed in Beckman (Ready Safe) liquid scintillation fluid (4 mL) in polyethylene scintillation vials and radioactivity determined using a scintillation counter (Packard Tricarb 1600). A blank consisting of four μCi of aqueous ^{14}C sodium incorporation used to determine total activity. Productivity ($\text{mg C g}_{dw}^{-1} \text{ hr}^{-1}$) was calculated as per Parsons *et al.* (1984) using the following equation:

$$\text{Photosynthesis (g C g}_{dw}^{-1} \text{ hr}^{-1}) = ((R_s - R_B) \times A \times 1.05 \times V) / (R \times t \times dw) \quad (\text{Equation 5})$$

where R_s was the sample count (disintegrations per minute) corrected for the filtered seawater blank; R_B was the dark count (disintegrations per minute); A was the alkalinity of water in mg C L^{-1} ; 1.05 was the correction for differential uptake of C isotopes; R was the total

activity (disintegrations per minute) of radioactive bicarbonate added; t was the incubation time in hours and dw was the dry weight (g) of the sample.

NUTRIENT ADDITIONS

The effect of various nutrient additions on productivity was tested using both PAM fluorometry and ^{14}C incorporation. Based on previous research (Paerl *et al.*, 1987; Duffy & O'Neil, submitted) iron, molybdenum, phosphorus, and ammonium were added at a concentration of 5 μM . Iron was added to treatments as iron chloride (FeCl_3) and a chelating agent added to treatments as equimolar ethylenediaminetetra-acetic acid (EDTA). Molybdenum was added as sodium molybdate ($\text{Na}_2\text{MoO}_4(\text{H}_2\text{O})_4$), phosphorus as sodium dihydrogen orthophosphate (Na_2HPO_4) and ammonium as ammonium chloride (NH_4Cl). Experiments were conducted using treatments of 5 μM FeCl_3 , 5 μM Fe + 5 μM EDTA, 5 μM EDTA, 5 μM Mo, 5 μM PO_4^{3-} , 5 μM NH_4^+ and a seawater control for comparison.

PAM fluorometry experiments were conducted under approximately 50 % incident light for 7 d. Fifty cm^3 of *Lyngbya majuscula* were placed in one litre glass beakers containing site water, pre-washed with 10 % HCl and 10 % Nitric Acid. Beakers were incubated in a water bath at ambient temperature (27°C). Pilot studies determined that dissolved nutrient concentrations returned to background concentrations within 3 d following addition. As a result, nutrients were added initially and every 2 d to each of 3 replicate treatments. Treatments were analogous to the ^{14}C incorporation experiments with an additional treatment containing a mix of all nutrients. Rapid light curve measurements and photochemical efficiency estimates were conducted at approximately equal daily irradiances on day 1, day 2, day 4 and day 7.

SALINITY

The effect of salinity was examined using both PAM fluorometry and ^{14}C incorporation. Seawater (36 ppt) was diluted with distilled water to generate a salinity gradient consisting of treatments 0 ppt, 9 ppt, 18 ppt, 27 ppt and 36 ppt. Fifty cm^3 of *Lyngbya majuscula* were placed in one litre glass beakers containing diluted site waters treatments for PAM fluorometry. *Lyngbya majuscula* samples were maintained in these treatments for 24 hours,

with measurements taken at times 0, 8 and 24 hours using PAM fluorometry. In order to assess recovery, samples from the 9 ppt treatment were removed and placed in the 36 ppt treatment for an additional 8 hours and re-measured.

LIGHT

A range of light intensities were used to generate photosynthesis- irradiance curves (PI) using ^{14}C incorporation and rapid light curve techniques. Three replicate samples of *Lyngbya majuscula* were incubated in shade bags of 0, 12, 25, 50 and 100% incident light and incubated *in situ* for 2 h in surface water under ^{14}C incorporation treatment. Three replicate samples were placed in each bag under ^{14}C incorporation treatment. Approximately 50 cm³ of *L. majuscula* was placed in shade bags of 50 and 100% incident light. After an initial 30 min, rapid light curves were generated on *L. majuscula* samples incubated at the varying intensities. Ambient surface light was monitored over the incubation period, remaining consistently around 1550 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. From the resulting PI curves, the maximum electron transport rate (ETR_{MAX}) and the associated saturating irradiance I_k were calculated using hyperbolic tangent modelling.

HUMIC ADDITIONS

^{14}C incorporation was used to examine the effect of humic acids on the productivity of *Lyngbya majuscula*. Humics acids were extracted and concentrated from adjacent waters using an XAD-8 resin column extraction at pH equal to 2 (Leeneer, 1981). An initial experiment was set up to examine the effect of different concentrations of humics on ^{14}C incorporation. Treatments with dissolved organic carbon concentrations of 2, 10 and 20 mg L⁻¹ were used to reflect natural ranges of dissolved organic carbon (Brown, 1987; Pettersson *et al.*, 1997).

Another experiment was set up using combinations of nutrients and humic acid extract to investigate their effect on ^{14}C incorporation by *Lyngbya majuscula*. Treatments of iron (5 μM), phosphorus (5 μM) and humic acid extract (20 mg/L) were used, as well as different combinations of the three were investigated.

TEMPERATURE

Water baths were maintained over a range of temperatures; 5°C, 15°C, 27°C, 35°C and 50°C. Four replicate samples of *Lyngbya majuscula* were placed in each temperature treatment and incubated using ¹⁴C incorporation for 2 h.

STATISTICAL ANALYSIS

There was no significant ($p < 0.05$) difference between nutrient concentrations at the 3 sites on a given day, therefore water column nutrients of the bloom area were pooled to compare between sampling dates with greater statistical power. Seagrass and sediment iron content values were also based on one homogenous sample, however, Prange (1999) demonstrated negligible variation between replicate seagrass samples within sites in Moreton Bay. Where applicable standard errors were used in all graphical representation.

Cochran's test for homogeneity of variance was performed on all data sets prior to analysis. A one-way ANOVA followed by a post-hoc Tukey's HSD means test was used to determine significant differences ($p < 0.05$) between both sampling dates and between experimental treatments.

RESULTS

FIELD OBSERVATIONS AND MONITORING

BLOOM DYNAMICS

The bloom of *Lyngbya majuscula* in Northern Deception Bay initiated around the 10th January 2000, and persisted till early April 2000, covering an area of approximately 8 km² at the time of peak abundance in late February (Figure 2). The bloom began in the shallow waters off Pebble beach, extending to the extremities of the bank and in a westerly direction towards Godwin beach, eventually covering the entire bank in these areas. The bloom appeared restricted to shallow waters and seagrass meadows.

BIOMASS

The biomass of *Lyngbya majuscula* was highly variable between study sites, ranging from 23 to 1181 g_{dw} m⁻² (Table 1). There was no apparent preference of *L. majuscula* for a particular seagrass species as it was associated with monospecific *Syringodium isoetifolium* and *Zostera capricorni* beds as well as mixed seagrass beds of *Z. capricorni*, *Halophila ovalis* and *H. spinulosa*. However, the effect of *L. majuscula* on seagrass biomass was variable and appeared to be species dependent. The area of bloom initiation was dominated by monospecific *S. isoetifolium* beds, which also had the highest decline in leaf and root/ rhizome biomass. *Lyngbya majuscula* growing on *Z. capricorni* beds also resulted in a significant decline in leaf biomass at two sites, however, root/ rhizome biomass was only affected significantly at one site. The mixed seagrass sites showed no apparent decline in any seagrass parameter.

The variable effects that *Lyngbya majuscula* had on seagrass biomass could largely be attributed to the variation in *L. majuscula* biomass. The highest *L. majuscula* biomass was found on *Syringodium isoetifolium*, which consequently had the most severe decline in biomass. There was also little variation in *L. majuscula* biomass associated with *S. isoetifolium*, indicating a consistent density and even cover of *L. majuscula*, smothering the seagrass. Biomass of *L. majuscula* in the mixed seagrass beds, however, was lower and

highly variable, indicating a variable density and patchy distribution of *L. majuscula* associated with these seagrass beds.

ABIOTIC ENVIRONMENTAL MONITORING

Ambient surface light ranged from 3.8 to 53.65 mol quanta m⁻² d⁻¹ for the period 5th October 1999 to 5th February 2000, with a mean of 34.51 mol quanta m⁻² d⁻¹ (Figure 3A). A period of prolonged high incident light was recorded between the period 2nd - 22nd January, averaging 44.7 mol quanta m⁻² d⁻¹ which coincided with the bloom initiation. Water temperature, salinity and rainfall were monitored from the 14th September 1999 to 5th April 2000. Average daily water temperature ranged from 18.4 to 28 °C over this period (Figure 3B). An increase of approximately 2 °C was recorded between the month of December and January, with the monthly average increasing from 23.3 to 25.3 °C. This increase coincided with the initiation of the bloom.

Salinity remained relatively constant ranging from 33.2 to 36.2 ppt, with a mean of 34.8 ppt over the monitoring period (Figure 3C). There was no apparent difference in salinity preceding or during the bloom, remaining consistently in the upper salinity range. Daily rainfall ranged from 0 to 88 mm in the monitoring period (Figure 3D). Rainfall was minimal to zero around the bloom initiation period with the total January rainfall recorded at 51mm. However the bloom was preceded by substantial rainfall in the month of December, totalling 270 mm of rain.

Wind patterns over the period preceding the *Lyngbya majuscula* bloom and during bloom initiation (21st December 1999- 31st January 2000) were predominantly east and northeast winds (Figure 4). The *L. majuscula* bloom site, and northern Deception Bay in general, are sheltered from winds originating from these directions due to the landmasses of Bribie Island and the adjacent mainland. This results in reduced fetch, and resulting wind-generated water motion is minimal.

WATER COLUMN CHARACTERISTICS

Levels of dissolved inorganic and organic nutrients, total nutrients and water column total iron concentrations did not significantly ($p < 0.05$) differ from the 15th September 1999 to 5th April 2000 sampling (Figure 5A, Table 3), with the exception of nitrate. Nitrate

concentrations over the three sites ranged from 0.1 to 0.5 μM , averaging $0.3 \pm 0.04 \mu\text{M}$, with significant differences between certain sampling dates, however, there is no apparent difference preceding or during the bloom. Average nutrient concentrations were calculated from all sites over the study period. Ammonium levels at the three sites ranged from 0.1 to 1.1 μM , averaging $0.5 \pm 0.1 \mu\text{M}$. Phosphorus concentrations ranged from 0.2 to 0.4 μM , averaging $0.28 \pm 0.02 \mu\text{M}$ at the three sites. Dissolved organic nitrogen concentrations ranged from 9.3 to 12.9 μM , averaging $10.8 \pm 0.6 \mu\text{M}$. Total nitrogen ranged from 10 to 13.8 μM , with an average concentration of $12 \pm 0.4 \mu\text{M}$ over the sampling periods (Figure 5B). Total phosphorus averaged $0.7 \pm 0.03 \mu\text{M}$ over the sampling period, ranging from 0.6 to 1 μM . Total water column iron was variable over the sampling period ranging from 1 to 7.1 μM , with an overall average of $2.65 \pm 0.5 \mu\text{M}$ (Figure 5C).

The creeks and waterways adjacent to the *Lyngbya majuscula* bloom area were sampled for dissolved and total nutrients to ascertain possible nutrient sources for sustaining the bloom. The concentrations of dissolved inorganic nutrients in these creeks and waterways were relatively consistent across all runoff creeks and waterways sampled (Table 2). Average concentrations of ammonium, nitrate and phosphorus for adjacent waters were 1.8 ± 0.3 , 0.6 ± 0.1 and $0.1 \pm 0.01 \mu\text{M}$ respectively. Total phosphorus levels averaged $0.6 \pm 0.01 \mu\text{M}$ over the respective runoff sites. Concentrations of dissolved organic and total nitrogen were considerably elevated at some sites, averaging 52.35 ± 14 and $56.33 \pm 12 \mu\text{M}$, ranging from 14.29 to 100 μM and 16.43 to 92.86 μM respectively. Total water column iron concentrations were highly variable ranging from 2.69 to 80.57 μM , with an overall average of $23.7 \pm 10 \mu\text{M}$. Dissolved organic carbon (DOC) concentrations were highly variable ranging from 2.45 to 24.7 mg L^{-1} , with an average of $10.36 \pm 3.4 \text{mg L}^{-1}$. There was considerable variation in the pH in these waterways, ranging from 3.8 to 7.8.

SEAGRASS AND SEDIMENT METALS

Seagrass and sediment metal concentrations were monitored in an attempt to identify pulses or increases in bioavailable iron concentrations. Seagrass leaf iron levels ranged from 1.09 to 4.08 $\text{mg g}_{\text{dw}}^{-1}$ (Figure 6). There was an apparent elevation in iron content of seagrass leaves near the time of *Lyngbya majuscula* bloom onset at each of the 2 sites. Sediment iron concentrations were variable over the study period ranging from 0.64 to 3.47 $\text{mg g}_{\text{dw}}^{-1}$.

Seagrass root/ rhizome iron levels were highly variable ranging from 1.89 to 13.11 mg g_{dw}⁻¹ with no apparent difference preceding or during the bloom, however, there was an increase in iron content at both sites in the latter stages of the *Lyngbya majuscula* bloom. There was no apparent difference in sediment iron levels preceding or during the bloom (Figure 7).

Samples of *Lyngbya majuscula* were analysed for elemental content and $\delta^{15}\text{N}$ signature (Table 5). The concentrations of tissue carbon, nitrogen, phosphorus and iron of *Lyngbya majuscula* were calculated to be 17.93 mmol C g_{dw}⁻¹, 1.405 mmol N g_{dw}⁻¹, 0.1 mmol P g_{dw}⁻¹ and 0.177 mmol Fe g_{dw}⁻¹, respectively. The $\delta^{15}\text{N}$ signature of *L. majuscula* was 1.72 ppt.

LYNGBYA MAJUSCULA ECOPHYSIOLOGY

NUTRIENT AND HUMIC ADDITIONS

Three techniques were used to assess the response of *Lyngbya majuscula* to added nutrients:

- 1) short-term estimates of photosynthesis were conducted using ¹⁴C incorporation (Figure 8)
- 2) photochemical efficiency estimates following 15 min dark adaptation using a PAM fluorometer (Figure 12A) and
- 3) rapid light curves generated in ambient light (Figure 12B).

The response time for the different methods was 2h and 7 d for ¹⁴C incorporation and PAM fluorometry, respectively.

Short-term ¹⁴C photosynthesis experiments demonstrated elevated ¹⁴C uptake rates in all treatments relative to the control (Figure 8). However, there was significant elevation of ¹⁴C uptake only in treatments of 5 μM Fe, 5 μM EDTA and 5 μM PO₄³⁻ treatments (Figure 9).

Elevated rates of ¹⁴C photosynthesis were recorded in all additions of humic acid extract (2, 10 and 20 mg L⁻¹), however, these increases were not significant ($p < 0.05$) relative to the control (Figure 10). With the addition of iron and humic acid extract together, ¹⁴C incorporation were also elevated relative to the control, however not significantly (Figure 11). ¹⁴C incorporation was not increased with a combination treatment of PO₄³⁻ and humic acid extract.

The long-term fluorometry experiments were subjected to other varying environmental factors, such as light, over the course of incubation. The variability in these other factors

could affect the nutrient response, in turn confounding results. Elevated Fv/Fm responses were seen in all treatments, however, only the 5 μM Fe and 5 μM FeEDTA treatments increased significantly ($p < 0.05$) in relation to the control (Figure 8A). Similar responses were observed using rapid light curves with elevated maximum electron transport rates in all treatments relative to the control, with the exception of the 5 μM PO_4^{3-} treatment (Figure 8B). However, there were no statistically significant increases in any treatments.

TEMPERATURE

The effect of temperature was examined by measuring ^{14}C incorporation over a range of temperatures from 5 to 50 $^\circ\text{C}$ (Figure 13). Incorporation rates were significantly reduced in treatments of 5 and 50 $^\circ\text{C}$, with rates of 0.19 ± 0.04 and 0.11 ± 0.04 $\text{mg C g}_{\text{dw}}^{-1} \text{h}^{-1}$, respectively. Incorporation rate was significantly increased ($p < 0.05$) nearly two fold in the 35 $^\circ\text{C}$ treatment (4.84 ± 1.27 $\text{mg C g}_{\text{dw}}^{-1} \text{h}^{-1}$) above the ambient treatment, 27 $^\circ\text{C}$. A slight reduction in ^{14}C incorporation, compared to ambient water temperature, was evident in the 15 $^\circ\text{C}$ treatment, however it was not significant ($p > 0.05$).

LIGHT

The effect of light was examined using rapid light curve and ^{14}C incorporation measurements over a range of light intensities from 0 to 1400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Figure 13). The rate of ^{14}C incorporation in the absence of light was negligible (0.01 ± 0.01 $\text{mg C g}_{\text{dw}}^{-1} \text{h}^{-1}$). Rates of ^{14}C incorporation were greatest under intensities of 700 and 1400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, with incorporation rates of 1.68 ± 0.35 and 1.72 ± 0.2 $\text{mg C g}_{\text{dw}}^{-1} \text{h}^{-1}$, respectively. A polynomial regression best described the relationship between light and ^{14}C incorporation.

There was no significant difference ($p > 0.05$) in maximum electron transport rates between 50 and 100% incident light treatments using the PAM fluorometer (Figure 14). However, contrary to the ^{14}C incorporation response, treatments incubated under full light (1400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) had a reduced maximum photosynthetic rate (18.7 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$) compared with the 50% irradiance treatment (43.4 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$). This was also reflected in the saturating irradiance (I_k) of the rapid light curves. The saturating irradiance in the 100% incident light treatment (105 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) was less than half that of the 50% incident light treatment (306 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$).

SALINITY

The response of *Lyngbya majuscula* to a range of salinities (0 to 36 ppt) was investigated (Figure 15). Photosynthesis of *L. majuscula* showed a broad salinity tolerance, with substantial photosynthetic activity from 9 to 36 ppt. ^{14}C incorporation was negligible in the 0 ppt treatment ($0.18 \pm 0.02 \text{ g C g}_{\text{dw}}^{-1} \text{ h}^{-1}$), however at 9 ppt, there was a lower but measurable rate of incorporation ($1.7 \pm 0.43 \text{ g C mg}_{\text{dw}}^{-1} \text{ h}^{-1}$) (Figure 15A.). Incorporation of ^{14}C was greatest in treatments of 18 and 27 ppt, with rates of $4.85 \pm 0.24 \text{ mg C g}_{\text{dw}}^{-1} \text{ h}^{-1}$ and $5.19 \pm 0.63 \text{ mg C g}_{\text{dw}}^{-1} \text{ h}^{-1}$, respectively.

Both fluorometry techniques demonstrated similar response to the range of salinity (Figure 15B and 15C). The photochemical efficiency of samples in 0 ppt and 9 ppt were significantly reduced in comparison to the other 3 treatments, with values of 0.025 ± 0.015 and 0.076 ± 0.02 , respectively. There was negligible difference in the upper range treatments (18, 27, 36 ppt), with photochemical efficiency values ranging from 0.219 ± 0.02 to 0.289 ± 0.02 .

Maximum electron transport rates were also negligible in the 0 ppt treatment ($1 \pm 0.5 \mu\text{mol e m}^{-2} \text{ s}^{-1}$). After transfer to the 36 ppt treatment, samples from the 9 ppt treatment showed substantial recovery in both the maximum electron transport rate and photochemical efficiency, with the maximum electron transport rate and Fv/Fm values increasing to $29.1 \pm 6.6 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ and 0.33 ± 0.03 , respectively. However, the increase in the maximum electron transport rate was not significant ($p > 0.05$).

DISCUSSION

ENVIRONMENTAL CONDITIONS

ABIOTIC PARAMETERS

The initiation of the *Lyngbya majuscula* bloom coincided with a suite of environmental conditions that appear critical to the formation of the bloom: prolonged high surface light preceded by a rainfall event, elevated water temperature, calm weather conditions and upper range salinity. Similar environmental conditions have been observed during the formation of other cyanobacterial blooms, both marine and freshwater. Cyanobacterial blooms in freshwater systems commonly coincide with periods of high water temperatures, calm weather and prolonged high irradiance following a rainfall event (Bowling, 1994; Codd *et al.*, 1994). Blooms of the pelagic marine cyanobacterium, *Trichodesmium*, are often reported during periods of high incident light and calm weather, and although rainfall does not appear critical in the formation of these, they are often found in waters that have passed islands or landmasses where runoff and/ or upwelling might be apparent (Carpenter, 1983; Capone *et al.*, 1997).

Elevated water temperature appears critical for the formation of *Lyngbya majuscula* blooms. Prior to the bloom event, an increase in water temperature to between 24 and 25°C was recorded in northern Deception Bay. All previously recorded blooms of *L. majuscula* in northern Deception Bay have occurred in late summer when water temperatures are in excess of 22 °C (Dennison *et al.*, 1999). The thermal optimum for cyanobacteria has been described as being around 25°C or greater (Fogg *et al.*, 1973; Robarts & Zohary, 1987), and water temperatures above 21°C have been reported as critical for bloom formation in *Trichodesmium* (Sellner, 1992).

The average daily salinity remained relatively constant near that of full strength seawater (36 ppt) over the period of the *Lyngbya majuscula* bloom. High salinity preference in marine cyanobacteria has been well documented. For example, *Lyngbya* sp. and *Calothrix* sp. have shown optimal salinity to be 35ppt (Jones, 1992) and blooms of *Trichodesmium* are generally restricted to fully saline waters (Capone *et al.*, 1997). Under experimental conditions *L.*

majuscula demonstrated a high salinity preference but euryhaline tolerance, which is not surprising given its coastal and estuarine range.

Calm weather conditions, resulting in a stable water column, occurring prior to and during the initiation period of the *Lyngbya majuscula* bloom. Predominantly north to northeast winds were apparent during bloom initiation resulting in minimal wind-generated water motion. In addition, neap tides were present during the bloom initiation period (O.T.T.B.S.G., 1999), aiding in water column stability. Blooms of *Trichodesmium* are often reported during periods of calm weather, resulting in high light penetration and some degree of stratification (Carpenter, 1983; Capone *et al.*, 1997). The role of vertical and horizontal water column stability has been reported in the initiation and persistence of marine cyanobacterial blooms (Fogg, 1969; Ganf & Horne, 1975; Reynolds & Walsby, 1975; Paerl, 1988).

A prolonged period of high light was recorded for the initial period of bloom development (2nd- 22nd January 2000). The average daily surface light (44.7 mol quanta m⁻² d⁻¹) was considerably higher than that recorded for Moreton Bay from late January to April (36.7 mol quanta m⁻² d⁻¹) (Longstaff *et al.*, 2000). Light attenuation coefficient (K_d) during this period was calculated from secchi depth measurements, ranging from 0.59 to 1.3 m⁻¹, with a mean of 0.78 m⁻¹. These values are comparable to K_d values, which varied only slightly from 0.5 m⁻¹ from January to June, recorded in eastern Moreton Bay, where light penetration is consistently high and water very clear (Longstaff *et al.*, 2000).

Mean sea level (M.S.L) in the bloom area is 1.08 m above lowest annual tide (L.A.T.)(O.T.T.B.S.G., 1999). The average water depth overlying the *Lyngbya majuscula* bloom area can be estimated at 1.5 m from the mean sea level, assuming maximum depth range of *L. majuscula* on these banks did not exceed 0.5 m below the lowest annual tide limit. Given the calculated mean K_d value, average water depth and surface light, the fraction of surface light reaching the benthic community can be estimated using equations described in Kirk (1994). An estimated 32% of surface light was calculated to reach the benthic community during bloom initiation. This fraction is comparable with the fraction of surface light recorded in the highly productive seagrass beds in eastern Moreton Bay (Longstaff *et al.*, 2000), indicating light conditions during the *L. majuscula* bloom initiation were both elevated and prolonged.

NUTRIENTS

The presence of relatively high water column iron levels may be important in sustaining a bloom of *Lyngbya majuscula*. Total water column iron concentrations were relatively high and constant over the study period, averaging $3.1 \pm 0.8 \mu\text{M}$. Previous studies have reported total water column iron concentrations ranging from 0.3 to 2.6 μM (Smolders & Roelofs, 1995) and 1.25 to 4.5 μM (Sadek *et al.*, 1986) in coastal bays and estuaries. Sadek *et al.* (1986) reported total iron concentrations of $0.07 \pm 0.25 \mu\text{M}$ in mullet aquaculture ponds with nuisance *L. majuscula* blooms.

Average concentration of total water column iron in creeks and waterways adjacent to the bloom area was $23.1 \pm 10.7 \mu\text{M}$, which could indicate a potential source of iron to the bloom area. Given only a relatively small amount of rain, 30 mm in two days, preceded this sampling event, it could be expected that iron levels in these creeks and waterways might reach higher concentrations with more substantial rainfall events. Dennison & Abal (1999) previously recorded concentrations of up to 231 μM total iron in these creeks during the 1998 *Lyngbya majuscula* bloom. A land use report investigating the creeks and waterways of the northern end of Pumicestone Passage recorded average total water column iron concentrations of $39.4 \pm 4.5 \mu\text{M}$ (NSR Environmental Consultants Pty. Ltd., 1999), highlighting the potential of adjacent creeks and waterways as sources for large amounts of iron. However, the bioavailability of iron critically depends on its speciation, and not necessarily its total concentration. In seawater, iron has a strong tendency to hydrolyse to thermodynamically stable ferric iron (Fe^{3+}) resulting in the formation of sparingly soluble hydroxides and oxides (Sunda, in prep; Waite, In Prep), rendering it mostly unavailable for biological uptake.

The reduction of Fe^{3+} in seawater leads to production of ferrous iron (Fe^{2+}), which is highly soluble and more kinetically labile with respect to chelation, forming much weaker organic chelates and complexes (Sunda, 1988). Fe^{2+} oxyhydroxides are highly soluble and the Fe^{2+} formed is less strongly bound to organic ligands, ultimately resulting in the dissolution of Fe^{2+} oxyhydroxides or the dissociation of iron chelates (Sunda, in prep). Three major processes have been identified that lead to Fe^{3+} reduction: 1) uptake of iron through the bioreduction of Fe^{3+} at cell surfaces (Jones *et al.*, 1987); 2) photoreduction of iron chelates, iron hydroxides and iron oxides (Waite & Morel, 1984; Waite *et al.*, 1984; Wells & Mayer,

1991; Wells *et al.*, 1991; Miller & Kester, 1993; Waite & Szymczak, 1994); and 3) reduction in highly reducing micro- and macro-environments through chemical or microbial pathways (Chambers & Odum, 1990). However, in most systems, the dissolved inorganic Fe^{2+} undergoes rapid reoxidation by O_2 or photochemically produced H_2O_2 (Moffet & Zika, 1987), the resultant Fe^{3+} is then rechelated by organic ligands or precipitated as iron hydroxides (Sunda, in prep).

Preliminary investigations of iron chemistry in the seawater of the bloom area indicate a large fraction of the total water column iron pool is organically bound (Rose, 1999). Dissolved organic carbon, primarily in the form of humic and fulvic acids, has been shown to chelate trace metals, such as iron, retaining them in solution (Prakash *et al.*, 1973; Prakash, 1975; Matsunaga *et al.*, 1984; Matsunaga *et al.*, 1998). This can provide a source of biologically available iron or retain iron in solution, which through photoreduction, can become bioavailable for uptake (Franko & Heath, 1982; Sunda & Huntsman, 1995). In addition, through complexation with other trace metals such as copper, humic material has been shown to mitigate the toxic effect of these metals, stimulating production and growth (Brand *et al.*, 1986).

Substantial amounts of dissolved organic carbon (DOC) are present in the *Lyngbya majuscula* bloom area. The average concentration of dissolved organic carbon in the bloom area was $1.96 \pm 0.17 \text{ mg L}^{-1}$, during the *L. majuscula* bloom. Dissolved organic carbon concentrations were previously measured in the bloom area during a flood event in early 1999, ranging from 1.8 to 10 mg L^{-1} (Rose, 1999). Brown (1987) recorded levels of dissolved organic carbon (DOC) in the marine waters of southern Florida ranging from 0.15 to 0.86 mg L^{-1} for 5 water bodies, indicating a substantial amount of dissolved organic carbon (DOC) was present during the recent *L. majuscula* bloom and in previous years. Levels of dissolved organic carbon (DOC) in creeks and waterways adjacent to the *Lyngbya majuscula* bloom area ranged from 2.45 to 24.7 mg L^{-1} , indicating the potential of a large source of DOC in adjacent watercourses. These concentrations are comparable to DOC levels reported in rivers discharging from highly organic forested areas, ranging from 8 to 30 mg L^{-1} (Pettersson *et al.*, 1997). The vegetated fraction of the Pumicestone Passage catchment region is approximately 70%, dominated by commercial pine plantation (Danaher DNR, pers. comm.), identifying a potential source of dissolved organic carbon to the region.

The growth of cyanobacteria in coastal ecosystems is often directly related to phosphorus loading (Paerl *et al.*, 1987). Phosphorus has been shown to enhance rates of nitrogen fixation in *Lyngbya majuscula* (Duffy & O'Neil, submitted) and inter-tidal cyanobacterial mats (Paerl *et al.*, 1987). In addition, elevated phosphorus concentrations have been implicated in cyanobacterial blooms in the estuaries of North Carolina (Seitzinger, 1991).

Phosphorus has a tendency to precipitate from solution through adsorption onto ferric oxides, ferric hydroxides and clay particles (Giordani *et al.*, 1996). Due to the low solubility and lability of these complexes in aerobic waters, they eventually settle in the sediments (Valiela, 1995). In anaerobic sediments, with high organic loading and sulphate content, the reduction of iron and sulphate can lead to the dissolution of bound phosphate (Chambers & Odum, 1990), providing a localised source of phosphorus to the water column. A diurnal pattern in oxygen stoichiometry has been observed in sediment associated with *Lyngbya majuscula* (see *Lyngbya* and seagrass interactions), with high concentrations of dissolved phosphorus and ammonium fluxing from the benthos.

ECOPHYSIOLOGICAL RESPONSE

PHOTOSYNTHESIS/ LIGHT RELATIONSHIP

Lyngbya majuscula exhibited an apparent tolerance for elevated irradiances, but a degree of photoinhibition was observed at full irradiance. The rate of ^{14}C incorporation was greatest in treatment incubated under full ($1440 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) and half ($700 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) irradiance at the waters surface. Given that the incorporation rates were almost identical, the full surface light treatment would appear photoinhibited. Rapid light curve assessment also demonstrated photoinhibition in *L. majuscula* samples incubated at full irradiance, indicated by a reduction in the saturating irradiance (I_k) of the photosystem (White & Critchley, 1999). Strong photoinhibition of *Trichodesmium* was observed by Lewis *et al.* (1988) at irradiances above $1000 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, and studies by McCarthy & Carpenter (1979) demonstrated a depression in carbon assimilation above $600 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. This is comparable to *L. majuscula*, where photoinhibitory effects were observed at irradiances above $700 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Similarly, an irradiance level above $950 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ has been recorded as inhibitory to benthic *Microcoleus* sp. (Pentecost, 1985).

The saturating photosynthetic irradiance (I_k) of *Lyngbya majuscula* was comparable to that demonstrated in *Trichodesmium*. Optimal irradiance for *Lyngbya majuscula* was between 230 and 480 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, based on estimates of saturating irradiance (I_k) generated from fluorometry and ^{14}C uptake PI curves, respectively. The saturating photosynthetic irradiance (I_k) of *T. erythraeum* and *T. thiebautii* has been calculated as 324 and 687 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively (Carpenter *et al.*, 1993). During the latter stages of blooms, *L. majuscula* can rise off the benthos through the accumulation of gas bubbles derived from photosynthesis, resulting in the formation of large mats at the waters surface (Diaz *et al.*, 1990; Dennison *et al.*, 1999). Given the substantial photosynthetic activity at high irradiances, it is apparent that these mats are photosynthetically functional and therefore the potential exists for these mats to recolonise new areas.

Comparatively high rates of light saturated photosynthesis were measured in *Lyngbya majuscula* (Table 4). Rates of ^{14}C incorporation were 2 orders of magnitude greater than recorded phytoplankton rates and were comparable to carbon fixation rates recorded in higher marine plants.

PHOTOSYNTHESIS/ TEMPERATURE RELATIONSHIP

Lyngbya majuscula appears eurythermal, with substantial photosynthetic activity between 15 and 35°C. Marine cyanobacteria are generally eurytherms, with temperature optimums typically between 25 and 35°C (Fogg *et al.*, 1973). The maximum photosynthetic rate of *L. majuscula* was observed at 35°C using ^{14}C incorporation, whereas at 50°C there was negligible measurable photosynthetic activity, which would indicate a thermal optimum somewhere between these two temperatures. Determining photosynthetic responses over a finer temperature range at these upper limits might yield a more definite temperature optimum of *L. majuscula*. However, the temperature optimum for nitrogen fixation in a marine *Lyngbya* sp. has been previously reported as 35°C (Jones, 1992), which adds substance to the temperature optimum observed in this study.

PHOTOSYNTHESIS/ NUTRIENT RELATIONSHIP

Experimental results suggest that iron, particularly iron speciation, and phosphorus availability are limiting to photosynthesis in *Lyngbya majuscula*. Enhanced rates of photosynthesis were recorded in *L. majuscula* with the addition of iron and EDTA in both ^{14}C

incorporation and PAM fluorometry experiments. Increased photosynthetic rates of *Lyngbya majuscula* treated with total iron could indicate the presence of a natural chelation process increasing iron bioavailability.

Ethylenediaminetetra-acetic acid (EDTA) is a low molecular weight synthetic compound that can chelate either ambient iron making it bioavailable, or other trace metals, mitigating their toxicity (Anderson, 1979; Paerl *et al.*, 1994). Therefore, elevated photosynthetic rates of *Lyngbya majuscula* under treatment of ethylenediaminetetra-acetic acid chelated iron (FeEDTA) could demonstrate iron stimulation and highlight the importance of bioavailable iron species. The stimulation of photosynthesis through FeEDTA addition has been previously recorded in *L. majuscula* (Gross & Martin, 1996) and has been well documented in *Trichodesmium* and other marine cyanobacteria (Rueter, 1988; Rueter *et al.*, 1990; Rueter & Unsworth, 1991; Geider & Laroche, 1994). In addition, stimulation of nitrogen fixation through FeEDTA addition has also been demonstrated in *Lyngbya majuscula* (Duffy & O'Neil, submitted) and *Trichodesmium* (Paerl *et al.*, 1987; Rueter *et al.*, 1992; Paerl *et al.*, 1994).

Iron appears to be a key nutrient for cyanobacteria growth and proliferation, with evidence present to suggest that iron, particularly iron species, may be limiting the growth of *Lyngbya majuscula*. A significant increase in photochemical efficiency (Fv/Fm) was also recorded for *L. majuscula* treated with elevated levels of iron and EDTA. This emphasises the importance of iron as a stimulant for *Lyngbya majuscula* growth and further demonstrates the importance of a bioavailable iron species.

¹⁴C incorporation rates were enhanced in *Lyngbya majuscula* treated with additions of humic acid extract, albeit not significantly. This could be attributed to the high background DOC levels recorded in the waters of the bloom area masking the response of the *L. majuscula* to the humic extract addition. Enhanced productivity of phytoplankton has been previously demonstrated with humic additions, most probably a result of an increase in bioavailable iron species or the mitigation of the toxic effect of other trace metals (Prakash *et al.*, 1973; Prakash, 1975; Heil, 1996).

Photosynthetic rates were also enhanced with phosphorus additions in both ¹⁴C incorporation and PAM fluorometry. Diurnal rhythms in both nutrient and oxygen stoichiometry have been

observed in *Lyngbya majuscula* mats, possibly identifying a unique pathway for nutrient acquisition. Given such high rates of photosynthesis in *Lyngbya majuscula*, it is logical to assume high rates of respiration, and low dissolved O₂ levels have been recorded during the night in benthic cores of *L. majuscula*, associated seagrass and sediment (Oberlin, 2000). This demonstrates a diurnal cycle in O₂ levels most probably controlled by the photosynthesis- respiration cycle in *L. majuscula*, which could lead to the breakdown of the sediment oxic-anoxic boundary and the flux of phosphorus and ammonium from the sediments to the water column. High concentrations of both phosphorus and ammonium have been measured fluxing from the benthos during the night in benthic cores containing seagrass, sediment and *L. majuscula* (Oberlin, 2000) and in decomposing *L. majuscula* mats in seagrass beds (Zimmerman & Montgomery, 1984), identifying the potential for sediments to provide a localised supply of phosphorus for uptake.

Seagrass beds are typically high in organic material through the accumulation of detritus (Udy & Dennison, 1997), aiding in the breakdown of the sediment oxic-anoxic boundary through organic decay, which could result in the release of ammonium (Capone, 1997). This ammonium could also be utilised by *Lyngbya majuscula* during the night as a nitrogen supply. However, the $\delta^{15}\text{N}$ signature of *L. majuscula* is close to atmospheric stable isotope ratios (Heaton, 1986), indicating a reliance on nitrogen fixation as its primary nitrogen source. The formation of localised anoxic zones could also result in a supply of dissolved iron through the release of Fe²⁺ from sediments following the reduction of Fe³⁺ compounds (Chambers & Odum, 1990).

LYNGBYA AND SEAGRASS INTERACTIONS

The effect of *Lyngbya majuscula* on seagrass biomass appears to species specific. The variable effect of *L. majuscula* on seagrass beds can be attributed to both physical and chemical alterations. Physical effects could be mostly attributed to smothering of seagrass, reducing light and water circulation to the seagrass beds, and would be more pronounced at increased *L. majuscula* densities.

Seagrass beds appear to have a unique assemblage of microbes, providing reducing substrate or oxygen to the sediment and in return receiving fixed nitrogen (Perry, 1997). *Syringodium*

isoetifolium is typically associated with an aerobic microbial assemblage, generated through increased O₂ supply to the rhizosphere, while *Zostera capricorni* assemblages appear to be characteristically anaerobic, most probably due to the high detrital loading associated with this species (Perry, 1997).

It is hypothesised that by physically smothering seagrasses, *Lyngbya majuscula* decreases photosynthetic rates in the seagrass through a reduction in light and circulation. As a result, O₂ transport to the roots would also be reduced, which might lead to an unfavourable change in the associated microbial community. In addition, the resulting seagrass decline would also increase organic loading, which could further exacerbate the adverse conditions for the seagrass, favouring high reducing conditions and anoxia.

BLOOM DYNAMICS

A suite of environmental conditions appear critical for the formation of *Lyngbya majuscula* blooms in northern Deception Bay. Calm weather conditions, prolonged high light following a rainfall event, elevated water temperature and constant near full strength salinity coincided with the observed *L. majuscula* bloom. This resulted in stability and high light penetration of the water column, possibly following nutrient input through runoff. Elevated total water column iron and dissolved organic carbon concentrations were observed in adjacent creeks and waterways, conceivably associated with forest and hydric soil disturbance.

The *Lyngbya majuscula* bloom in northern Deception Bay covered an area of approximately 8 km² at its peak around the 24th February, a conservative 55 days after the bloom initiation. This equates to a linear expansion rate of 100 m² min⁻¹, which is not surprising given its comparatively high rates of photosynthesis and nitrogen fixation (Duffy & O'Neil, submitted).

Photosynthesis was enhanced through total iron addition, which could indicate a natural chelation process present in the waters of the bloom area. A large fraction of the water column iron in the bloom region is organically bound, identifying the potential for dynamic iron cycling through organic chelation and photoreduction, increasing iron bioavailability.

Phosphorus additions also stimulated photosynthesis in *Lyngbya majuscula*. Phosphorus uptake in *L. majuscula* could be constrained by the supply of phosphorus from the sediments and runoff from adjacent creeks and waterways. The molar Fe: P ratio of *L. majuscula* was calculated at 1.77, compared with the average molar Fe:P ratio in associated seawater of 4.8, which could indicate a phosphorus limitation in *Lyngbya majuscula*. Which, given the high rates of nitrogen (Duffy & O'Neil, submitted) and carbon fixation, seems likely. However, this molar ratio does not take into account bioavailability of iron which could greatly reduce the Fe:P ratio in the water column.

Fe:P molar ratios in coastal and marine cyanobacteria have been reported to range from 0.002 to 0.04, 2-3 orders of magnitude lower than Fe:P molar ratios described in *Lyngbya majuscula*, indicating a very high requirement for iron.

Blooms of the marine cyanobacterium, *Lyngbya majuscula*, have been reported in northern Deception Bay in increasing severity for almost a decade. This study has identified critical environment conditions for bloom formation including elevated water temperature, calm weather conditions, high irradiance following a rainfall event and near full strength salinity. Iron and phosphorus were identified as key nutrients for *Lyngbya majuscula* growth with high total iron concentrations measured in the bloom area. High concentrations of dissolved organic carbon were also measured in the bloom area, potentially resulting in a dynamic water column chelation process increasing bioavailable iron. Adjacent creeks and waterways were identified as large sources of both iron and dissolved organic carbon during runoff events.

Blooms of *Lyngbya majuscula* in northern Deception Bay have had serious detrimental effects on the ecology and economy of the local region. Given the adverse impacts of these blooms, this study highlights the need for further investigation of potential nutrient sources and critical assessment of land use practices in adjacent areas in order to revise management strategies and potentially mitigate future *L. majuscula* blooms.

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TABLE 1 Seagrass biomass over the period 20th January to 5th April, and mean *Lyngbya majuscula* biomass. Mixed species beds comprise assemblages of *Zostera capricorni*, *Halophila ovalis* and *Halophila ovalis*. Percent decline in seagrass species and associated species assemblage. Parentheses represent standard error.

Site ID.	Bloom Appearance	Ave. <i>Lyngbya</i> (g _{dw} m ⁻²)	Seagrass Type	Seagrass Biomass (g _{dw} m ⁻²)					
				Leaf			Root/ Rhizome		
				Initial	Final	% Decline	Initial	Final	% Decline
2	13/02/00	23 (10)	Mixed species bed	85 (10)	127 (44)	0	681 (19)	520 (57)	18
3	01/02/00	294 (106)	<i>Zostera capricorni</i>	116 (3)	86 (9)	26 *	355 (43)	202 (21)	39 *
4	25/02/00	48 (20)	<i>Zostera capricorni</i>	61 (5)	50 (8)	14	256 (70)	220 (52)	14
5	13/02/00	120 (41)	<i>Zostera capricorni</i>	57 (25)	27 (7)	30 *	434 (68)	255 (44)	28
6	01/02/00	257 (88)	Mixed species bed	22 (5)	21 (8)	5	453 (16)	440 (74)	3
7	20/01/00	433 (81)	<i>Syringodium isoetifolium</i>	58 (6)	27 (2)	54 **	434 (47)	255 (14)	41 *
8	20/01/00	1181 (82)	<i>Syringodium isoetifolium</i>	88 (9)	21 (5)	76 **	437 (50)	205 (7)	53 *

*p < 0.05

**p < 0.01

TABLE 2 Water column characteristics of waterways adjacent to *Lyngbya majuscula* bloom site. Samples were collected on the 10th November 1999, following 30mm of rainfall in 48 hours.

Site ID	Site Name	Water Column Parameter									
		Sal.	pH	NH ₄ ⁺	NO ₃ ⁻	PO ₄ ³⁻	Total Nitrogen	Dissolved Organic Nitrogen	Total Phosphorus	Total Iron	Dissolved Organic Carbon
		(ppt)		(μM)	(μM)	(μM)	(μM)	(μM)	(μM)	(μM)	(mg L ⁻¹)
9	Shirley Ck.	0	6.8	2.30	0.60	0.16	78.6	100.0	1.0	38.5	10.2
10	Bribie Waterways Canal Estate	33	7.8	1.40	0.60	0.13	16.4	14.3	0.6	2.7	nd
11	Dux Ck.	0	4.3	1.70	0.10	< 0.06	62.1	69.3	0.5	18.8	24.7
12	Pacific Harbour Entrance	31	7.7	0.90	0.40	0.16	17.9	15.7	0.7	4.8	2.5
13	Pacific Harbour North Channel	30	7.1	2.70	0.60	< 0.06	20.0	16.4	0.5	9.0	nd
14	Wright's Ck.	0	5.5	2.80	0.80	0.16	78.6	85.7	1.0	80.6	4.1
15	Pine Plantation	0	3.8	0.60	< 0.1	< 0.06	92.9	92.9	0.3	7.2	nd

nd= no data

TABLE 3 Summary of average water column parameters over the study period. ^{a,b}values indicate significant differences over sampling dates at p<0.05.

Date	Water Column Parameter								
	Secchi (m)	NH ₄ ⁺ (μM)	NO ₃ ⁻ (μM)	PO ₄ ³⁻ (μM)	Total Nitrogen (μM)	Dissolved Organic Nitroaen (μM)	Total Phosphorus (μM)	Total Iron (μM)	Dissolved Organic Carbon (mg L ⁻¹)
15/09/99	3.0	0.4 ^a	0.5 ^a	0.3 ^a	11.0 ^a	9.3 ^a	0.7 ^a	1.0 ^a	nd
06/10/99	2.5	1.1 ^a	0.4 ^{ab}	0.3 ^a	13.3 ^a	12.4 ^a	0.7 ^a	4.7 ^a	nd
27/10/99	1.4	0.3 ^a	0.2 ^{ab}	0.3 ^a	0.4 ^a	9.5 ^a	0.6 ^a	2.0 ^a	nd
20/01/00	2.5	0.1 ^a	0.2 ^b	0.4 ^a	11.4 ^a	10.2 ^a	0.9 ^a	3.0 ^a	2.4 ^a
25/02/00	2.5	0.1 ^a	0.1 ^b	0.2 ^a	11.4 ^a	9.3 ^a	0.7 ^a	7.1 ^a	1.9 ^a
20/03/00	nd	0.1 ^a	0.1 ^b	0.2 ^a	13.8 ^a	11.2 ^a	0.6 ^a	2.3 ^a	2.0 ^a
05/04/00	2.6	0.5 ^a	0.3 ^{ab}	0.3 ^a	13.3 ^a	12.9 ^a	0.8 ^a	1.7 ^a	1.6 ^a

nd= no data

TABLE 4: Comparative rates of ^{14}C incorporation of selected plant communities.

Community	Carbon uptake rate $\text{mg C m}^{-2} \text{ h}^{-1}$	Reference
Cyanobacteria		
<i>Lyngbya majuscula</i>	350	This Study
Cyanobacterial mat	17- 113	Bauld <i>et. al.</i> , 1979
<i>Synechococcus sp.</i>	210	Carr and Whitton, 1973.
Phytoplankton		
	0.06- 15	O' Donohue <i>et. al.</i> . 1997
	3.8- 5	Chapman and Chapman, 1973.
Macroalgae		
<i>Sargassum sp.</i>	290	Luning, 1990.
<i>Laminaria sp.</i>	100- 140	Luning, 1990.
<i>Macrocystis sp.</i>	90- 150	Luning, 1990.
<i>Fucus sp.</i>	30- 70	Luning, 1990.
Seagrass		
<i>Zostera Capricorni</i>	14- 63	Larkum <i>et. al.</i> , 1989
<i>Halophila ovalis</i>	30	Larkum <i>et. al.</i> . 1989
<i>Thalassia</i>	240- 740	Larkum <i>et. al.</i> , 1989

TABLE 5: Elemental composition and $\delta^{15}\text{N}$ signature and of *Lyngbya majuscula*.

Carbon ($\text{mmol C g}_{\text{dw}}^{-1}$)	Nitrogen ($\text{mmol N g}_{\text{dw}}^{-1}$)	Phosphorus ($\text{mmol P g}_{\text{dw}}^{-1}$)	Iron ($\text{mmol Fe g}_{\text{dw}}^{-1}$)	$\delta^{15}\text{N}$ (ppt)	C: N: P : Fe
17.93	1.41	0.1	0.177	1.72	179: 14: 1: 1.8

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FIGURE 1: Map of the study region and associated sites within Deception Bay

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FIGURE 4: Midday wind rose for the Brisbane airport over the three weeks prior to the bloom of *Lyngbya majuscula*.

FIGURE 5: Water column nutrient characteristics (μM) averaged over the three water quality sites over the period 15th September 1999 to 5th April 2000 A) Dissolved inorganic parameters, ammonium, nitrate and phosphorus, B) Total and dissolved organic nitrogen and total phosphorus, and C) Total Iron. (^{a, b} values indicate significant differences at $p < 0.05$)

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FIGURE 14: Rapid light curves generated from *Lyngbya majuscula* incubated under varying light intensities (0- 1400 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) using PAM fluorometry. (^a, ^b values indicate significant differences at $p < 0.05$)

FIGURE 15: Effect of varying salinities (0- 36 ppt) on photosynthesis using A.) ^{14}C incorporation, B.) photochemical efficiency indicated by PAM fluorescence and C.) Maximum ETR indicated by PAM fluorescence. Additional column on B.) and C.) demonstrates recovery effect of 9ppt treatment after transfer to full salinity. (^a, ^b values indicate significant differences at $p < 0.05$)

FIGURE 1

FIGURE 2

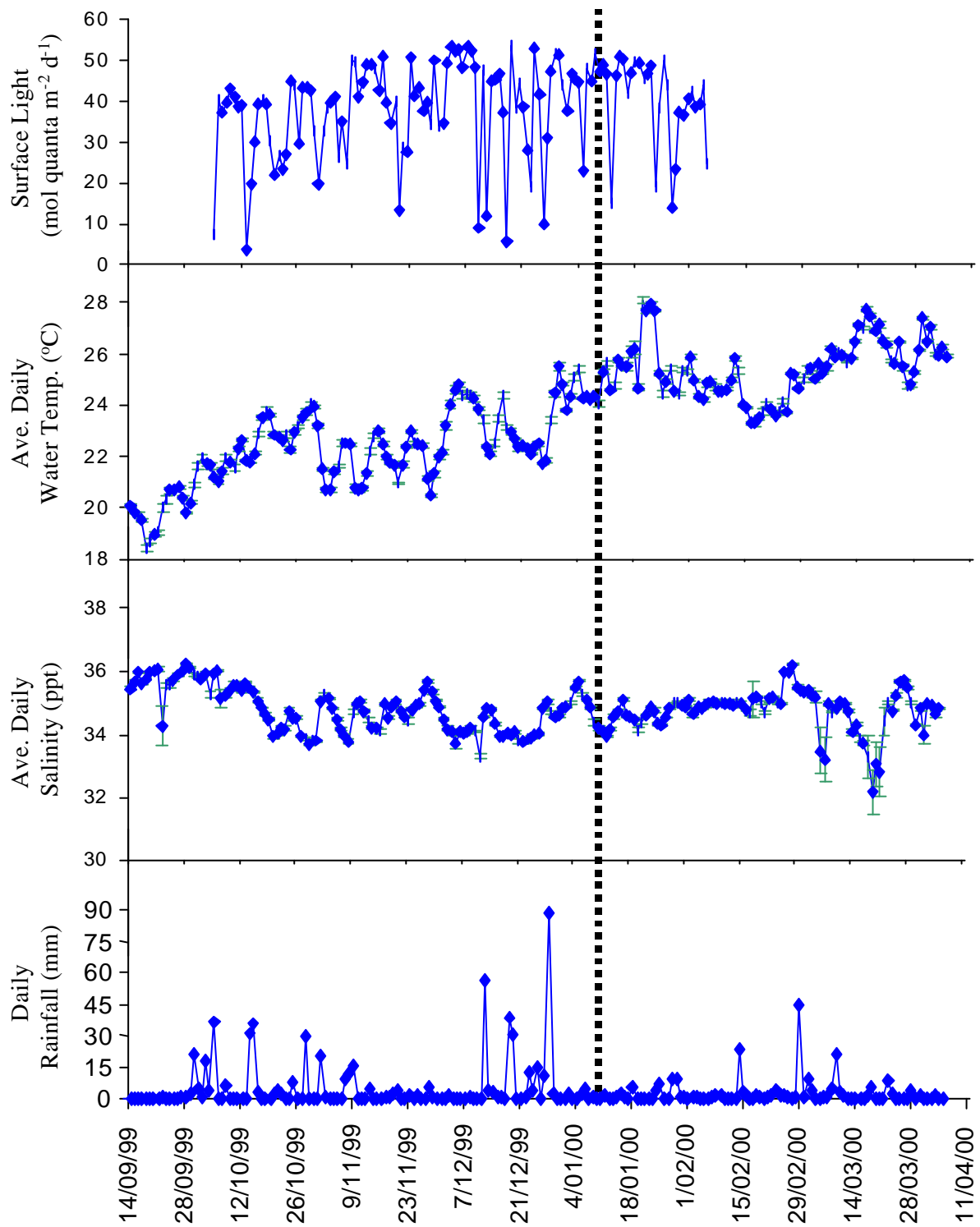


FIGURE 3

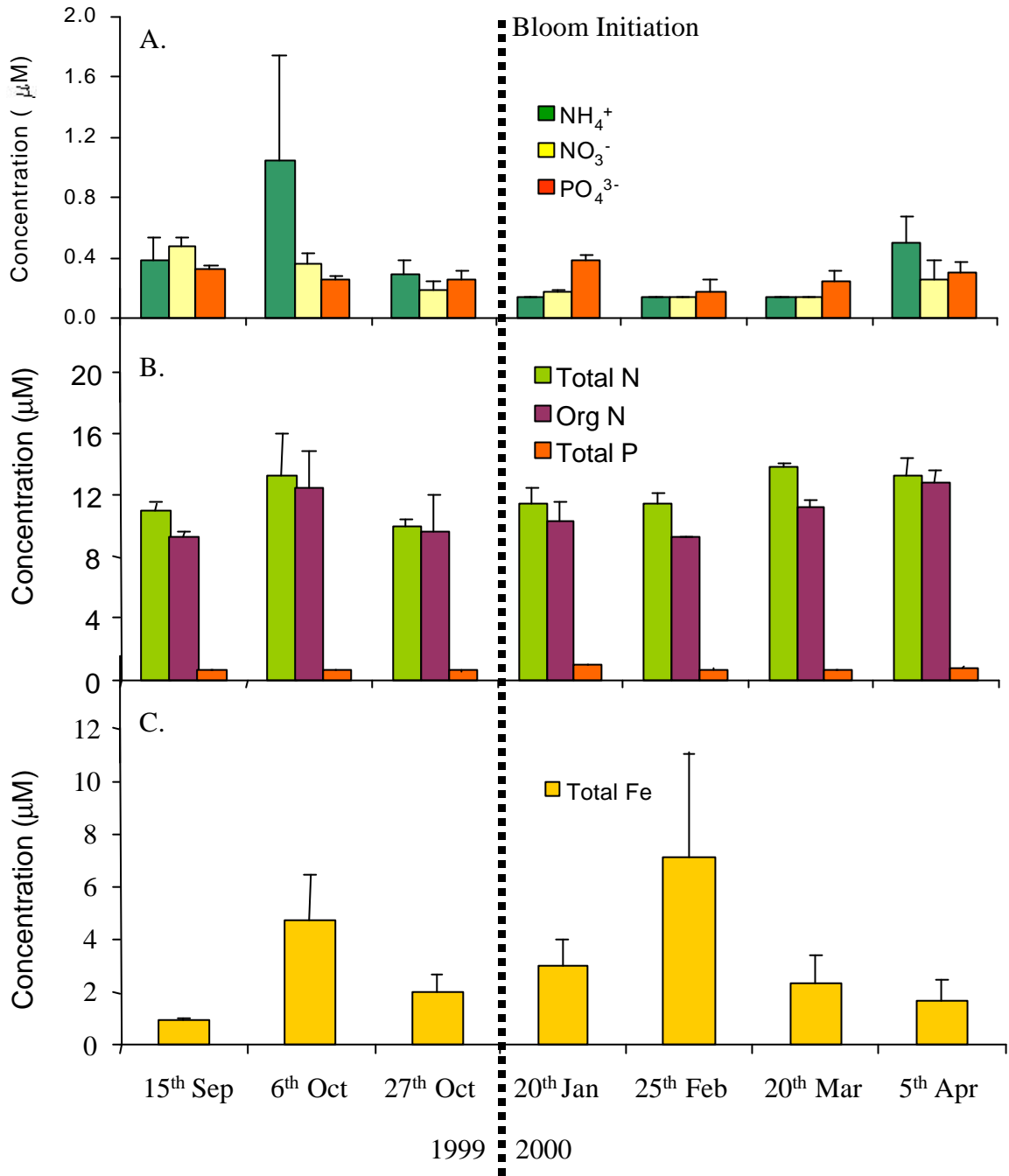


FIGURE 5

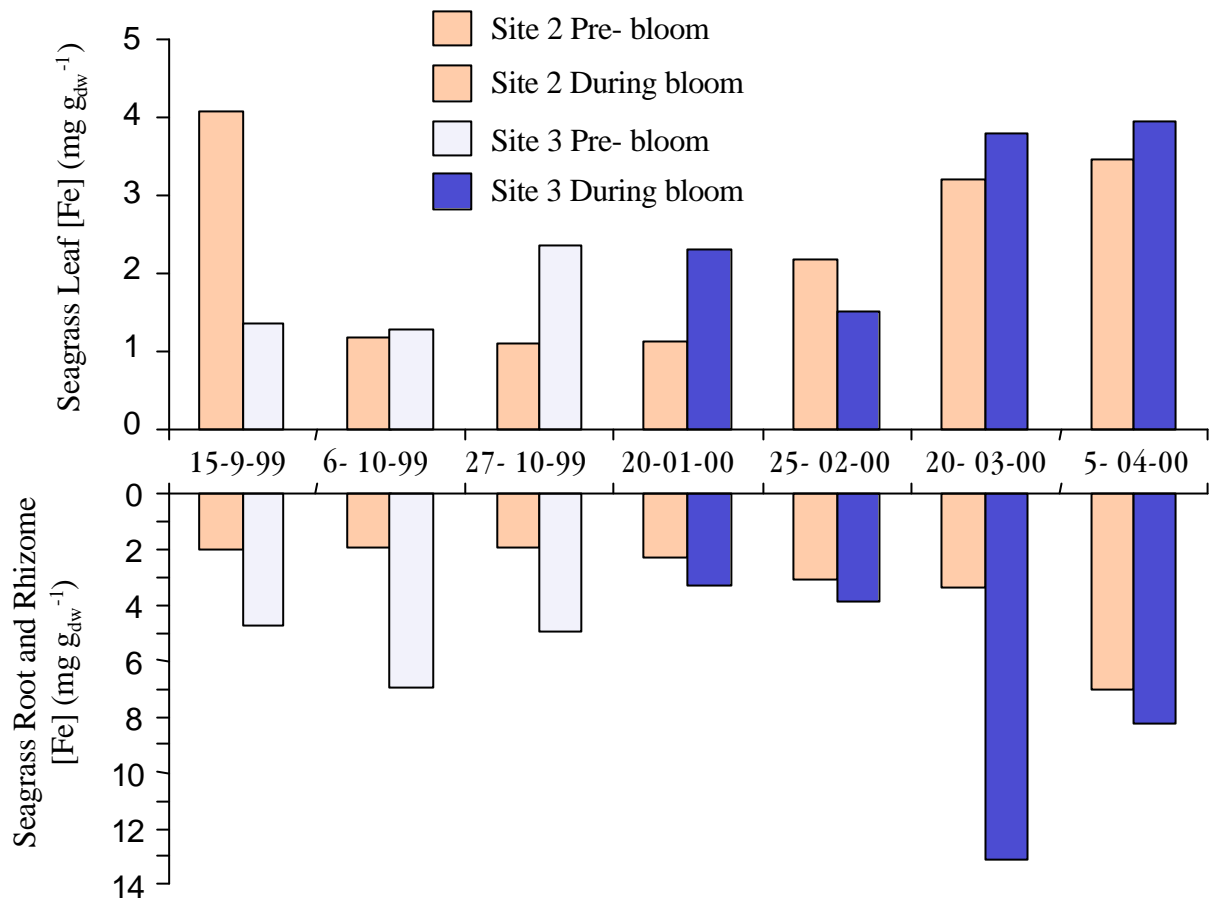


FIGURE 6

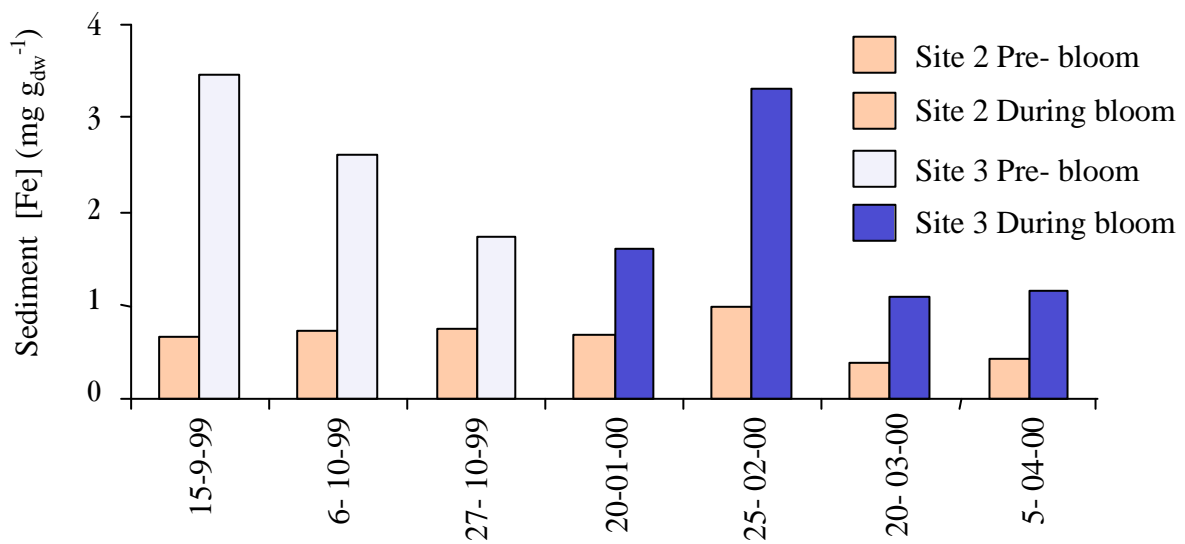


FIGURE 7

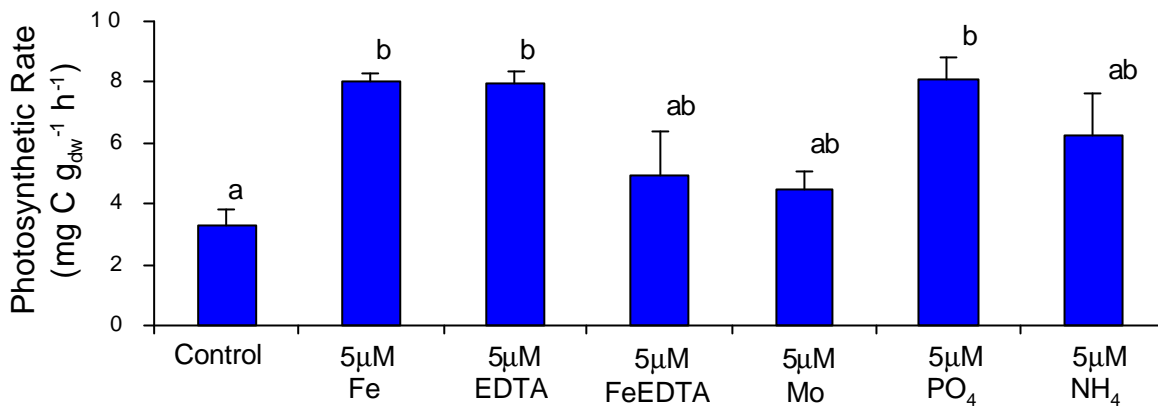


FIGURE 8

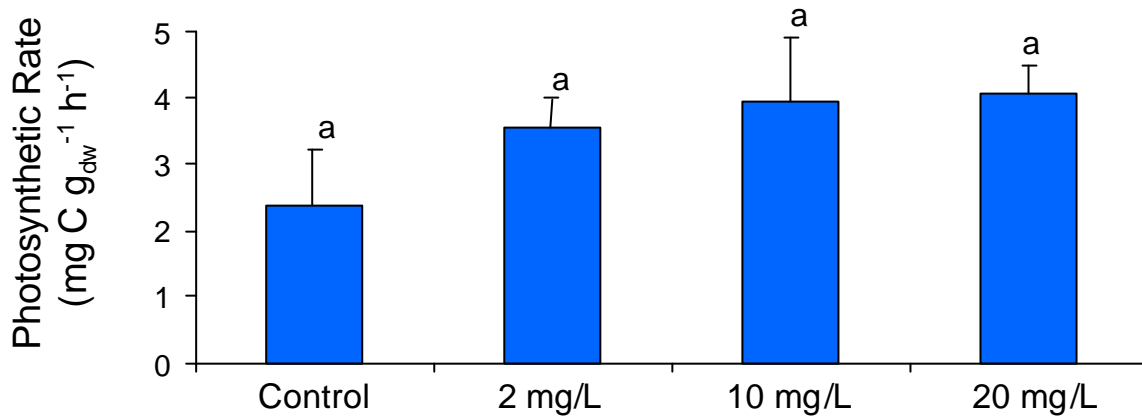


FIGURE 9

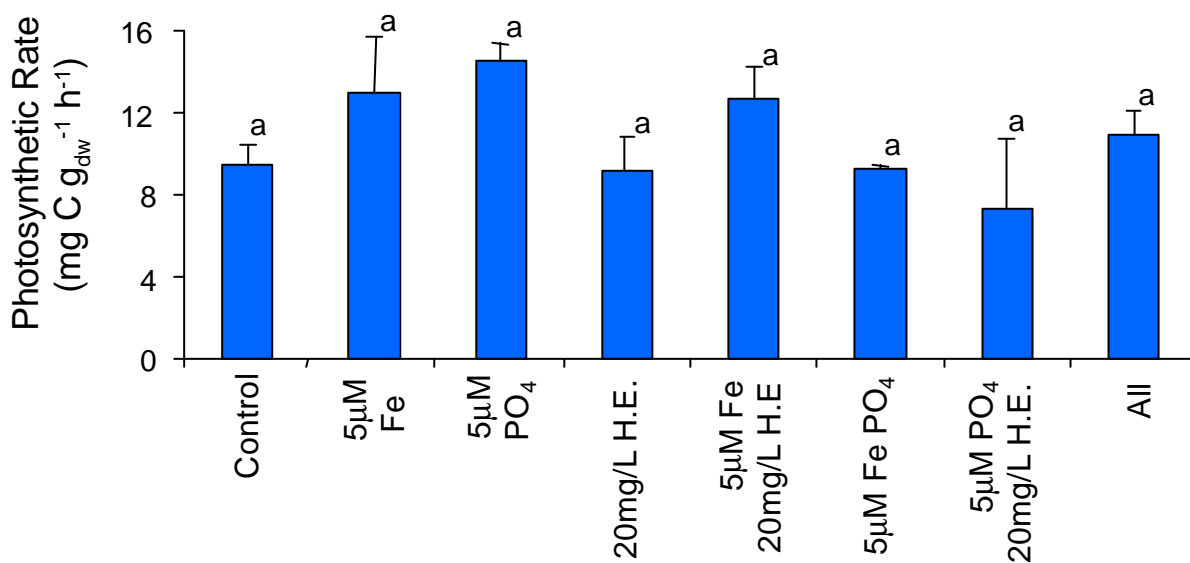


FIGURE 10

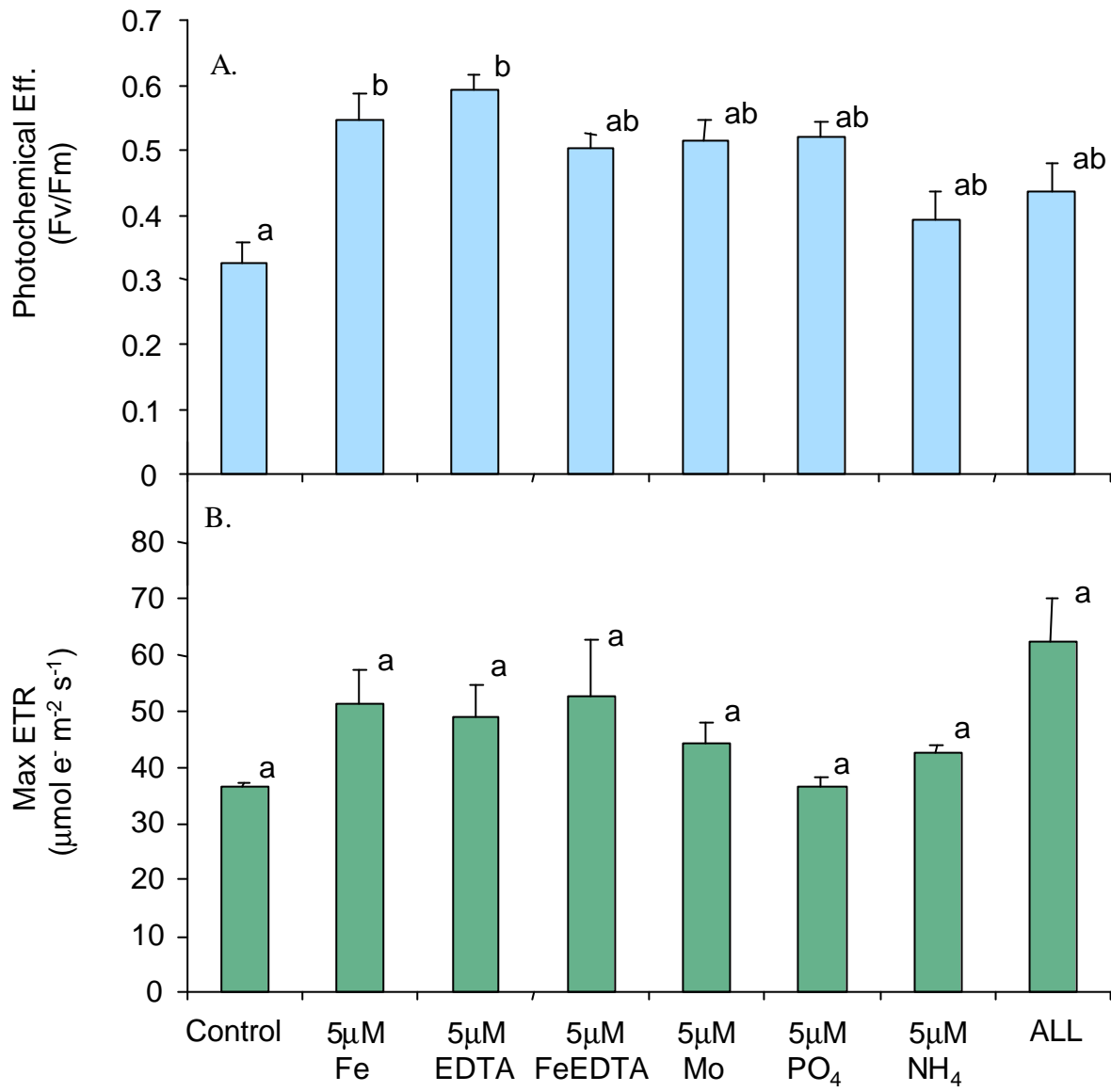


FIGURE 1 I

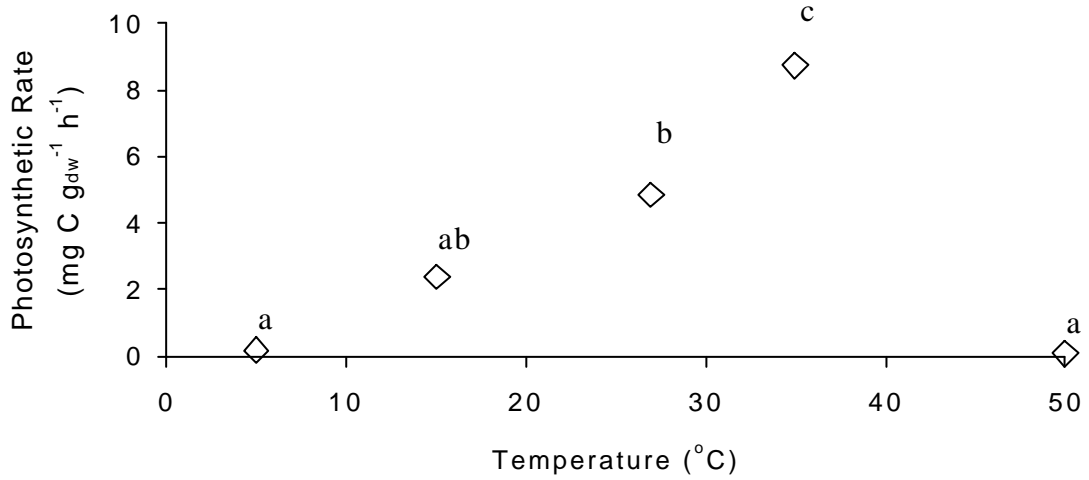


FIGURE 1 2

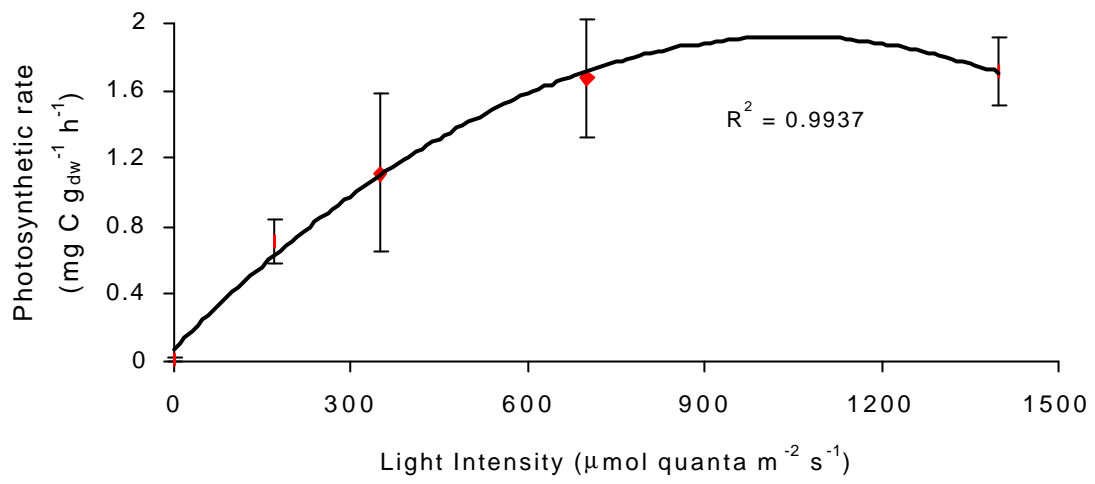


FIGURE I 3

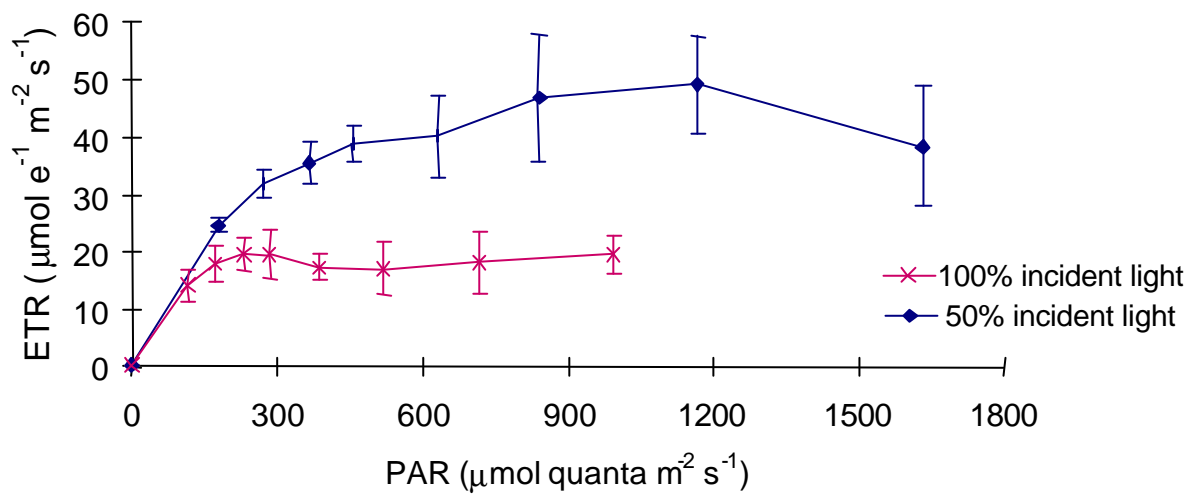


FIGURE I 4

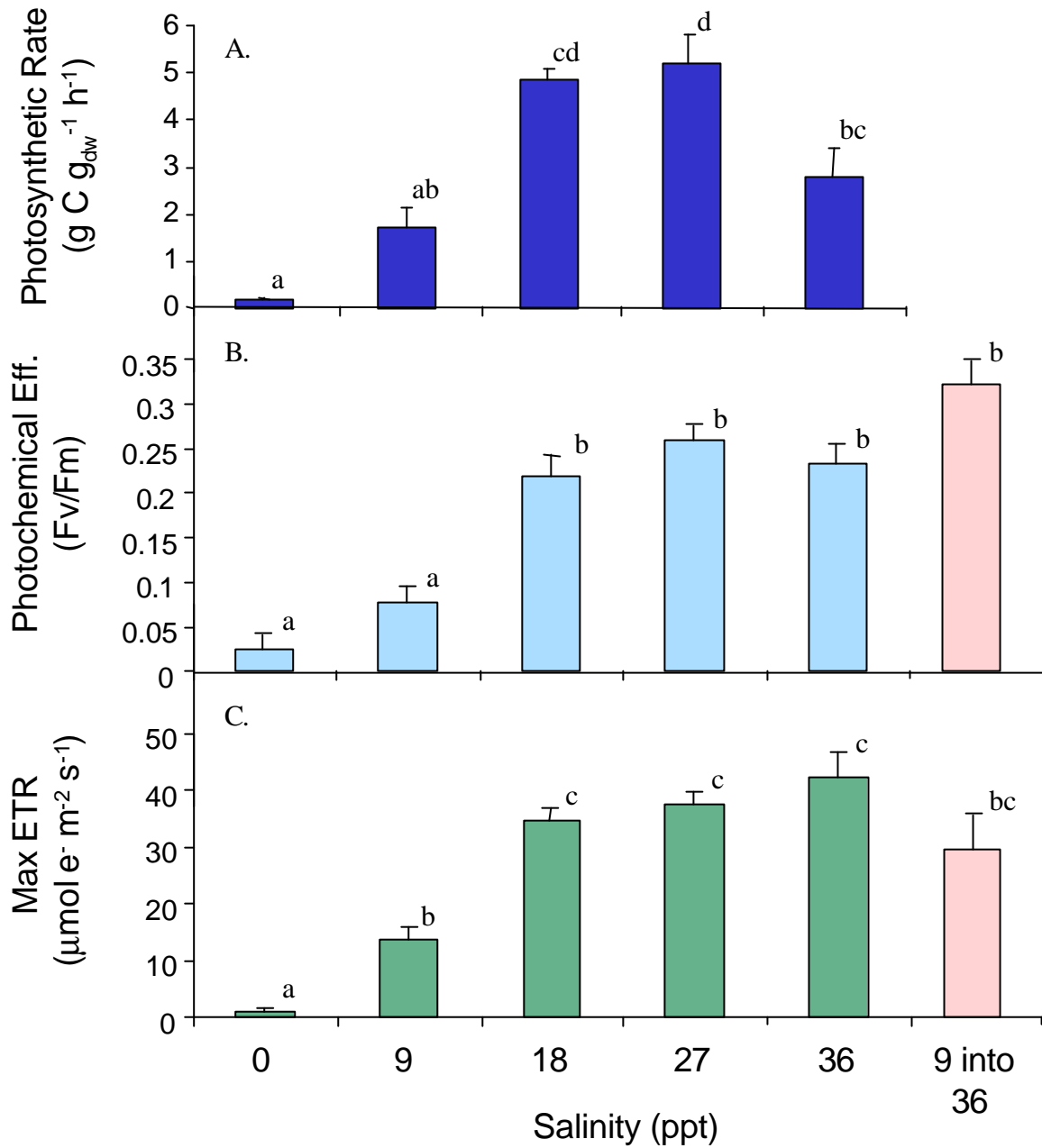


FIGURE 15

