



Influence of inorganic and organic nutrient enrichment on blue-green algal activity and relative biomass in a eutrophic southwest Montana reservoir
by Thomas David Miller

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Biological Sciences
Montana State University
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Abstract:

Nutrient enrichment experiments were conducted over two ice-free seasons in a blue-green algal dominated southwest Montana reservoir to determine seasonal trends in nutrient deficiency. Additional experiments examined the influence of inorganic N, phosphate and dissolved organic carbon (mannitol) on the relative activity of blue-green and non-blue-green algal components of the community. Results showed that the whole phytoplankton community (i.e., all size classes) was generally N-deficient. Phosphorous addition alone stimulated growth and photosynthesis of the N₂-fixing *Anabaena* or *Aphanizomenon* component of the community in only two of ten experiments. The non-blue-green algal component was most consistently stimulated by N. Activity of nitrogenase, the enzyme catalyzing atmospheric nitrogen fixation, was stimulated by P and by mannitol on several occasions whereas N addition consistently reduced nitrogenase activity. Nitrogenase activity was found to have a positive relationship with temperature and total P, and a negative relationship with the DIN:SRP ratio. A multiple linear regression model showed that the relative abundance of nitrogen fixing blue-green algae was positively correlated with the dissolved inorganic N to soluble reactive P ratio, and to total N. This study provides evidence that P is not always the primary nutrient that controls productivity of lakes and reservoirs, and that N must also be considered when making water quality decisions, even in systems dominated by N₂-fixing blue-green algae.

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APPROVAL

of a thesis submitted by

Thomas David Miller

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

15 August 1991
Date

John C. Prusac
Chairperson, Graduate Committee

Approved for the Major Department

26 August 1991
Date

Robert S. Moore
Head, Major Department

Approved for the College of Graduate Studies

August 28, 1991
Date

Henry S. Parsons
Graduate Dean

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ABSTRACT

Nutrient enrichment experiments were conducted over two ice-free seasons in a blue-green algal dominated southwest Montana reservoir to determine seasonal trends in nutrient deficiency. Additional experiments examined the influence of inorganic N, phosphate and dissolved organic carbon (mannitol) on the relative activity of blue-green and non-blue-green algal components of the community. Results showed that the whole phytoplankton community (i.e., all size classes) was generally N-deficient. Phosphorous addition alone stimulated growth and photosynthesis of the N₂-fixing Anabaena or Aphanizomenon component of the community in only two of ten experiments. The non-blue-green algal component was most consistently stimulated by N. Activity of nitrogenase, the enzyme catalyzing atmospheric nitrogen fixation, was stimulated by P and by mannitol on several occasions whereas N addition consistently reduced nitrogenase activity. Nitrogenase activity was found to have a positive relationship with temperature and total P, and a negative relationship with the DIN:SRP ratio. A multiple linear regression model showed that the relative abundance of nitrogen fixing blue-green algae was positively correlated with the dissolved inorganic N to soluble reactive P ratio, and to total N. This study provides evidence that P is not always the primary nutrient that controls productivity of lakes and reservoirs, and that N must also be considered when making water quality decisions, even in systems dominated by N₂-fixing blue-green algae.

INTRODUCTION

Phosphorous has traditionally been thought to limit phytoplankton productivity in lakes (Hecky and Kilham 1988; Schindler 1977). This view has been challenged in recent years by studies showing nitrogen deficiency for many freshwater systems (e.g. Canfield et al. 1989; Dodds et al. 1989; Elser et al. 1988; Prepas and Trimbee 1988; Priscu and Priscu 1984; Vincent et al. 1984; White et al. 1985). Elser et al. (1990) reviewed phytoplankton nutrient enrichment experiments and found nitrogen to be more important than previously recognized. Competition for nutrients in limited supply also plays a significant role in determining phytoplankton community structure (Reynolds 1984). Consequently, a better understanding of nutrient deficiencies will provide water quality managers with important information on the development of bloom formation by nuisance algal species.

The ability of scum-forming blue-green algae (cyanobacteria, e.g., Anabaena and Aphanizomenon) to outcompete other groups in nitrogen deficient systems, or systems with low nitrogen to phosphorus ratios, allows them to dominate many lakes and reservoirs (McQueen and Lean 1987; Tilman et al 1986; Priscu 1987). Nitrogen deficiency can result in blue-green algal blooms that proliferate to

nuisance levels. Systems dominated by these nuisance organisms experience diminished natural resource value. Aesthetic quality and recreational use are hampered by unsightly surface scum and odor. Fish populations are affected by oxygen depletion following collapse of blue-green algal blooms (Ayles et al. 1976; Barica 1975) and by inefficient transfer of primary production to higher trophic levels (Carpenter et al. 1987; Shapiro 1980). Neuro- and hepata-toxins produced by blue-green algae (Gorham and Carmichael 1988) pose a serious hazard to animals and occur more frequently than usually perceived (Sonzogni et al. 1988). Recreation in waters with blue-green algal scum has resulted in cases of contact dermatitis. Algal extracellular products can pose other health problems in municipal water supplies in addition to taste and odor problems. These organics can act as precursors of trihalomethanes (THM's), carcinogenic chemicals formed during chlorination (Cooke 1986). The consequences of blue-green algal blooms underscore the importance of understanding factors regulating blooms to aid in management.

Researchers have reported various factors that contribute to blue-green algal dominance in lakes. Characteristics of blue-green algae that promote their dominance include bouyancy (Reynolds et al. 1987; Klemer and Konopka 1989), immunity to grazing (Porter 1977; Sterner 1989; Holm et al. 1983; Nizan et al. 1986), excretion of

iron chelators that inhibit the growth of other algae (Murphy and Lean 1976; Keating 1978), possession of accessory photosynthetic pigments (Carr and Whitton 1982; Tilzer 1987), ability to exist at low CO₂ levels (Shapiro 1973; Pearl and Ustach 1982), elevated water temperatures (Tilman and Kiesling 1984; Tilman et al. 1986; Smith et al. 1987), water column stability (Reynolds 1984; Priscu 1987), and low underwater light availability (Mur et al. 1978; Tilzer 1987; Smith 1986, 1990). Although blue-green algal picoplankton are abundant in many oligotrophic waters (Stockner 1988), blue-green algae, particularly filamentous, scum forming species, generally contribute more to the phytoplankton biomass in eutrophic waters (Trimbee and Prepas 1987; Wetzel 1983). Most eminent, in terms of management, is the generalization that the relative abundance of nuisance blue-green algae is promoted by increased total P (Trimbee and Prepas 1987) and low N to P ratios (i.e., N-deficiency) in the lake water (Schindler 1977; Smith 1983).

The capability of heterocystous blue-green algae (e.g., Aphanizomenon and Anabaena) to fix N₂, via the enzyme nitrogenase, plays a key role in allowing them to dominate N-deficient systems (Schindler 1977; Carr and Whitton 1982; Wetzel 1983). It follows that the factors regulating nitrogenase activity influence this ability to outcompete other phytoplankton in N-deficient waters. The roles of

macronutrients (N and P), micronutrients (Fe, Mo and Cu) and O₂ have been reviewed by Horne and Commins (1987), Rueter and Petersen (1987) and Pearl (1990), respectively.

Influences of nutrients include inhibition by dissolved inorganic N, Cu and O₂, and stimulation by P, low N:P, Mo and Fe. Nitrogenase activity is light (energy) and temperature dependent (Carr and Whitton 1982; Priscu 1987). Pearl (1990) also associated dissolved organic carbon (DOC) and bacteria with increased nitrogenase activity.

Because of the importance of nutrients in the literature, and our developing ability to manage nutrient inputs from point and non-point sources in the watershed, this study focused on the influence of nutrients on blue-green algae. The objectives of this study were:

1. To determine seasonal phytoplankton nutrient deficiency in a blue-green algal dominated reservoir.
2. To examine the influence of N, P and organic-C enrichment on relative blue-green algal abundance.
3. To examine the influence of N, P and organic-C enrichment on the relative activity of blue-green and non-blue-green algal components of the phytoplankton community.
4. To investigate the influence of N, P and organic-C on blue-green algal nitrogenase activity.

METHODS

Study Site

The reservoir chosen for this study was Hebgen Lake, Montana (lat. $44^{\circ}51'51''$, long. $111^{\circ}20'09''$, elev. 1991 m), the first impoundment on the Madison River (Fig. 1). Hebgen Lake is a storage reservoir operated by Montana Power and Gas Company for hydroelectric production downstream. The reservoir has a storage capacity of $476.5 \times 10^6 \text{ m}^3$, maximum depth of 25 m at the dam, average annual discharge of $891 \times 10^6 \text{ m}^3$, and a 2344 km^2 drainage basin which lies largely within Yellowstone National Park (U.S.G.S. 1984). Permanent sampling stations visited during routine trips are marked on Figure 1 as: 1, Grayling Arm; 2, Madison Arm; 3, Mid-Lake; 4, Dam. Water for all nutrient enrichment experiments was collected from the Grayling Arm at station 1. The Grayling Arm is a hydrologically distinct lobe of Hebgen Lake with its own inflows and a single outflow through a narrow channel connecting it with the main lake. Ground water fluxes have not been quantified. The Grayling Arm has a surface area of 7.8 km^2 , volume of $29.9 \times 10^6 \text{ m}^3$, mean depth of 3.8 m and maximum depth of 8 m at full pool. It is polymictic, but thermally stratifies occasionally. This lobe of the lake experiences dense blooms of often toxic N_2 -fixing blue-green algae which dominate the phytoplankton

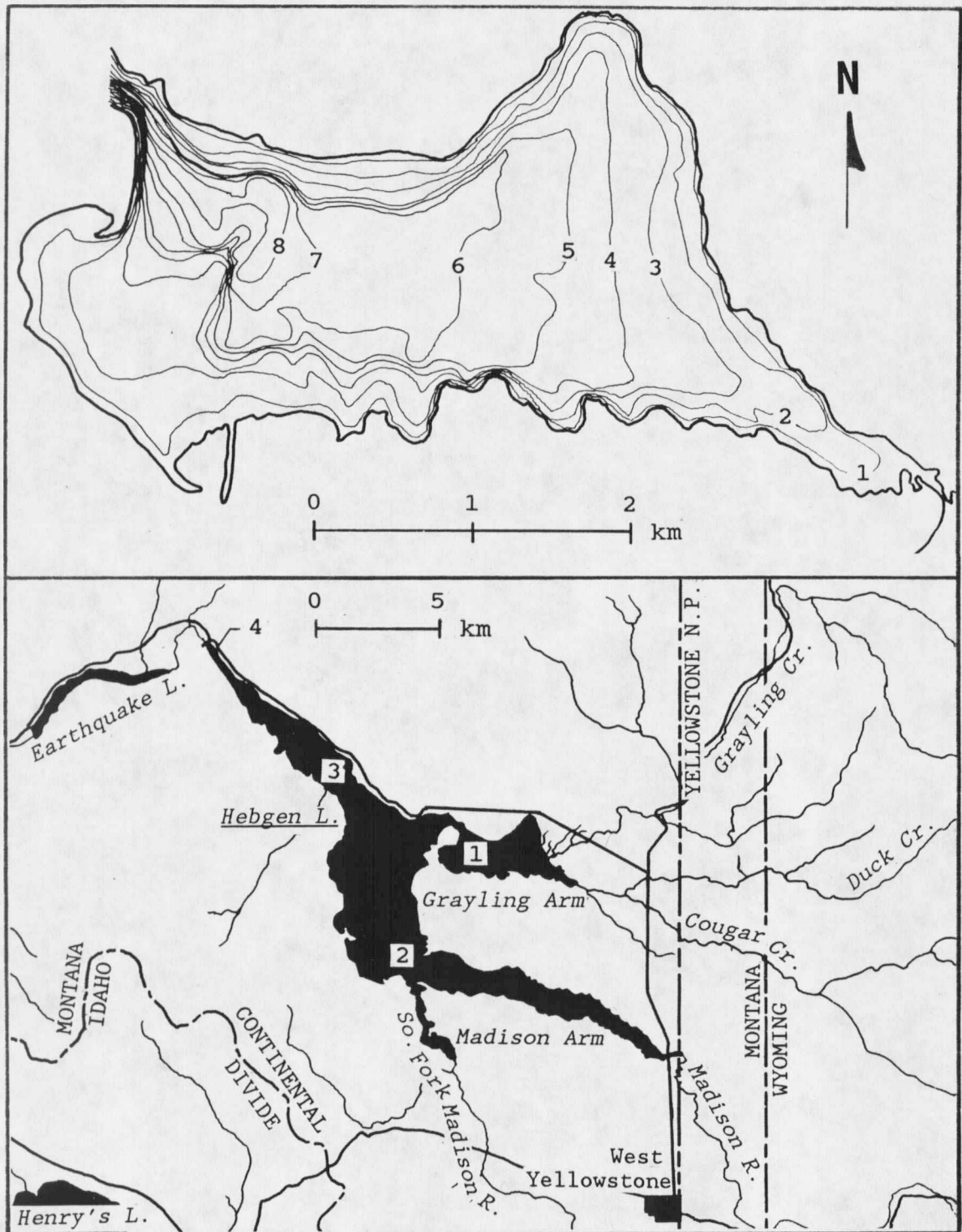


Figure 1. Map of Hebgen Lake, Montana, with permanent sampling stations marked 1, 2, 3 and 4 (bottom) and contour map of Grayling Arm (top). Contour interval 1 m.

community during most of the ice-free season. A detailed description of Hebgen Lake was presented by Martin (1967) and Martin and Arneson (1978).

Field Monitoring

Routine sampling trips to Hebgen Lake were conducted approximately biweekly during the ice-free seasons (mid-May through early November) of 1988 and 1989. In 1988 data were collected at buoys set at the four permanent sampling locations marked on Figure 1. Sampling focused on the Grayling Arm in 1989, with the addition of three sampling dates at the Mid-Lake station. Water samples for nutrient chemistry, phytoplankton enumeration, and measurement of phytoplankton photosynthesis and nitrogenase activity were collected with a 4-l Van Dorn sampler from discrete depths (0, 1, 3, 5, 10, 15 and 20 m) at each station. These profiles extended down to 5 m at station 1, 10 m at station 2, and 15 or 20 m at stations 3 and 4, depending on water level. Secchi depth and dissolved oxygen profiles were recorded during each site visit. Samples for dissolved and particulate nutrient analyses and phytoplankton enumeration were stored in clear high density polyethylene bottles on ice until returned to the laboratory.

One light and one dark bottle for phytoplankton carbon uptake and one bottle for nitrogenase activity (also a kill from 1 m) were incubated for 4 to 6 hours during midday at the depth and location of sampling (see phytoplankton

photosynthesis measurement and nitrogenase activity assay sections below). Photosynthesis was terminated by putting carbon uptake bottles into a light-proof box for transport to the laboratory. Extremely rough water precluded incubations at the Dam station on several occasions and retrieval of incubation bottles from the Madison Arm twice during 1988. Leakage of the acetylene flask precluded nitrogenase activity incubations at station 1 on 23 August 1989.

Dissolved Nutrient Analyses

Soluble reactive phosphorous (SRP) was determined by the molybdate method modified for AsO_4^- interference (Downes 1978) and total dissolved phosphorous (TDP) by the acid hydrolysis procedure (Solórzano and Sharp 1980) followed by orthophosphate determination (Stainton et al. 1977). Concentrations of NH_4^+ were measured by the phenol hypochlorite method (Solórzano 1969), NO_3^- by cadmium reduction (Eppley 1978), and total dissolved nitrogen by persulfate digestion (D'Elia et al. 1977) followed by determination of NO_3^- by cadmium reduction. These analyses were performed on samples that had been prefiltered through Whatman GF/C filters and frozen in acid-washed high density polyethylene bottles before analysis. Dissolved organic carbon (DOC) was measured with a Dohrmann Carbon Analyzer on acidified ($\text{pH} < 3$) samples. Dissolved inorganic carbon (DIC) was determined using the bromocresol green - methyl red

titration method for alkalinity (A.P.H.A. 1971). Alkalinity ($\text{mg CaCO}_3 \text{ l}^{-1}$) was converted to DIC (mg C l^{-1}) by multiplying the former by 0.24, based on the molecular weights and milli-equivalencies of C and CaCO_3 .

Particulate Matter Analyses

The Whatman GF/C filters used for dissolved nutrient sample filtration were retained for particulate analyses. Pheophytin corrected chlorophyll a (CHL a) was determined by fluorometry (Strickland and Parsons 1972), standardized with known amounts of pure CHL a (Sigma). The acid hydrolysis procedure (Solórzano and Sharp 1980) with subsequent orthophosphate measurement on the digest (Stainton et al. 1977) was used to measure particulate phosphorus (PP). Particulate carbon (PC) and particulate nitrogen (PN) were determined with a Carlo Erba model 1106 elemental analyzer calibrated with acetanilide. Total nitrogen (TN) and total phosphorus (TP) are the sum of the total dissolved and particulate fractions of each respective element.

Phytoplankton Enumeration

Phytoplankton species and numbers were determined from samples preserved with Lugol's solution. Uttermohl chambers (Uttermohl 1958) were filled with an appropriate amount of sample (5-25 ml), depending upon algal density, and settled for at least 4 h cm^{-1} of water in the chamber. The settled

phytoplankton were identified and counted with a calibrated Zeiss inverted microscope (Lund et al. 1957) and measured for biovolume determination. Equations for volumes of geometric shapes that approximated each cell type and appropriate average dimensions for each species were used to determine biovolume, which was converted to biomass under the assumption that the specific gravity of phytoplankton equals that of water. Data were grouped by divisions: Cyanophyta (blue-green algae), Chrysophyta, Cryptophyta, Pyrrophyta, Chlorophyta and LRGT (not an algal division but a group representing all "Little Round Green Things" less than 2 μm in diameter).

Phytoplankton Photosynthesis Measurement

During field monitoring and mesocosm and limnocorral experiments, the rate of phytoplankton photosynthetic C-uptake (primary productivity = PPR) was determined by adding [^{14}C]- NaHCO_3 as a tracer (final activity of about 0.05 $\mu\text{Ci ml}^{-1}$) to 150 ml aliquots of sample. Sterile aqueous [^{14}C]- NaHCO_3 solution with a specific activity of 50 mCi mmol^{-1} (ICN Radiochemical Inc.) was diluted with sterile, deionized water to a final working activity of about 6 $\mu\text{Ci ml}^{-1}$. After adjusting the pH to 10.8 with NaOH, the working stock was ampulated in 5 or 10 ml volumes and autoclaved. The final activity was determined by internal standardization with a ^{14}C -toluene standard (ICN). Standardization was done in the presence of ethanolamine (Fisher Scientific) to avoid loss

of CO₂ to the atmosphere. One light and one dark bottle were used for each depth for field monitoring. Three light bottles and one dark bottle from each treatment were incubated for the experiments. Darkened samples were included to correct for non-photosynthetic ¹⁴C uptake. Bottles were incubated in-situ for approximately 4 h near midday. Photosynthesis was terminated by placing samples in dark boxes and subsequent filtration onto Whatman GF/C filters, followed by three washes with 10 ml deionized water. The filters were placed into 20 ml scintillation vials and acidified with 250 μl of 3.0 N HCl to eliminate unincorporated [¹⁴C]-NaHCO₃; after drying, activity remaining on the filter was determined with standard liquid scintillation spectrophotometry (Beckman LS-100C scintillation counter). Efficiency (used to convert CPM to DPM) was computed by the external standard channels ratio, with a quench curve using acetone as a quenching agent and ¹⁴C-toluene as the standard radiation source. Disintegrations per minute (DPM) of ¹⁴C tracer was converted to carbon uptake using the following equation.

$$\mu\text{g C l}^{-1}\text{h}^{-1} = ((\text{LtDPM} - \text{DkDPM}) \times \text{DIC} \times 1.06) /$$

$$(\mu\text{Ci} \times 2.2 \times 10^6 \times \text{time})$$

LtDPM-DkDPM : light bottle DPM - dark bottle DPM

DIC : dissolved inorganic carbon (based on
alkalinity titration) (μg C l⁻¹)

1.06 : isotope discrimination factor to correct for the slight preference for ^{12}C during photosynthesis.

μCi : ^{14}C activity of tracer added to bottle in $\mu\text{Curries}$.

2.2×10^6 : converts DPM to μCi
 ($2.2 \times 10^6 \text{ DPM } \mu\text{Ci}^{-1}$)

time : incubation time (h)

Size Fractionation

On one day during each experiment (except June 1988 mesocosm experiment), separation of the phytoplankton community into blue-green algal and non blue-green algal size fractions was accomplished to investigate the relative photosynthetic activity of each. Size fractionation of each treatment was done by pouring a subsample from each light or dark bottle through 20 μm , 100 μm or 210 μm Nitex netting and collecting the phytoplankton that passed through on a GF/C filter. The radioactivity on the filter was used to compute what is reported herein as DPM ml^{-1} , or $\mu\text{g C}(1 \times \text{h})^{-1}$ for the < 20, < 100, or < 210 μm (non-blue-green algal) fraction, depending on which mesh size was chosen to effectively exclude the filamentous N_2 -fixing blue-green algae from the rest of the community. This activity was subtracted from the activity calculated for an aliquot of whole sample from the same bottle to yield activity in the > 20, > 100, or > 210 μm (blue-green algal) fraction.

Nitrogenase Activity Assay

Rates of atmospheric nitrogen fixation were estimated using the acetylene reduction method to measure nitrogenase activity (NA) (Flett et al. 1976). This technique employs the ability of the nitrogenase enzyme to reduce acetylene (C_2H_2) to ethylene (C_2H_4). For each treatment, four 55 ml aliquots were decanted into 70 ml serum vials and sealed with rubber septa. Formalin (2.5 ml) was added to one of the vials (kill) for background ethylene determination. Injection of 6.0 ml high purity acetylene was followed by notation of start time and gentle shaking for 10 s to equilibrate acetylene with the aqueous phase. After approximately 4 h incubations, vials were shaken vigorously for 30 s to equilibrate gases between the aqueous and gaseous phases. The stop time was noted when 1.5 ml of headspace gas was transferred to a 4 ml vacuutainer. Analysis of 0.5 ml gas from each vacuutainer was made with a Carle 100-AGC gas chromatograph fitted with a flame ionization detector connected to a Shimadzu model C-R3A integrator. The system was calibrated with high purity ethylene (Matheson Gas Co.) to yield nmol ethylene injection⁻¹. Rates of acetylene reduction (ethylene production) were calculated using the following equation.

$$\text{nmol } C_2H_4 \text{ ml}^{-1} \text{ h}^{-1} = ((\text{nmol } C_2H_4 \times 15 \text{ ml}) / (0.5 \text{ ml} \times \text{time} \times 55 \text{ ml} \times \text{trans. coeff.})) \times 3.31$$

nmol C_2H_4 : C_2H_4 produced in sample - formalin kill
15 ml : volume gas phase
0.5 ml : volume injected into gas chromatograph
55 ml : volume aqueous phase
3.31 : correction for dilution in vacutainers
trans. coeff. : transfer coefficient of ethylene
from aqueous to vapor phase

The transfer coefficient of ethylene from aqueous to vapor phase is actually the proportion of ethylene transferred to the vapor phase, which is temperature dependent and varied from 0.620 at 9 °C to 0.690 at 20 °C for this study. This coefficient depends upon the bunsen absorption coefficient for ethylene, water temperature, and aqueous:vapor phase ratio (Kellar et al. 1980). On 17 August 1988 a concentrated sample ($117 \mu\text{g CHL a l}^{-1}$) of Anabaena from the Grayling Arm was used to check the validity and optimization of my incubation times and acetylene injection size. Hourly samples over a 12 h incubation showed a linear response, indicating that 4 h incubation times should not deplete acetylene. even during dense blooms. The response of samples injected with 1,2,3..., or 12 ml of acetylene indicated that the 6.0 injection elicited rates of acetylene reduction near maximum.

Experimental Procedures

02JUN88 Experiment

On 02 June 1988 water collected from 0.5 m in the Grayling Arm was put into four 1-l polyethylene bottles. Three bottles were inoculated with either $200 \mu\text{g l}^{-1} \text{NH}_4^+\text{-N}$, $20 \mu\text{g l}^{-1} \text{PO}_4^{3-}\text{-P}$, or both; the fourth sample served as an unammended control. The inoculated samples were placed in a laboratory incubator at the temperature and light period of collection under cool-white fluorescent light ($150 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Twelve hours after nutrient inoculation, triplicate subsamples from each treatment were incubated with ^{14}C -bicarbonate for 4 h at the same light and temperature as above. The ^{14}C -labeled samples were size fractionated with $20 \mu\text{m}$ mesh onto GF/C filters and counted.

River Water Experiments

Bioassays designed to investigate the influence of the river inputs to the Grayling Arm on phytoplankton were conducted in May (16-21), July (26-30) and October (09-13) of 1989. The inclusion of N and P additions facilitated interpretation of the responses. A 4 l bulk sample of water from 0.5 m was transported to the laboratory and inoculated with [^{14}C]- NaHCO_3 (final activity of about $0.008 \mu\text{Ci ml}^{-1}$). Zooplankton were not removed. Aliquots of the ^{14}C -inoculated lake water and ammendments were added to three 250 ml polycarbonate flasks for each treatment as follows:

- control (200 ml lake water)
 +N (200 ml lake water + 200 $\mu\text{g l}^{-1}$ $\text{NH}_4^+\text{-N}$)
 +P (200 ml lake water + 50 $\mu\text{g l}^{-1}$ $\text{PO}_4^{3-}\text{-P}$)
 N+P (both +N and +P)
 25%R.W. (150 ml lake water + 50 ml river water)
 50%R.W. (100 ml lake water + 100 ml river water)

The river water used for these additions was a 1:1:1 mix of water from Grayling Creek, Duck Creek and Cougar Creek, the three inflows to Grayling Arm. Nutrient concentrations and alkalinity were determined for the lake and river water at the inception of each experiment.

The 250 ml flasks were incubated at the temperature and light period of collection under cool-white fluorescent light ($150 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Subsamples from each replicate were filtered on days 2 and 4 (days 3 and 5 in May) for determination of ^{14}C -uptake by algae. Size fractionations ($20 \mu\text{m}$ in May and July; $210 \mu\text{m}$ in October) were accomplished on the last day of each experiment. In-vivo fluorescence of the phytoplankton in each replicate was determined on 3 ml aliquots with a Turner 112 fluorometer (fitted with standard filters for CHL a analysis) to yield a relative measure of biomass. Results from river water treatments were corrected for dilution of biomass and isotope. A fourth replicate was included with each treatment during the October river water experiment in which nitrogenase activity and CHL a were measured on day 5.

Limnocorral Experiments

Two experiments employed larger scale (2000 l) polyethylene tubes (limnocorrals) suspended by rafts anchored at the Grayling Arm station. The 1.15 x 2.3 m tubes, constructed out of clear Canvex™ (Raven Industries), were closed at the bottom and shaped with integral PVC rings placed at 1 m intervals. Limnocorrals were filled with 0-1 m water at the station by submersing the top and allowing them to fill as the bottom sank. Thorough mixing after nutrient additions and before each sampling was accomplished with a Secchi disk. Samples were collected from limnocorrals at 1 m with an opaque Van-Dorn bottle. Background nutrient samples were collected at the inception of these experiments. Incubations for carbon uptake and nitrogenase activity were conducted in the same manner as for mesocosm experiments.

Limnocorral experiment 1 (03-19 June 1989) included two enclosures: control (no amendments) and +N (c.a. $200 \mu\text{g l}^{-1} \text{NH}_4^+\text{-N}$). Additions were made initially and on days 5 and 10 of this 16-day experiment. Samples for photosynthetic C-uptake, phytoplankton enumeration, and CHL a were collected on days 1, 3, 5, 10 and 16, and for nitrogenase activity on days 1, 5 and 10. Photosynthetic C-uptake was size fractionated ($20 \mu\text{m}$) on days 3, 5 and 16.

Limnocorral experiment 2 (29 June-13 July 1989) employed four limnocorrals, each with a different N:P ratio. Three limnocorrals were enriched with $280 \mu\text{g l}^{-1} \text{NH}_4^+\text{-N}$. P

was added to each limnocorral at 56, 14 or 7 $\mu\text{g l}^{-1}$ $\text{PO}_4^{-3}\text{-P}$, respectively, yielding N:P enrichment ratios of 5:1, 20:1 and 40:1, respectively. The fourth limnocorral was not enriched and served as a control. Nutrient additions were made at the beginning of the experiment and on days 5 and 10. Samples for phytoplankton photosynthetic C-uptake, size fractionated (20 μm) C-uptake, nitrogenase activity, CHL a concentration and phytoplankton enumeration were collected on days 5, 10 and 14 from each limnocorral.

Mesocosm Experiments

Five day time-course nutrient enrichment bioassays were conducted in June, August and October of 1988 and 1989 in the Grayling Arm. Water was collected from 0.5 m at a station located in the deepest part of the Grayling Arm and prescreened through 280 μm Nitex netting to remove large zooplankton (except in October 1988 and August and October 1989 when prescreening would have removed a large number of filamentous blue-green algal aggregates). For the June and August experiments, one 20 l collapsible polyethylene carboy (mesocosm) for each treatment was filled with sample and suspended in-situ at the station for the duration of the experiment. Water for the October experiments was transported 150 km to our laboratory (due to logistical constraints) where one mesocosm was incubated for each treatment in an incubator at the temperature and light period of collection under cool-white fluorescent light (150

$\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$). Experiments began on 22 June, 21 August and 23 October in 1988, and on 19 June, 07 August and 19 October in 1989. Seven nutrient treatments and an unamended control were included with each experiment (Table 1). Nutrient additions using NH_4Cl , KH_2PO_4 , mannitol (MANN) and Na_2MoO_4 (1988 only) were made as a single pulse at the beginning (day 0) of 1988 experiments and on days 0, 1, 2, 3 and 4 of 1989 experiments. Multiple additions were made in 1989 to ensure that the added nutrients were not depleted during the incubation period. Chlorophyll *a* (CHL *a*), nutrient concentrations, and phytoplankton primary productivity ($^{14}\text{CO}_2$ uptake) were determined for all treatments on each day of the experiments. Bottles for $^{14}\text{CO}_2$ uptake (3 light, 1 dark) were incubated for about 4 h near midday alongside the treatment microcosms. Phytoplankton samples were taken from the water used for the experiment (day 0) and from each treatment at the end of each experiment.

Statistical Models

Monitoring data were used to build multiple linear regression models to facilitate determination of key environmental factors regulating N_2 -fixing blue-green algal dominance and nitrogenase activity in the Grayling Arm. A statistical text (Neter et al. 1985) was consulted for appropriate methods and interpretations. The dependent variables were the arcsine of the square root of the percent

Table 1. Nutrient amendments ($\mu\text{g l}^{-1}$) to water from Hebgen Lake (Grayling Arm, 0.5 m) for mesocosm experiments.

TREATMENT	JUNE 1988	AUGUST 1988	OCTOBER 1988
Control	-- --	-- --	-- --
$\text{NH}_4^+\text{-N}$	100.0	100.0	100.0
$\text{NO}_3^-\text{-N}$	100.0	100.0	100.0
Mo	9.6	9.6	9.6
$\text{NO}_3^-\text{-N} +$ Mo	100.0 9.6	100.0 9.6	100.0 9.6
$\text{PO}_4^{-3}\text{-P}$	50.0	50.0	50.0
Mannitol (0.5 MAN)	91.1	91.1	91.1
Mannitol (1.0 MAN)	182.2	182.2	182.2

TREATMENT	JUNE 1989	AUGUST 1989	OCTOBER 1989
Control	-- --	-- --	-- --
$\text{NH}_4^+\text{-N}$	140.0	140.0	140.0
$\text{NO}_3^-\text{-N}$	140.0	140.0	140.0
$\text{PO}_4^{-3}\text{-P}$	93.0	93.0	93.0
$\text{NH}_4^+\text{-N} +$ $\text{PO}_4^{-3}\text{-P}$	140.0 93.0	140.0 93.0	140.0 93.0
Mannitol (MAN)	91.1	91.1	91.1
Mannitol + $\text{NH}_4^+\text{-N}$ (M+N)	91.1 140.0	91.1 140.0	91.1 140.0
Mannitol + $\text{PO}_4^{-3}\text{-P}$ (M+P)	91.1 93.0	91.1 93.0	91.1 93.0

N_2 -fixing blue green algae (%FXBG) and the natural log of the biomass specific rate of nitrogenase activity. One primary model was constructed for each dependent variable using Grayling Arm data only, because my experiments all manipulated Grayling Arm water. A secondary model for each dependent variable included pertinent data points (those with values for the dependent variable greater than 0, see Appendix 1) from all stations to supplement the Grayling Arm data. This allowed me to check the validity of the primary models. The natural log of the following independent variables were used in the modelling process.

TEMP	TP	TDN	NH_4^+ -N:SRP
SRP	NH_4^+ -N	DON	NO_3^- -N:SRP
TDP	NO_3^- -N	TN	DIN:SRP
DOP	DIN	DOC	TN:TP

Data used in the models consisted of an areal values resulting from trapezoidal integration of each factor over the epilimnion (0-5 m) of the water column (see Appendix 1: integrated values). The only exception was the use of the mean temperature of the epilimnion. A forward stepwise regression procedure (Statgraphics version 1.2) was employed to select variables that are most closely associated with either dependent variable based on an F-ratio equivalent to $p=0.05$. A linear model was produced using the selected variables. Residual plots were examined for random distribution to assure that proper transformations were

used. The ridge trace procedure was then employed to produce standardized partial regression coefficients that were adjusted for potential intercorrelation between independent variables. These standardized coefficients offer a means of comparing the relative importance of each independent variable in the model.

RESULTS

Field Monitoring Data

Data collected during routine sampling trips are presented in Appendix 1 (lake station data) and Appendix 2 (inflow and outflow data). These data were collected primarily for statistical modelling, and to interpret experimental results in the proper ecological perspective. These data will not be discussed in detail here. Grayling Arm and Grayling Arm inflow data are summarized in Table 2. During 1988, blue-green algae (Cyanophyta) in the Grayling Arm (Fig. 2) were predominated by the N₂-fixing species Anabaena spiroides until September, when Aphanizomenon flos-aquae became dominant. The majority of blue-green algae were Anabaena circinalis during June of 1989. In July of 1989, Aph. flos aquae became dominant and remained so into October. Anabaena flos-aquae, Lyngbya bergei, Microcystis aeruginosa and Gomphospheria sp. were also identified in Grayling Arm samples. When N₂-fixing blue-green algae were present at other stations, they were mainly Aph. flos-aquae, otherwise L. bergei were the predominant blue-green algal genera.

Table 2. Mean (range) of all data collected (0 and 1 m only for PPR and NA) from routine field monitoring trips for Grayling Arm and the three Grayling Arm inflows (Grayling, Duck, and Cougar Creeks). BLD = below limit of detection (approx. $1 \mu\text{g l}^{-1}$ for those noted). Phyto = total phytoplankton biomass. All in units $\mu\text{g l}^{-1}$ unless noted.

	GRAYLING ARM		INFLOWS	
SECCHI (m)	1.4	(0.7-2.0)	-	-
°C	13.2	(0.9-20.6)	-	-
CHL <u>a</u>	16.3	(1.1-275.2)	-	-
Phyto	6808	(190-98800)	-	-
PPR ($\mu\text{gC l}^{-1}\text{h}^{-1}$)	26.0	(2.6-134.7)	-	-
NA ($\text{nmol l}^{-1}\text{h}^{-1}$)	297.1	(0-2582.8)	-	-
$\text{NH}_4^+\text{-N}$	11.4	(0.3-57.2)	6.9	(BLD-34.9)
$\text{NO}_3^-\text{-N}$	27.1	(1.8-93.5)	11.9	(2.6-63.0)
DIN	38.6	(2.5-128.9)	18.7	(4.3-90.6)
TDN	249.1	(98-530)	115.8	(18-300)
PN	202.1	(22-1535)	29.5	(BLD-157)
TN	453.2	(153-2014)	143.7	(38-308)
SRP	13.0	(1.0-60.4)	10.4	(1.3-41.5)
TDP	33.7	(5.2-129.8)	20.4	(4.9-43.6)
PP	23.4	(6.3-166.8)	8.6	(1.8-31.8)
TP	57.1	(19.4-14.8)	24.8	(7.0-57.8)
DOC	7370	(2276-73400)	1820	(1020-2920)
PC	1536	(450-15285)	662	(256-1938)
DIN:SRP (g:g)	3.8	(0.4-16.8)	2.3	(0.6-7.5)
TN:TP (g:g)	8.4	(4.0-18.3)	7.0	(2.0-19.7)
PN:PP (g:g)	7.8	(1.6-21.3)	-	-

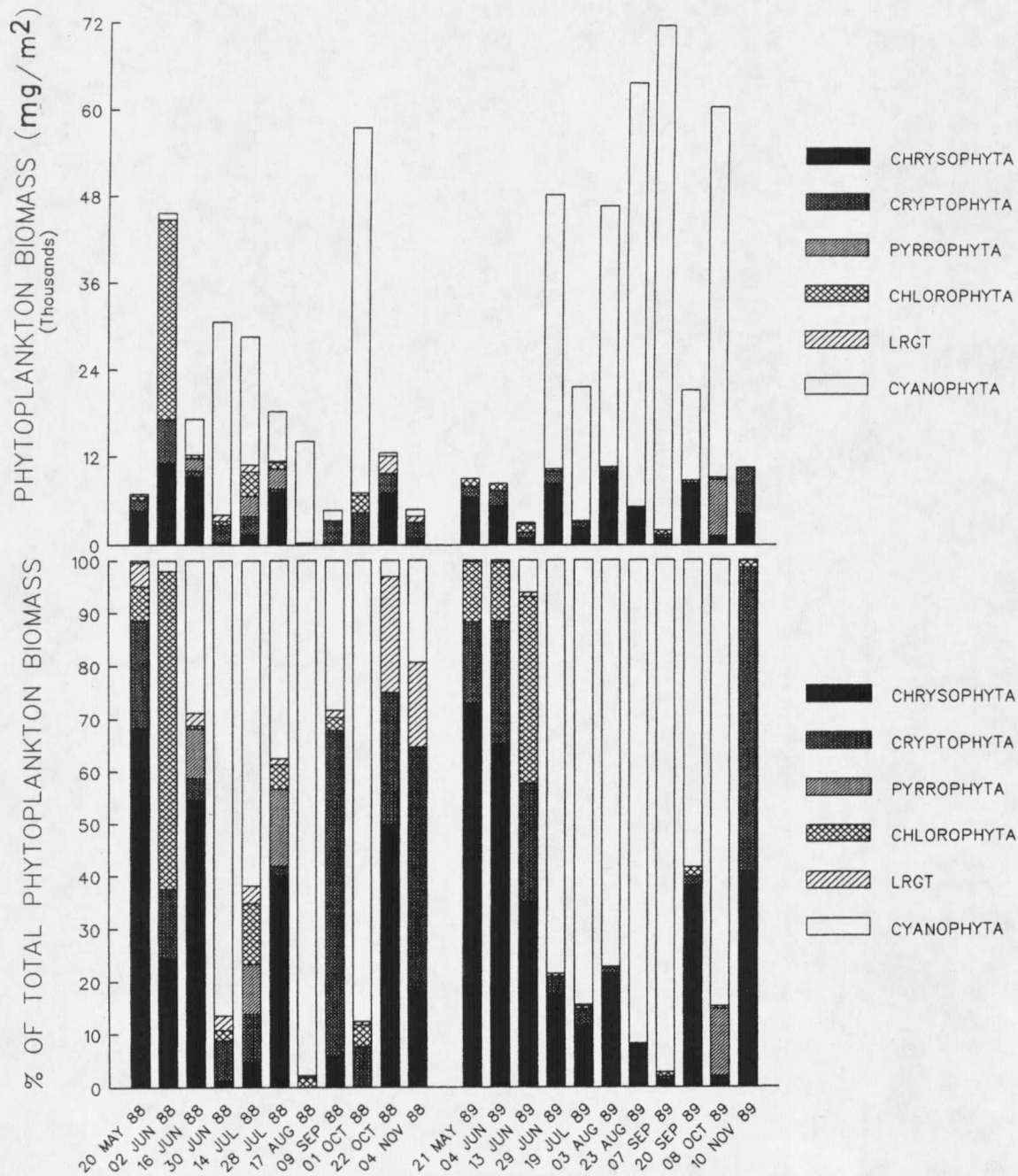


Figure 2. Phytoplankton biomass and relative distribution by divisions for the Grayling Arm of Hebgen Lake during the study period.

Experimental Results

03JUN88 Experiment.

At the time of this bioassay, Chlorococcus was the dominant phytoplankton genus; the 2.0 % blue-green algae (Cyanophyta) reported for 02JUN88 (Fig. 2) was due to a few cells of Anabaena at 5 m. Temperature was 11 °C, CHL a 6.3 $\mu\text{g l}^{-1}$, and PN:PP (g:g) was 5.96 (see Appendix 1, 02 June 1988 Grayling Arm for other parameters). Uptake of ^{14}C (DPM ml^{-1}) was stimulated significantly ($p < 0.01$) by NH_4^+ and NH_4^+ plus PO_4^{-3} in both the $> 20 \mu\text{m}$ and $< 20 \mu\text{m}$ fractions. PO_4^{-3} did not significantly ($p > 0.05$) increase activity.

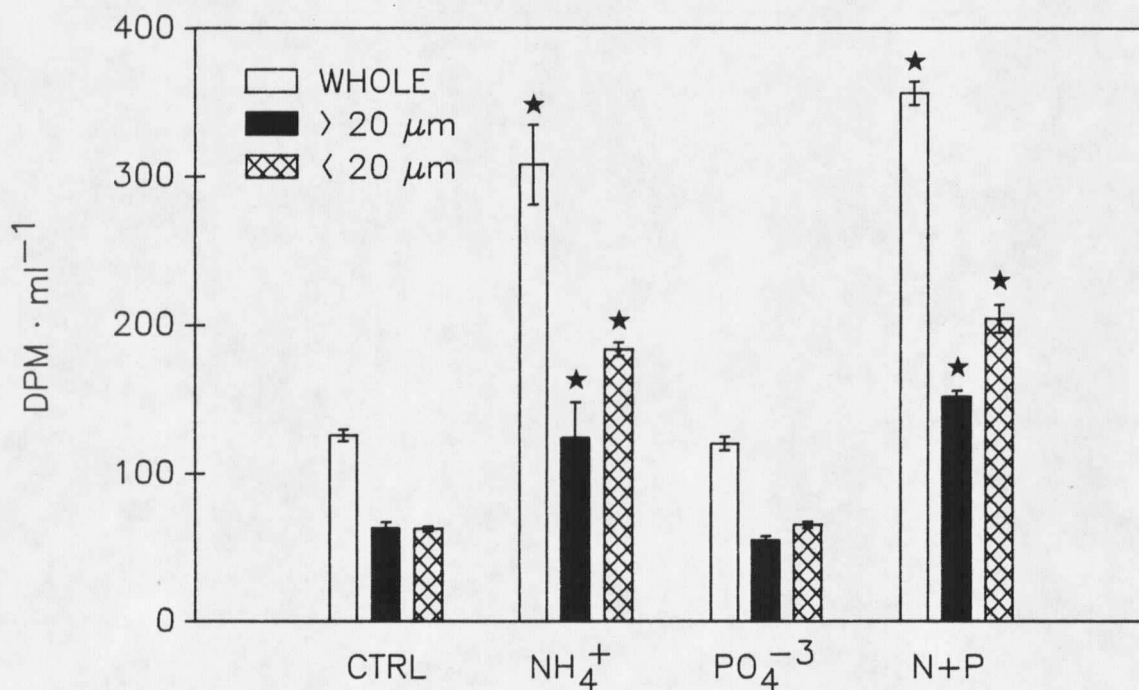


Figure 3. 03JUN88 experiment: C-uptake for whole community and size fractions. ANOVA results (compared to control):
★ = $p < 0.01$.

River Water and Limnocorral Experiments

Initial Conditions. Conditions at the inception of each 1989 river water and limnocorral experiment are presented in Table 3. River water conditions are given for comparison.

May 1989 River Water Experiment. Blue-green algae were not found in the water used for this experiment. Enrichment with NH_4^+ , NH_4^+ plus PO_4^{-3} (N+P) and 25% and 50% river water stimulated ^{14}C uptake (Fig. 4) and in-vivo fluorescence (Table 4) significantly ($p < 0.01$). The same treatments stimulated the $< 20 \mu\text{m}$ size fraction photosynthetic activity (Fig. 4). The $> 20 \mu\text{m}$ fraction activity was increased by NH_4^+ and NH_4^+ plus PO_4^{-3} but not by river water. Addition of PO_4^{-3} alone had no effect ($p > 0.05$).

July 1989 River Water Experiment. Stimulation of ^{14}C uptake (Fig. 5) by PO_4^{-3} and NH_4^+ plus PO_4^{-3} was significant ($p < 0.01$). Unreplicated size fractionations showed that both the > 20 (blue-green algal) and $< 20 \mu\text{m}$ (mainly Chrysophyta) fractions were stimulated by PO_4^{-3} . In-vivo fluorescence (Table 4) was stimulated ($p < 0.01$) by NH_4^+ , PO_4^{-3} and NH_4^+ plus PO_4^{-3} . River water additions did not stimulate phytoplankton in this experiment.

October 1989 River Water Experiment. ^{14}C uptake (Fig. 6) was stimulated significantly by NH_4^+ , PO_4^{-3} , 50% river water ($p < 0.05$) and NH_4^+ plus PO_4^{-3} ($p < 0.01$). Uptake in the

Table 3. Ambient temperature ($^{\circ}\text{C}$), CHL a ($\mu\text{g l}^{-1}$) and nutrient concentrations ($\mu\text{g l}^{-1}$) of water collected from Grayling Arm for 1989 River Water (RW) and Limnocorral (LIMNO) experiments. Phyto (Ana = Anabaena; Aph = Aphanizomenon; Cyc = Cyclotella; Dino = Dinobryon) refers to the dominant (comprise $> 60\%$ of biomass) phytoplankton genus or combination of genera. Ratios at the bottom are g:g.

	MAYRW		JULRW		OCTRW		LIMNO1	LIMNO2
	L.W.	R.W.	L.W.	R.W.	L.W.	R.W.		
$^{\circ}\text{C}$	10.0	- -	21.0	- -	10.1	- -	10.4	15.4
CHL a	4.9	- -	88.2	- -	6.8	- -	2.8	17.8
Phyto	Cyc	- -	Aph	- -	Aph	- -	Dino	Ana
NH_4^+-N	3.3	6.6	2.5	23.0	32.7	9.0	9.7	5.0
NO_3^--N	47.1	59.0	5.0	33.1	91.3	10.4	6.5	6.7
TDN	134	173	132	96	336	414	158	131
PN	- -	- -	1014	47	93	33	92	268
TN	- -	- -	1146	143	429	447	250	399
SRP	13.5	41.5	2.3	25.6	24.1	16.3	8.4	3.7
TDP	22.4	40.4	14.4	52.0	57.2	24.4	14.0	11.9
PP	- -	- -	- -	- -	21.4	6.7	15.3	13.7
TP	- -	- -	- -	- -	78.6	31.1	29.3	25.6
PC	- -	- -	5704	860	826	330	670	1366
DIN:SRP	3.7	1.6	3.3	2.2	5.1	1.2	1.9	3.2
TN:TP	8.1*	- -	6.9*	- -	5.5	14.4	8.5	15.6
PN:PP	7.4*	- -	11.1*	- -	4.4	4.9	6.0	19.6
PC:PN	7.1*	- -	5.6	18.3	8.9	10.0	7.3	5.1
PC:PP	52.7*	- -	70.8*	- -	38.6	49.3	43.8	99.7

* = sample not from experiment but from same station within 5 days of experiment.

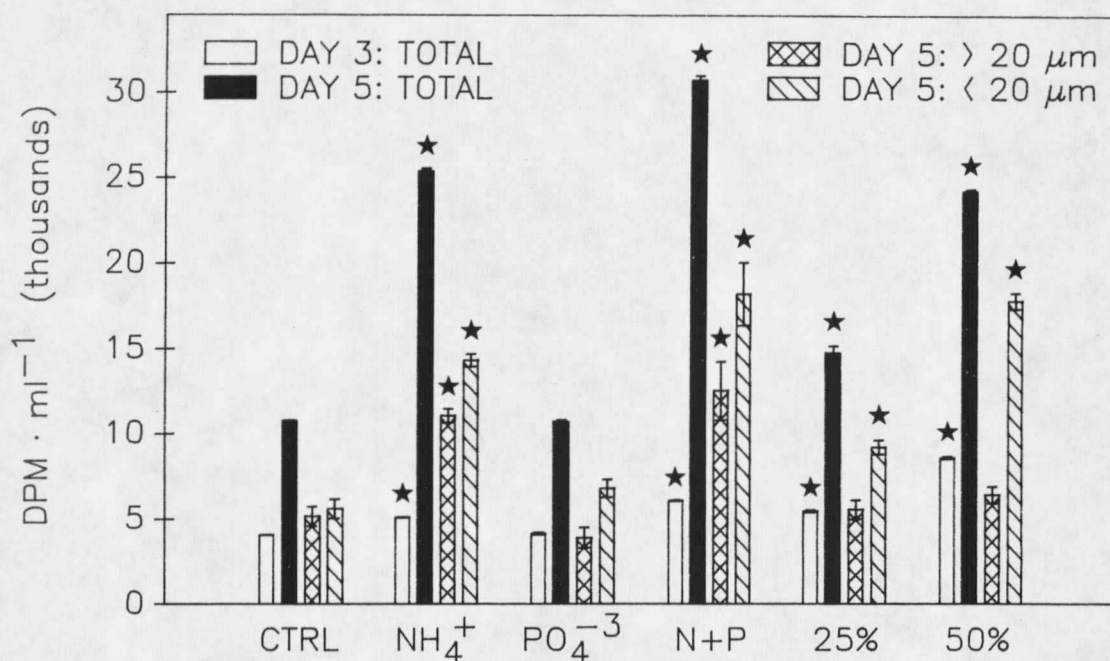


Figure 4. May 1989 river water experiment: C-uptake for whole community and size fractions. 25% and 50% refer to river water additions. ANOVA results (compared to control): ★= $p < 0.01$.

Table 4. In-vivo fluorescence results for May and July river water experiments, and chlorophyll *a* ($\mu\text{g l}^{-1}$) and nitrogenase activity ($\text{nmol C}_2\text{H}_4 \text{ (ml h)}^{-1}$) results from October river water experiment. Number in parenthesis = 1 SE. *=differs significantly from control ($p < 0.01$).

River Water Experiment	Treatment					
	CTRL	NH ₄ ⁺	PO ₄ ⁻³	N+P	25%RW	50%RW
<u>May</u>	21.6 (0.28)	38.6* (0.30)	21.9 (0.37)	44.3* (0.70)	25.5* (0.27)	32.7* (0.74)
<u>July</u>	34.1 (1.23)	52.1* (4.03)	49.9* (1.72)	54.0* (1.77)	44.0 (1.65)	40.1 (4.94)
<u>October</u> (CHL <i>a</i>)	8.9 (1.00)	12.6 (1.16)	11.7 (1.72)	12.9 (0.63)	12.1 (1.73)	7.1 (1.27)
(NA)	1.26 (0.18)	1.37 (0.30)	2.15* (0.25)	1.40 (0.11)	- -	0.77 (0.21)

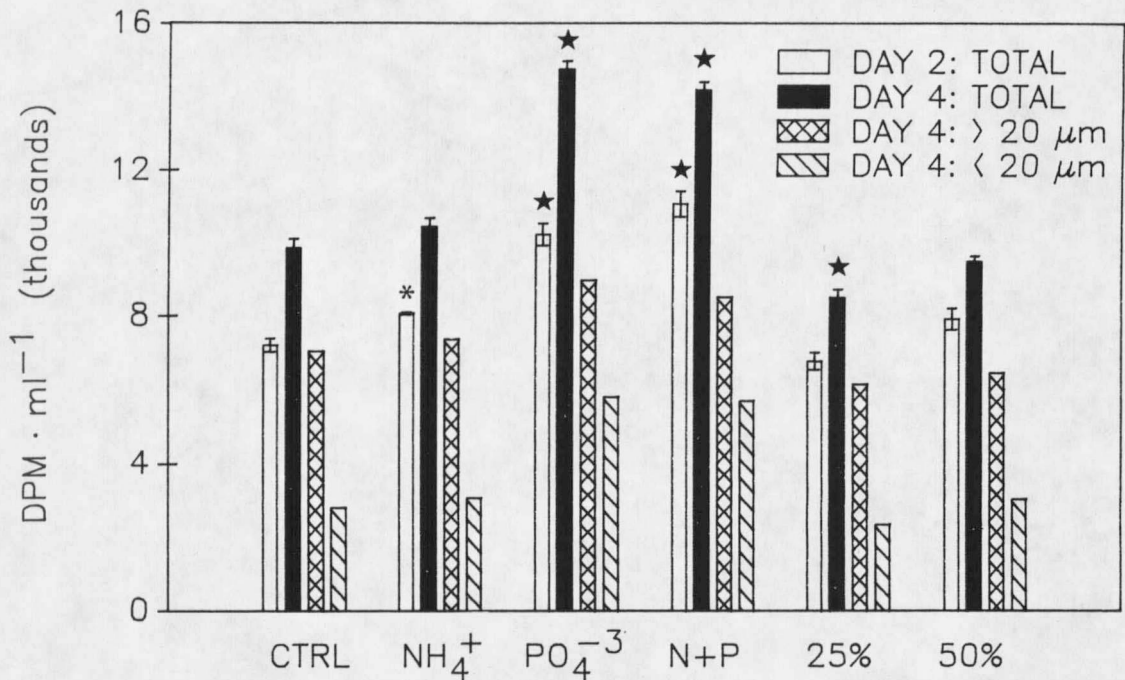


Figure 5. July 1989 river water experiment: C-uptake for whole community and size fractions. 25% and 50% refer to river water additions. ANOVA results (compared to control): *= $p < 0.05$; ★= $p < 0.01$.

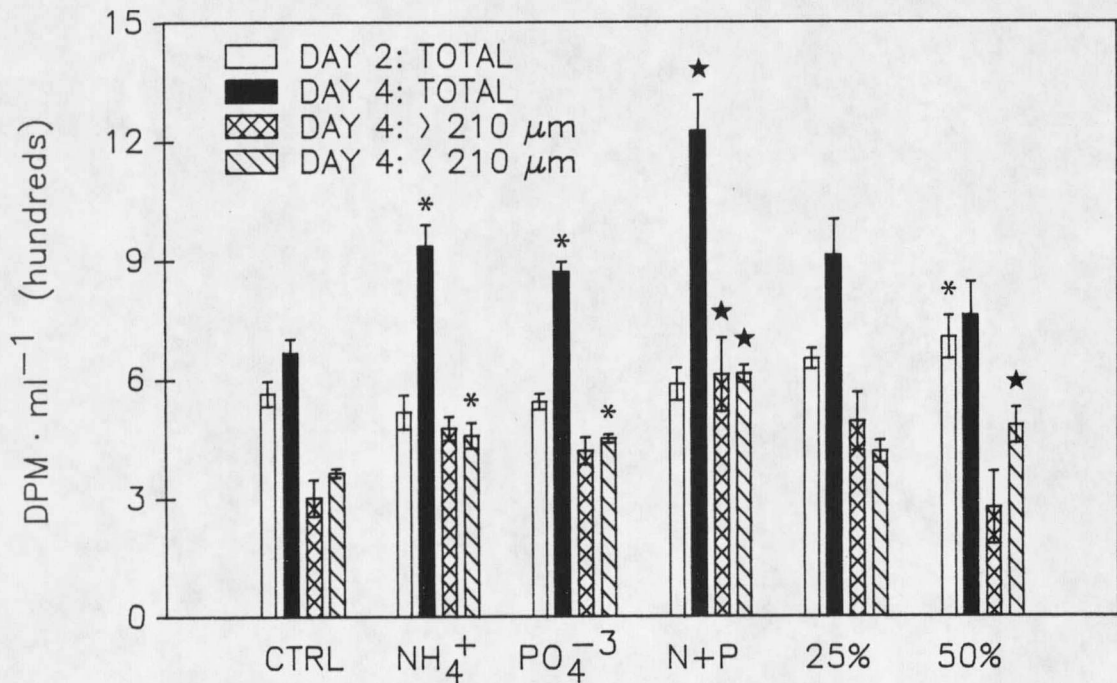


Figure 6. October 1989 river water experiment: C-uptake for whole community and size fractions. 25% and 50% refer to river water additions. ANOVA results (compared to control): *= $p < 0.05$; ★= $p < 0.01$.

NH_4^+ plus PO_4^{-3} treatment was significantly ($p < 0.05$) greater than either the NH_4^+ or PO_4^{-3} treatments. Only the $< 210 \mu\text{m}$ (mainly Chrysophyta and Chlorophyta) size fraction was stimulated significantly by addition of NH_4^+ , PO_4^{-3} ($p < 0.05$) and 50% river water ($p < 0.01$). Addition of both NH_4^+ and PO_4^{-3} was required to significantly ($p < 0.01$) increase uptake by the $> 210 \mu\text{m}$ (blue-green algal) fraction. CHL a was not enhanced ($p > 0.05$) by any of the treatments. PO_4^{-3} enrichment enhanced nitrogenase activity (Table 4) significantly ($p < 0.01$).

Limnocorral Experiment 1. Phytoplankton photosynthetic C-uptake (Fig. 7A) and CHL a (Fig. 7B) increased significantly ($p < 0.01$; $p < 0.05$ for day 10 CHL a) in the +N limnocorral through day 10. This enhancement was substantial, with community carbon uptake exceeding the control by six fold on day 3. Size fractionations on days 3 and 5 showed that both the > 20 (blue-green algal component) and $< 20 \mu\text{m}$ (non-blue-green algal) size fractions were stimulated by N. No difference from the control was detected by ANOVA ($p > 0.05$) on day 16. No nitrogenase activity was detected on day 1, but on day 5 (CTRL= 6.3 ± 1.26 , +N= 0.77 ± 0.14) and day 10 (CTRL= 12.8 ± 0.67 , +N= 4.9 ± 0.48) nitrogenase activity in the +N treatment was significantly less than control ($p < 0.01$). Addition of N elicited a relative increase in non-blue-green algal biomass compared to the control limnocorral (Fig. 8); the blue-green algal

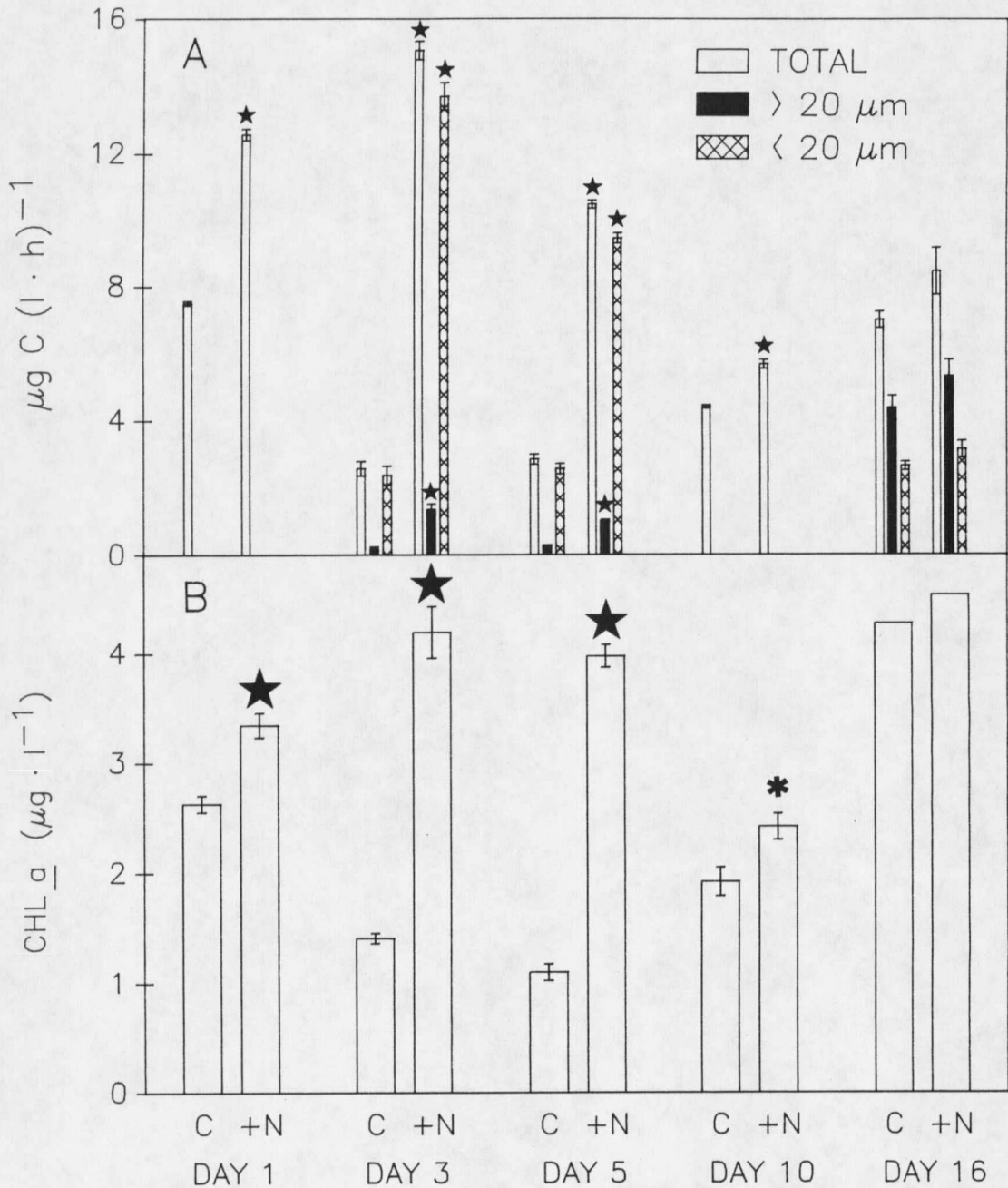


Figure 7. Limnocorral experiment 1 (June 1989): C-uptake for whole community and size fractions (A) and CHL \underline{a} concentrations (B). ANOVA results (compared to control): ★=p<0.05; * =p<0.01. C=control.

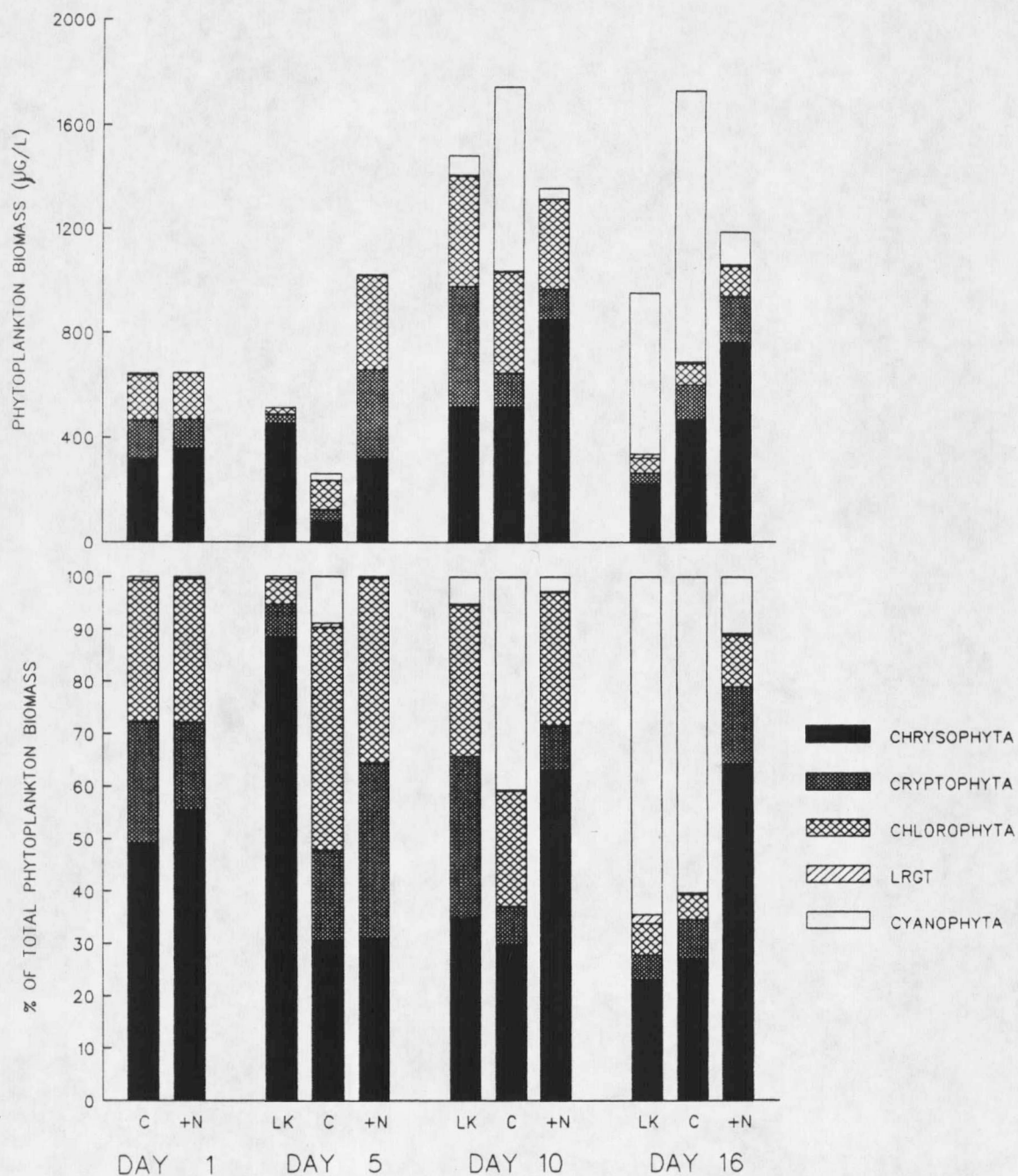


Figure 8. Limnocorral experiment 1 (June 1989): phytoplankton biomass and relative distribution by division. C=control; LK=lake sample.

biomass in the control yielded greater total phytoplankton biomass than in +N. The relative abundance of N_2 -fixing blue-green algae steadily increased over the experiment to 60% in the control, but increased to only 10% in the +N treatment. Phytoplankton samples taken at the same times from the lake just outside the limnocorrals showed that the control community structure was equivalent to the lake community by the end of the experiment, particularly with respect to the blue-green algal component.

Limnocorral Experiment 2. Addition of N and P at a 5:1 ratio (g:g) stimulated carbon uptake significantly ($p < 0.01$) on days 5 and 10 (Fig. 9A). The 5:1 treatment enhanced the $> 20 \mu m$ (blue-green algal component) fraction uptake significantly ($p < 0.01$) on days 5, 10 and 14; the $< 20 \mu m$ (mainly Chrysophyta and Cryptophyta) fraction was significantly more active than the control on day 5 ($p < 0.01$) but significantly less on day 14 ($p < 0.01$). The relative contribution of carbon uptake by the $> 20 \mu m$ size fraction was enhanced by the 5:1 treatment (Fig. 9B). CHL a was significantly greater than control ($p < 0.01$) on day 5 in the 5:1 limnocorral only (Fig. 10). The 20:1 and 40:1 treatments did not increase carbon uptake or CHL a on the days assayed. Nitrogenase activity was stimulated significantly by the 5:1 enrichment on day 5 ($p < 0.05$) and day 14 ($p < 0.05$) (Fig. 10). The 20:1 treatment inhibited nitrogenase activity on day 10 ($p < 0.01$). By day 10, total phytoplankton biomass had

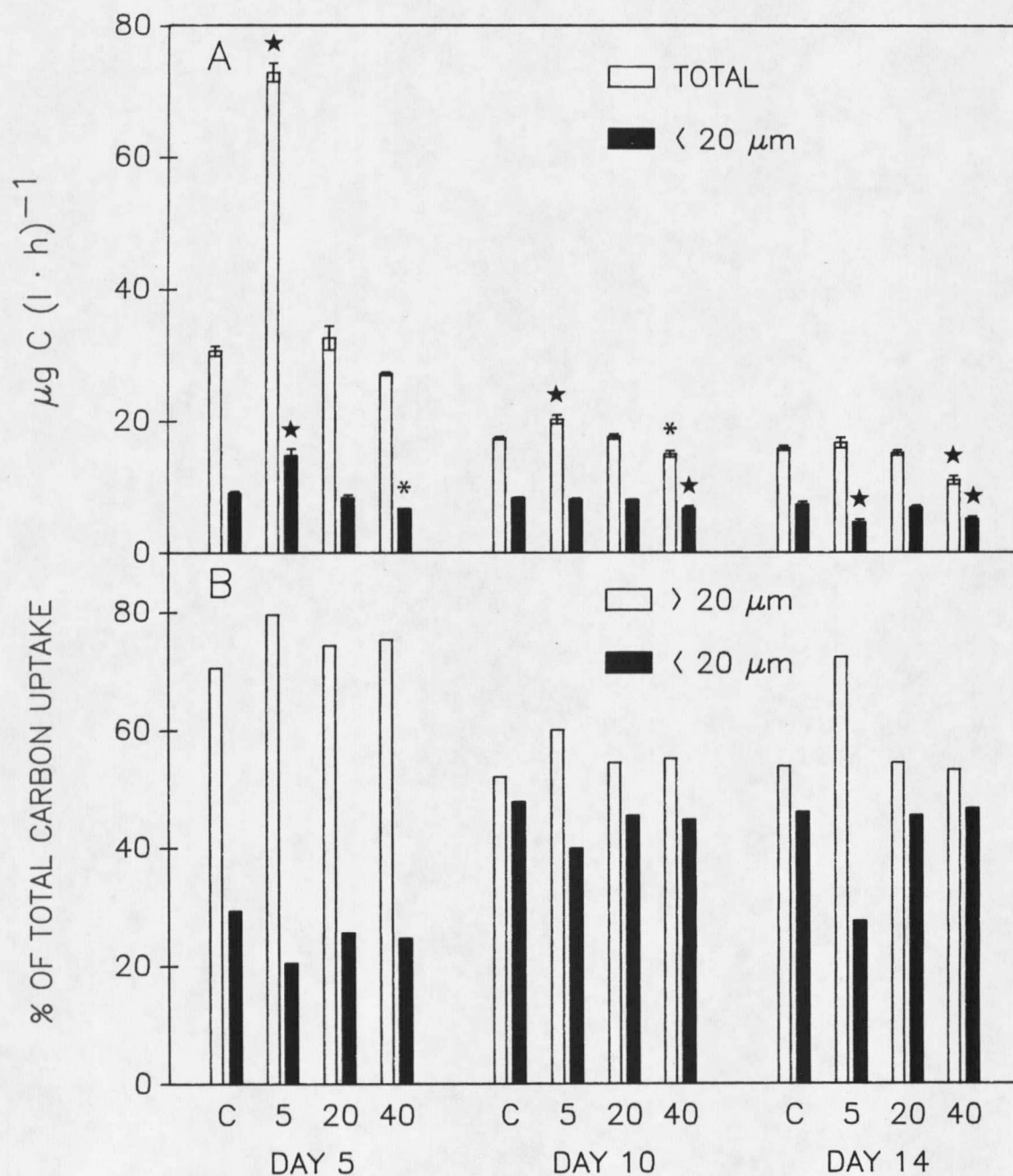


Figure 9. Limnocorral experiment 2 (July 1989): (A) C-uptake (ANOVA results: * = $p < 0.05$; ★ = $p < 0.01$) and (B) relative contribution of size fractions. C = control; 5, 20 and 40 refer to 5:1, 20:1 and 40:1 N:P (g:g).

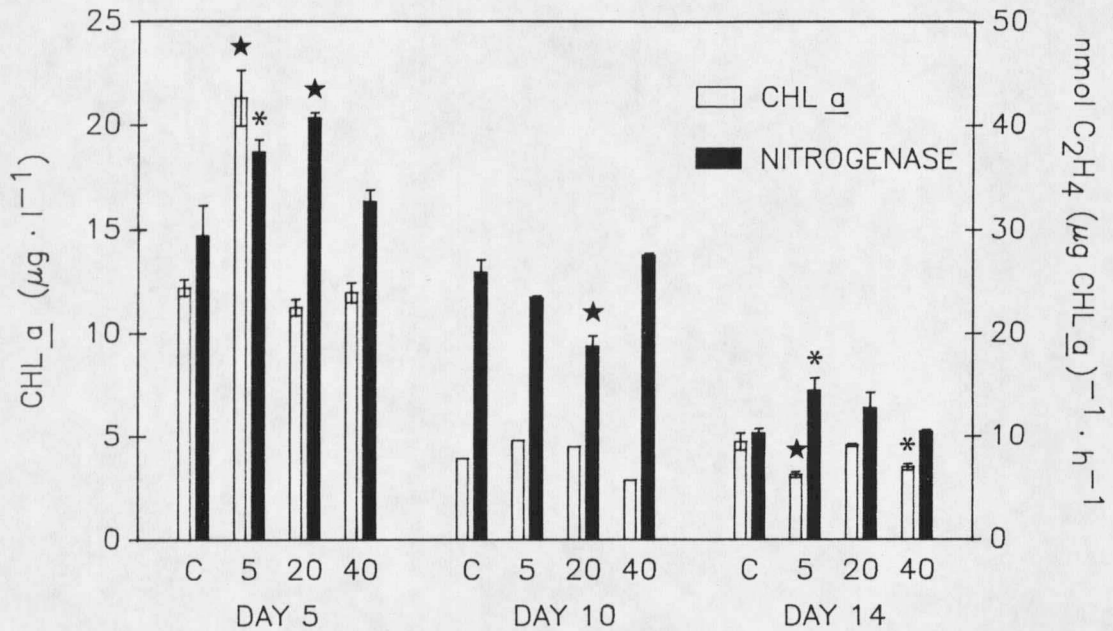


Figure 10. Limnocorral experiment 2 (July 1989): CHL \underline{a} and nitrogenase activity (ethylene production). ANOVA results: *= $p < 0.05$; \star = $p < 0.01$. C=control. 5, 20 and 40 refer to the 5:1, 20:1 and 40:1 treatments.

increased 130% in the 20:1 treatment and decreased 30% in the 5:1 treatment (Fig. 11). The relative abundance of blue-green algae exceeded the control by three fold in the 5:1 limnocorral and more than two fold in the 20:1 limnocorral on day 10. Differences in phytoplankton composition between treatments decreased by day 14.

1988 and 1989 Mesocosm Experiments

Mesocosm Initial Conditions. N_2 -fixing blue-green algae dominated the phytoplankton community at the beginning of all six mesocosm experiments, with Anabaena sp. in June 1988, August 1988 and June 1989, and Aphanizomenon sp. in October 1988 and August and October 1989 (Table 5). The

