Nitrogen transformations in Lake Bonney, Antarctica : dynamics in a non-turbulent environment
by Christopher Dee Woolston

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

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NITROGEN TRANSFORMATIONS IN LAKE BONNEY, ANTARCTICA: DYNAMICS IN A NON-TURBULENT ENVIRONMENT

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A thesis submitted in partial fulfillment
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Christopher Dee Woolston

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.

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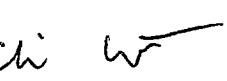
Signature 

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NITROGEN TRANSFORMATIONS IN LAKE BONNEY, ANTARCTICA

Christopher D. Woolston, 1994

ABSTRACT

This study was designed to determine sources of dissolved inorganic nitrogen (DIN) and microbial strategies for DIN utilization in a non-turbulent environment (perennially ice-covered Lake Bonney, Antarctica).

Water samples were inoculated with ^{15}N labeled DIN and incubated either in situ or in an incubator. Experiments were designed to test DIN uptake over a range of substrate concentrations, irradiances, and incubation periods. Additional experiments were designed to measure in situ NH_4^+ regeneration, as well as the effects of free amino acids, ambient NH_4^+ concentration , and phytoplankton on NH_4^+ regeneration rates.

Experiments designed to measure in situ NH_4^+ regeneration rates were inconclusive; apparently rates were below the detection limits of the method. The efficiency of conversion of serine to free NH_4^+ increased with depth. Removal of phytoplankton had no discernible effect on regeneration rates.

NH_4^+ was the preferred source of DIN at all depths. In situ uptake rates of NH_4^+ in the trophogenic zone generally exceeded the rate of supply of NH_4^+ by diffusion, indicating that NH_4^+ regeneration was a major source of DIN in the trophogenic zone. Three mechanisms for enhancing uptake of DIN (particularly NH_4^+) in relatively nutrient poor waters were observed: increased microbial affinity for NH_4^+, surge uptake of DIN within minutes of DIN enrichment, and increased affinity of DIN uptake for irradiance.

Microplankton in the shallow, relatively nutrient poor waters of Lake Bonney appear well acclimated and adapted to utilize the ambient supply of DIN.

INTRODUCTION

It has long been recognized that supply of dissolved inorganic nitrogen (DIN) often controls productivity of marine phytoplankton (e.g. Dugdale and Goering 1967). Recently, it has become increasingly evident that phytoplankton growth can be limited by nitrogen in freshwater systems as well (Dodds et al. 1991; Priddle et al. 1986; Priscu and Priscu 1984).

The ecological importance of DIN has been thoroughly documented, but the complexity of most environments has confounded attempts to isolate physiological responses to nutrient supply in natural systems (Smetacek et al. 1990). Turbulence and grazing pressure create conditions of disequilibrium which prevent maximal utilization of nutrients and add significant complexity to phytoplankton dynamics (Smetacek et al. 1990).

Even in some relatively quiescent Antarctic lakes (e.g. Heywood Lake, Signey Island) during ice-cover, minor turbulence and subsequent fluctuations in the light field greatly complicate determination of the factors that control productivity (Hawes 1983). Similarly, losses due to grazing must be considered in most investigations of aquatic nutrient cycles and phytoplankton productivity. Grazing rates must be quantified for accurate calculations of critical depth and nutrient control of primary productivity (Smetacek et al. 1990). Furthermore, Lynch and Shapiro (1981) found that grazing significantly affects community succession in response to nutrient additions. Like turbulence, but to

a lesser extent, grazing creates disequilibrium that inhibits phytoplankton acclimation or adaptation to nutrient resources.

The perennially ice-covered lakes in the McMurdo dry valley region of South Victoria Land, Antarctica, provide an opportunity to observe natural transformations of DIN in the absence of turbulence or grazing pressure. This study focuses on Lake Bonney, a meromictic lake located in the upper Taylor Valley.

Like other lakes in the McMurdo dry valleys, Lake Bonney is characterized by extreme hydraulic stability. In this lake, mixing occurs almost exclusively on a molecular scale and turbulent mixing is nearly non-existent (Spigel et al. 1990). There are two main causes for this stability. Firstly, the perennial 4 m ice-cover prevents direct wind-induced mixing. Secondly, chemical gradients within the lake lead to strong density stratification (Spigel et al. 1990).

Grazing pressure in Lake Bonney is limited by the near absence of upper trophic levels. Some zooplankton have been recorded, including ciliated zooplanktors of the genus *Strombidium* and *Sphaerophyra* (Parker et al. 1982). These organisms are primarily associated with benthic mats and are rare to absent in the pelagic environment (Parker et al. 1982).

In the absence of grazing pressure, phytoplankton production can be more directly related to temperature, light, and nutrient availability (Cullen 1990). These three factors have a complex regulatory effect on productivity, and much controversy has surrounded attempts to isolate one factor as the cause of seasonal succession of phytoplankton populations (McCombie 1960, Cullen 1990). At a physiological level, they are not mutually exclusive: all organisms require nutrients for biosynthesis, and temperature and

light govern the ability of phytoplankton to utilize available substrate. Observation of nutrient control of primary productivity could therefore most readily be accomplished in a system where light and temperature remain relatively constant over the doubling time of an organism.

For these reasons, Lake Bonney is an ideal site for monitoring phytoplankton utilization of nutrients. The hydraulic stability of Lake Bonney prevents rapid temporal variation in temperature. Due to the perennial 4 m ice-cover, which provides insulation as well as prevents turbulence, changes in temperature at a given depth are very gradual. Even in relatively turbulent marine habitats, seasonal temperature changes in Antarctic waters do not significantly affect microbial heterotrophic activity (Ellis-Evans 1982).

Furthermore, during the austral spring and summer, microplankton in Lake Bonney grow in a relatively constant light regime. In all aquatic systems, the light regime fluctuates according to first order effects (factors that govern solar flux, e.g. latitude, weather, and light extinction within the water column) and second order effects (e.g., turbulent displacement of phytoplankton and phytoplankton migration). In Lake Bonney, these effects combine to form temporal stability in the light regime over the life span of microplankton. At high latitudes, there is little diel variation in irradiance; four months of darkness and four months of daylight are separated by two month periods of twilight. The intensity of light that reaches the water column of Lake Bonney varies with cloud cover and the changing transparency of the ice, but these variations are minor compared to the diel light/dark cycles found in low latitude systems. Hydraulic stability minimizes second order effects

and thus provides phytoplankton a relatively constant light environment.

The stability of the water column has shaped the structure and composition of planktonic communities. Most of the phytoplankton in Lake Bonney are flagellates (principally *Chlamydomonas subcaudata*, *Cryptomonas* sp., and *Ochromonas* sp.) (Sharp 1993), while diatoms are rare (Parker and Simmons 1983; Parker et al. 1977, Sharp 1993). Apparently, natural selection strongly favors motile algae in this quiescent environment.

In addition to shaping community composition, the stability of the water column has allowed for specialized growth and survival strategies. As previously described, these strategies include motility and efficient photosynthesis at low irradiance (Priscu et al. 1990). Potentially, water column stability could also allow for efficient utilization of inorganic nutrients by phytoplankton and efficient regeneration of NH_4^+ by bacteria.

The purpose of this study was to assess transformations of dissolved inorganic nitrogen by several different microbial communities in Lake Bonney. The main body of this thesis has been divided into three parts. Part I describes the methods and results of routine data collection. These results are necessary for interpretation of all experiments discussed in Parts II and III. Part II includes experiments designed to assess sources of DIN as well as microbial strategies to enhance DIN utilization. Part III focuses on the role of irradiance in regulating DIN uptake and the degree to which microplankton have adapted to utilize irradiance for DIN uptake. Following Part III is an overall conclusion that briefly integrates the results of Part I, II, and III. The remainder of this

introductory section will discuss aspects relevant to all parts of the thesis: previous research on Lake Bonney and other dry valley lakes, hypotheses and objectives, and a description of the study site.

PREVIOUS RESEARCH ON LAKE BONNEY AND OTHER DRY VALLEY LAKES

Captain R.F. Scott's discovery of Lake Bonney in 1903 marked the beginning of dry valley lake research (Parker et al. 1982). Owing to their remoteness, thorough scientific investigation of the lakes did not begin until the early 1960's. Liquid water beneath the ice covers of Lake Bonney and Lake Vanda was sampled in 1961; this was the first attempt at limnological analysis of dry valley lakes (Angino and Armitage 1963).

Several early investigations of Lake Bonney focused on distribution of nutrients (Armitage and House 1962, Angino and Armitage 1963, Angino et al. 1963, Goldman 1964, Yamagata et al. 1967). The extreme salinity of Lake Bonney's deeper waters apparently caused methodological difficulties; reports of nutrient concentrations were inconsistent and often contradictory (Fortner et al. 1986, Sharp 1990).

The first studies of dynamic biological processes in dry valley lakes were conducted by Goldman (1964). Goldman's measurements of primary productivity within the lake indicated a high quantum yield of photosynthesis. He concluded that the phytoplankton had adapted to low ambient light levels.

In the 1970's, several studies documented the distribution of microplankton in dry valley lakes (Koob and Leister 1972, Parker et al. 1977, Parker et al. 1982). Koob and Leister (1972) reported three distinct zones of primary productivity and phytoplankton abundance.

Later studies attempted to correlate productivity in dry valley lakes with light and nutrient supply. Vincent (1981) suggested that nutrients played the primary role in regulation of microplankton growth. Specifically, he proposed that the phytoplankton of Lake Fryxell (located in the lower Taylor Valley) were nitrogen deficient and that regeneration of NH_4^+ in large part fueled productivity in the trophogenic zone. Green et al. (1989) noted that the allochtonous inputs of PO_4^{-3} to Lake Fryxell greatly exceeded NO_3^-, and nitrogen, compared to phosphorus, was more efficiently cycled within the lake.

Thorough investigation of the factors that control productivity in Lake Bonney began in 1989. Priscu et al. (1990) hypothesized that the photosynthetic apparatus of Lake Bonney phytoplankton should be precisely adapted to the relatively constant ambient light fields. Photosynthesis/irradiance experiments conducted initially by Priscu et al. (1988) and confirmed by Lizotte and Priscu (1992) demonstrated that phytoplankton were indeed shade adapted; however, phytoplankton in the relatively nutrient-poor surface waters appeared to harvest light less efficiently than deeper populations. Lizotte and Priscu (1993) determined quantum yield of photosynthesis through measurements of natural fluorescence, chlorophyll concentration, phytoplankton absorption spectra, photosynthetic efficiency, and spectral irradiance. Interestingly, quantum yields increased dramatically with increasing proximity to the chemocline. Models constructed in an attempt to predict primary productivity from natural fluorescence, irradiance, and quantum yields were unsuccessful. Apparently, productivity of the upper

trophogenic zone is largely influenced by nutrient supply (Lizotte and Priscu 1993).

Sharp (1993) formulated a one-dimensional light-dependent growth model of primary productivity. This model assumed that light availability, and not nutrient supply, limits growth. The model's predictions of gross chlorophyll specific growth rates did not correlate well with actual growth rates, particularly in surface waters. For example, chlorophyll specific growth rates within the chemocline exceeded growth rates in the relatively high irradiance surface waters. Once again, it was concluded that nutrient supply in the upper trophogenic zone limited photosynthesis (Sharp 1993). Nutrient bioassays conducted by Sharp (1993), designed to detect deficiency of nitrogen or phosphorus, were inconclusive; however, recent bioassays have shown that phytoplankton photosynthesis in the upper trophogenic zone can be enhanced significantly by nutrient enrichment (Priscu, unpublished data).

HYPOTHESES AND OBJECTIVES

Just as Priscu et al. (1990) hypothesized that phytoplankton should be precisely adapted to ambient light levels, I propose that the level of physiological adaptations for inorganic nitrogen uptake will reflect the nutritional status of the community. In situations where nitrogen limitation exists (if any), phytoplankton will have enhanced efficiency of inorganic nitrogen uptake, particularly uptake of NH_4^+. Specifically, this study was designed to test the following hypotheses:

1. NH_4^+ is the preferred source of DIN for all Lake Bonney planktonic communities.
2. Regeneration of NH_4^+ provides a major nitrogen source for phytoplankton in the upper trophogenic zone.
3. "Regenerated" NO_3^- and NO_2^- provide a minor source of nitrogen for phytoplankton in the upper trophogenic zone.
4. Nitrogen deficient communities are capable of rapid short term uptake of NH_4^+, and, to a lesser extent, $NO_3^-$and NO_2^-.
5. Nitrogen uptake is extremely "shade adapted," i.e., quickly saturated with respect to irradiance, particularly in nitrogen depleted surface waters.
6. Photoinhibition of nitrogen uptake, when present, occurs at an irradiance substantially above ambient levels.

The following objectives were undertaken to test the hypotheses:

1. Measure maximum uptake rates of NH_4^+, NO_3^-, and NO_2^- throughout the water column.
2. Measure the uptake response to different concentrations of NH_4^+, NO_3^-, and NO_2^-.
3. Measure rates of regeneration of NH_4^+.
4. Measure the time course uptake of NH_4^+, NO_3^-, and NO_2^-.
5. Quantify the irradiance requirements for uptake of NH_4^+, NO_3^-, and NO_2^-.

DESCRIPTION OF STUDY SITE

Geography and Geology

The McMurdo dry valleys lie between the MacKay and Koettlitz glaciers at latitude 77° 10'S to 77° 45'S, longitude 160° 20'E to 160° 00'E (Chinn 1993). At 3700 km^2, the valleys represent the largest ice-free area on the continent. The Taylor Valley was originally carved by the Taylor V glaciation approximately four million years ago, and numerous smaller glaciations have shaped the valley since then (Heywood 1984, Chinn 1993). The Taylor Valley has been largely ice-free for at least 100,000 years, and the basins of the present day Taylor Valley lakes (Lake Bonney, Lake Fryxell, Lake Hoare, Lake Chad, and Mummy Pond) were formed 100,000 to 500,000 years ago (Chinn 1993).

The major rock types found in the modern-day Taylor Valley include dolerites, marbles, granite, basalts, gneisses, schists, sandstones, and metasediments (Claridge and Campbell 1977; Heywood 1984). Ultramaphic dikes are prominent features of the land surrounding Lake Bonney, evidence of past vulcanism (Lawson, personal communication).

Climate

The McMurdo dry valleys constitute one of the driest ecosystems in the world. Precipitation in the form of snow may reach 10 cm a year, but most of the moisture is quickly lost to sublimation (Green et al. 1989). Potential ablation rates are

approximately 30 times greater than the average annual precipitation (Chinn 1993). Katabatic winds, which blow from the Polar Plateau and can exceed 130 km h^{-1}, maintain the relative humidity below 50% (Heywood et al. 1984). The annual mean temperature is between -20 to -25° C (Heywood et al. 1984).

Morphology

Lake Bonney, with a surface area of approximately 4 km^2 (Chinn 1993), is the largest lake in the Taylor Valley. It consists of two lobes (the east lobe and the west lobe) which are connected by a narrow (40 m wide), shallow (14 m deep) sill. The west lobe, the smaller of the two, is abutted by the Taylor Glacier. Both lobes have a maximum depth of approximately 40 m. Several streams (runoff from the Taylor, Hughes, LaCroix, Sollas, Rhone, Calkin, Matterhorn, and Marr glaciers) flow intermittently into the lake during late austral spring and austral summer. The lake has no outflows; water is lost through ablation and sublimation of the perennial 4 m thick ice-cover (Chinn 1993).

Part I

Routine Data Collection and Overview of ^{15}N Experiments

ROUTINE DATA COLLECTION METHODS

Parameters Analyzed

Routine collections were conducted in each lobe every 10 d throughout the 1992-1993 field season (hereafter referred to as the "1992 season") and every 14 d throughout the 1993 field season. Water samples were analyzed for chlorophyll a (CHL); primary production (PPR); NH_4^+, NO_3^-, and NO_2^- (DIN); soluble reactive phosphorus (SRP) and particulate carbon and nitrogen (PC and PN). An inventory of sampling dates and depths is presented in Table 1. All depths are from the piezometric level, the level of the water surface in the sampling hole (approximately 27 cm below the top of the ice).

Water Sampling Procedure

Early in each season, sampling holes were drilled with a 10 cm or 25 cm diameter ice auger. After drilling, holes were enlarged with a "hot finger," copper tubing through which hot glycol was circulated. The hot finger was reapplied to the holes periodically throughout the season to prevent narrowing or refreezing. After application of the hot finger, holes were allowed to cool for at least 24 h before sampling. A portable structure was placed over the sampling hole.

For routine data collection, samples were collected with a 2.2 liter Niskin sampling bottle with teflon-coated springs. The bottle was fastened to metered 1/8 " aircraft cable and was lowered and raised with a manual winch. The Niskin bottle was inverted several

times before removal of water to eliminate gradients within the bottle.

Water samples were collected through a short piece of rubber tubing attached to the nozzle of the Niskin. One l from each depth was transferred into a 1 liter HDPE bottle for analysis of DIN, SRP, CHL, and CHN. Once full, each 1 liter HDPE bottle was placed in a cooler for transport to laboratories on the lakeshore. A portion of the water remaining in the Niskin was used to fill primary productivity bottles (3 145 ml Pyrex glass screw-top bottles). Two of the bottles were clear and one was opaque. The tubing on the Niskin was inserted to the bottom of each bottle to prevent intrusion of gasses into the sample. Once full, the bottles were placed in a dark box until inoculated with $^{14}C\text{-}CO_3^{-2}$. All bottles were acid washed and rinsed three times with sample water before final collection.

Chemical and Biological Measurements

Chlorophyll a

Collection of CHL and nutrient samples commenced within 2 h of collection. The laboratory was cool and dim during filtration of CHL and nutrients. For the 1992 season, 2 200 ml aliquots were each filtered onto precombusted (450° C for 2 h) 25 mm Whatman GF/F glass fiber filters under low vacuum (< 0.5 atm). The filtrate was collected in an acid washed Ehrlemyer flask. A portion of the filtrate was used to pre-rinse a 125 ml HDPE nutrient bottle; the rest was transferred to the bottle for future nutrient analysis. During the 1993 season, two 100 ml aliquots were filtered for CHL analysis, and the filtrate was collected directly into the 125 ml HDPE nutrient

bottle through the use of vacuum chambers (bell jars). Each CHL filter was gently folded in half (CHL side in) and placed in a glassine envelope. The envelopes were wrapped in aluminum foil and kept frozen until analysis at Crary laboratory, McMurdo Station. Nutrient bottles were also kept frozen until analysis. Analysis of CHL and nutrients occurred within two months of collection.

For CHL analysis, the frozen filters were placed in 20 ml scintillation vials. Ten ml of 90% acetone were added to each vial, and the samples were vortexed for 1 min. Tests conducted in 1989 indicate that this is an appropriate method of CHL extraction for the phytoplankton in Lake Bonney (Lizotte and Priscu, unpublished data). CHL concentration of the extract was measured fluorometrically with a Turner Design model 10 AU fluorometer calibrated with standard concentrations of purified CHL (Sigma Chemical). Measurements were made before and after acidification (0.2 ml 1 N HCl) to correct for phaeopigment fluorescence.

Nutrients

Nutrient samples were thawed before analysis. All nutrient assays were conducted on 10 ml samples. For NH_4^+ analysis, samples from 15 m and deeper in the east lobe and 13 m and deeper in the west lobe were diluted 1:10 (1 ml lake water + 9 ml deionized water). This was done to prevent salt interference with the color forming reaction and to keep NH_4^+ concentrations within the linear range of the method (Sharp 1993). The blue indophenol reaction between NH_4^+, phenol, and hypochlorite at high pH was used to

determine NH_4^+ concentration (Solorzano 1969). Absorbance of the sample was measured using a 1 cm pathlength cell and a Beckman spectrophotometer.

For NO_2^- analysis, samples from 15 m and deeper in the east lobe were diluted 1:10 (1 ml lake water + 9 ml deionized water). Samples from the west lobe were not diluted. NO_2^- concentrations in the west lobe were consistently less than 1 μM; dilution of the sample could therefore decrease NO_2^- concentrations below the limit of detection. Repeated recovery tests indicate greater than 90% recovery of added NO_2^- at depths below 15 m. Concentration of NO_2^- was measured with the NED/sulfanilamide diazotization reaction (APHA 1985).

Samples for NO_3^- analysis from 15 m and deeper in the east lobe and 13 m and deeper in the west lobe were diluted 1:10. All of the NO_3^- in the sample was reduced to NO_2^- by shaking the samples with spongy cadmium and ammonium chloride EDTA buffer at pH 8.3 (Jones, 1984). The concentration of NO_2^- was then determined with the NED/sulfanilamide reaction.

Soluble reactive phosphorus samples from 15 m and deeper in the east lobe were diluted 1:2 (5 ml lake water + 5 ml deionized water). Samples from the west lobe were not diluted; recovery tests indicated greater than 90% efficiency of SRP detection in undiluted samples. SRP was measured with the ammonium molybdate/potassium antimonyl tartrate method (Downes 1978).

Following the methods of Sharp (1993), the reduction step to prevent arsenate interference was omitted.

Particulate Carbon and Nitrogen

Collection of PC and PN commenced after filtering of all CHL and nutrient samples. For each depth, one 500 ml aliquot was filtered on a precombusted 25 mm GF/F glass fiber filter at low vacuum (< 0.5 atm). Each filter was rinsed with approximately 20 ml of deionized water (DIW) to remove inorganic nitrogen from the filter. Filters were air dried for several d in aluminum weigh-boats before transport to Crary Laboratory. Upon arrival at the laboratory, filters were frozen (-45° C) until analysis. Samples from the 1992 season were transported frozen to MSU and analyzed within 6 months of collection. Samples from the 1993 season were analyzed at Crary laboratory within 3 months of collection.

Particulate filters were acidified before analysis to remove remaining inorganic carbon. Flash-combustion gas chromatography (Carlo Erba model 1500 elemental analyzer) was used to measure the nitrogen and carbon content of the filters wrapped in tin foil (combustion catalyst). Areas under chromatographic peaks were compared to areas obtained from standards of isothiourea (1992) or acetanilide (1993). Standards were weighed $\pm$ 1.0 μg with a Cahn model 35 electrobalance. All measurements were corrected for carbon and nitrogen contamination of the filters and the foil.

Primary Productivity

Photosynthesis was measured by in situ uptake of ^{14}C-CO_2. A Glison 1000 ml pipette was used to inoculate primary productivity

bottles with $^{14}C\text{-}CO_3^{-2}$ stock. Bottles were sealed tightly and inverted several times after addition of $^{14}C\text{-}CO_3^{-2}$ to mix the sample.

The productivity bottles were suspended for 24 h at the depth of collection. Studies conducted by Sharp (1993) indicated that measurements of daily net photosynthesis based on a single 24 h incubation did not differ significantly from measurements based on three 8 h incubations. The incubation hole was located away from the sampling hut and was covered to prevent excess irradiance from entering the water column. The bottles were placed in the hole between 0700 h and 0800 h local time, when the sun was behind mountains. This reduced the possibility that algal photosynthetic ability would be damaged by incidental exposure to sunlight before incubation.

The bottles were returned to the lakeshore laboratory immediately after removal from the lake. All bottles were kept cold and dark before and during filtration. Samples were filtered on precombusted 25 mm GF/F Whatman glass fiber filters at low vacuum (<0.5 atm). Filters were transferred to 20 ml scintillation vials and acidified with 0.5 ml 3 N HCl to remove unassimilated ^{14}C. The vials were dried (40-50° C) for 1 to 2 d and sealed for shipment to Crary laboratory, McMurdo.

Activity of the filters was determined by liquid scintillation spectroscopy at Crary Laboratory. Ten ml of CytoScint ES (ICN Pharmaceuticals) liquid scintillation cocktail was added to each vial before analysis. Counts per min were compared to a quench curve for

conversion to disintegrations per min (DPM). Acetone was used as the quenching agent and ^{14}C-toluene was the standard.

DPM was converted to rate of carbon uptake through the following equation:

$$\text{mg C m}^{-3}\ \text{day}^{-1} = \frac{(\text{DIC})^* (\text{DPM}_C - \text{DPM}_D) * 1.06 * \mu\text{Ci} * 10^3\ \text{liter}}{(2.2 * 10^6\ \text{DPM}) * (\mu\text{Ci added})^* \text{m}^3 * t} \quad (1)$$

where DIC (dissolved inorganic carbon) is expressed in mg l^{-1} , 1.06 is a correction for isotope discrimination, DPM_C is disintegrations per min in the clear bottles, DPM_D is disintegrations per min in the dark bottle, $2.2 * 10^6$ is the DPM per µCi, 10^3 converts liters to m^3, and t is the length of incubation (d).

Physical Parameters

Lake profiles of temperature, oxygen, and irradiance were collected on the day of each routine data collection. Irradiance was measured at 1 m intervals with a Li-Cor 4π quantum sensor attached to a Li-Cor model LI-1000 data logger. In addition, irradiance on the ice surface was continuously monitored by a Li-Cor 2π quantum sensor attached to a Campbell 21 x data logger. In situ irradiance was measured every 10 min at 10 m east lobe from 17 Jan 1993 to 31 Dec 1993 by a with a Li-Cor 4π quantum sensor attached to a Campbell 21x data logger. Irradiance readings from the Campbell were calibrated to correspond with Li-Cor 1000 readings.

Table 1. Dates and depths of collection for various parameters.
EAST LOBE

Sampling Dates	CHL	PPR	SRP	DIN	PC/PN
24 Nov 1992	B	A	B	B	B
4 Dec	C	A	C	C	C
14 Dec	C	A	C	C	C
24 Dec	C	A	C	C	C
4 Jan 1993	C	A	C	C	C
27 Oct	E	D	E	E	E
10 Nov	E	D	E	E	E
24 Nov	E	D	E	E	E
7 Dec	E	D	E	E	E
21 Dec	E	D	E	E	E

A= 4, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 20 m.

B= 4, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 20, 23, 26, 29, 32, 35 m.

C= 4, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 20, 22, 25, 30, 32, 35 m.

D= 4, 6, 8, 10, 12, 13, 15, 18, 20, 22 m.

E= 4, 6, 8, 10, 12, 13, 15, 18, 20, 22, 25, 30, 35, 38 m.

Table 1 , cont. Dates and depths of collection for various parameters.
WEST LOBE

Sampling Dates	CHL	PPR	SRP	DIN	PC/PN
26 Nov 1992	B	A	B	B	B
6 Dec	B	A	B	B	B
26 Dec	B	A	B	B	B
6 Jan 1993	B	A	B	B	B
7 Jan	B	NA	B	B	B
29 Oct	D	C	D	D	D
12 Nov	D	C	D	D	D
26 Nov	D	C	D	D	D
9 Dec	D	C	D	D	D
23 Dec	D	C	D	D	D

A= 4, 5, 6, 8, 10, 12, 13, 14, 15, 17 m.

B= 4, 5, 6, 8, 10, 12, 13, 14, 15, 17, 20, 22, 25, 30, 35 m.

C= 4, 6, 8, 10, 12, 13, 14, 15, 17, 20 m.

D= 4, 6, 8, 10, 12, 13, 14, 15, 17, 20, 22, 25, 30, 35, 38 m.

RESULTS

Nutrients

Profiles of NH_4^+, NO_3^-, NO_2^-, and SRP from 1993 routine data collections in each lobe are summarized in Figure 1. Concentrations of all nutrients are low above the first chemocline, and nutrient gradients are generally strong within and directly beneath the chemocline. SRP concentrations in each lobe and NO_2^- concentrations in the west lobe rarely exceed 1 μM.

Primary Productivity and Chlorophyll a

Primary productivity and CHL (Figure 2) were closely correlated in upper 20 m of each lobe. Below 20 m, productivity was undetectable despite the presence of chlorophyll.

In the east lobe, CHL typically reached its maximum concentration just below the ice (4-6 m). There was often another, less prominent, chlorophyll a peak within the first chemocline (10-13 m). Primary productivity followed the same general pattern, except that primary productivity peaks within the first chemocline were more prominent than the productivity peaks from 4-6 m. CHL concentrations in the upper 20 m of the west lobe consistently exceeded concentrations of the east lobe.

Particulate Carbon and Nitrogen

Representative profiles of PC:PN ratios are illustrated in Figure 3. PC:PN is always greater than the Redfield ratio of 6.6 (by atoms),

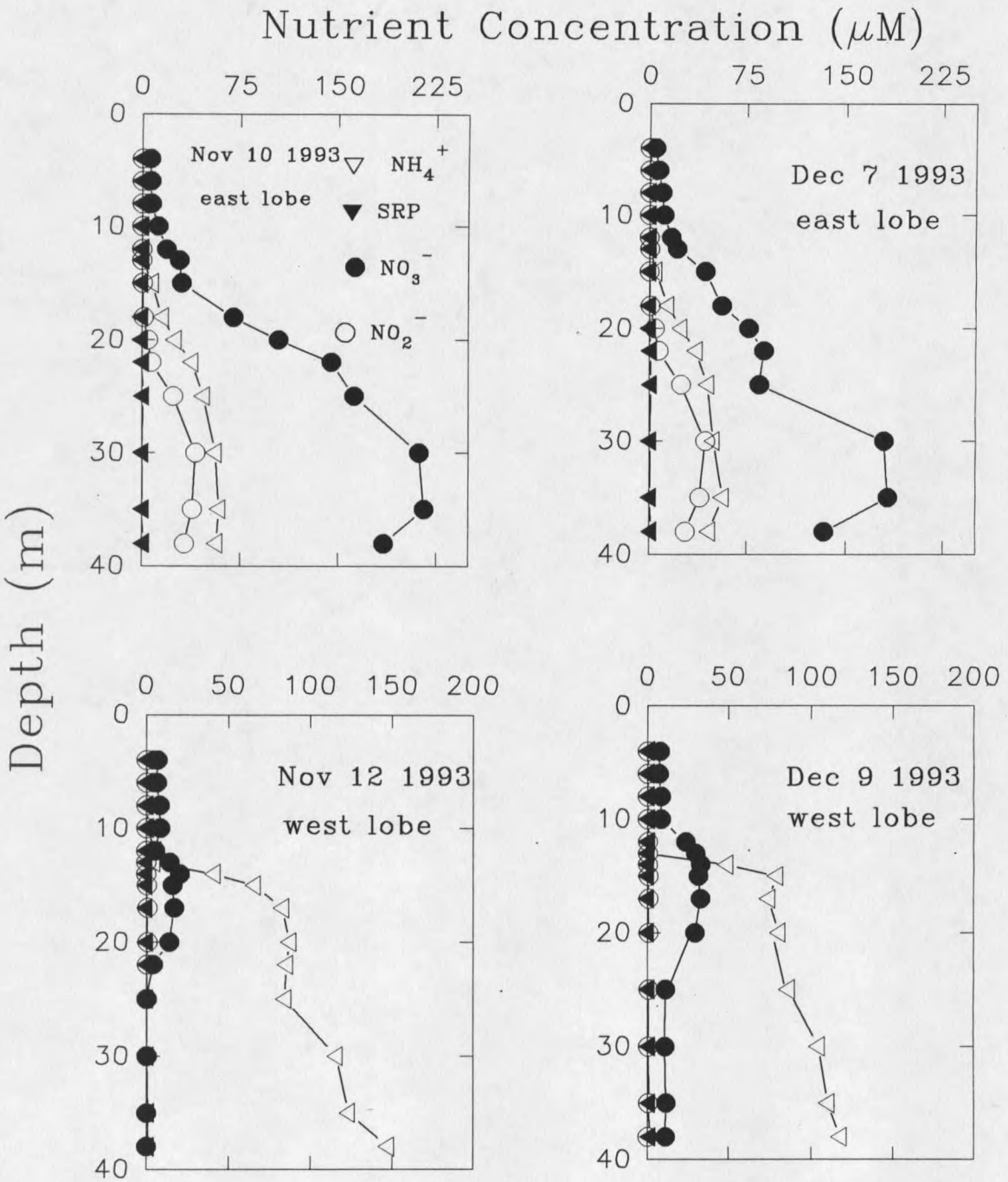

Figure 1. NH_4^+, NO_2^-, NO_3^-, and SRP concentrations (μM) in east and west lobes of Lake Bonney, 1993.

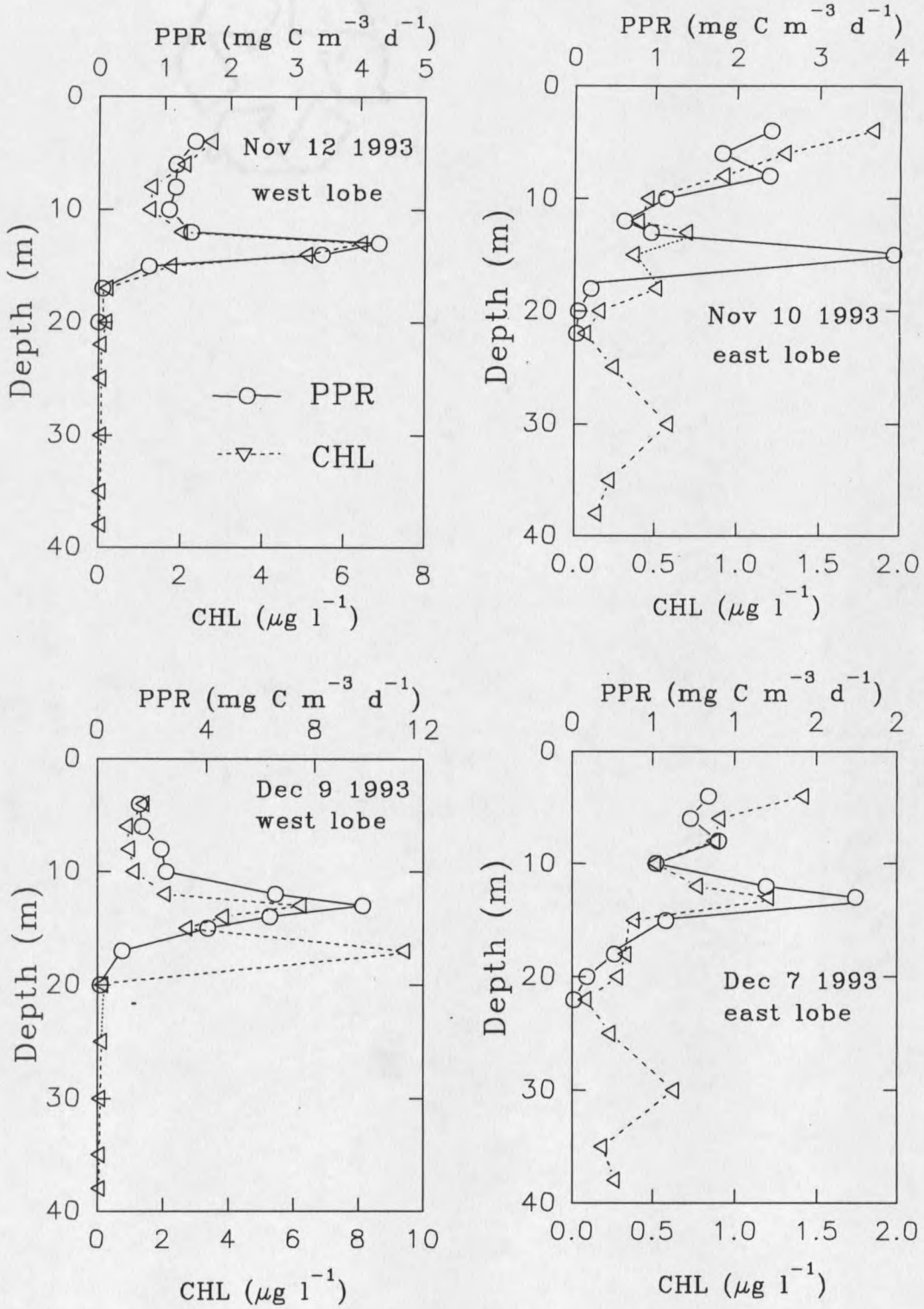

Figure 2. Chlorophyll a ($\mu g\ l^{-1}$) and primary productivity (mg C m^{-3} d^{-1}) in east and west lobes of Lake Bonney, 1993.

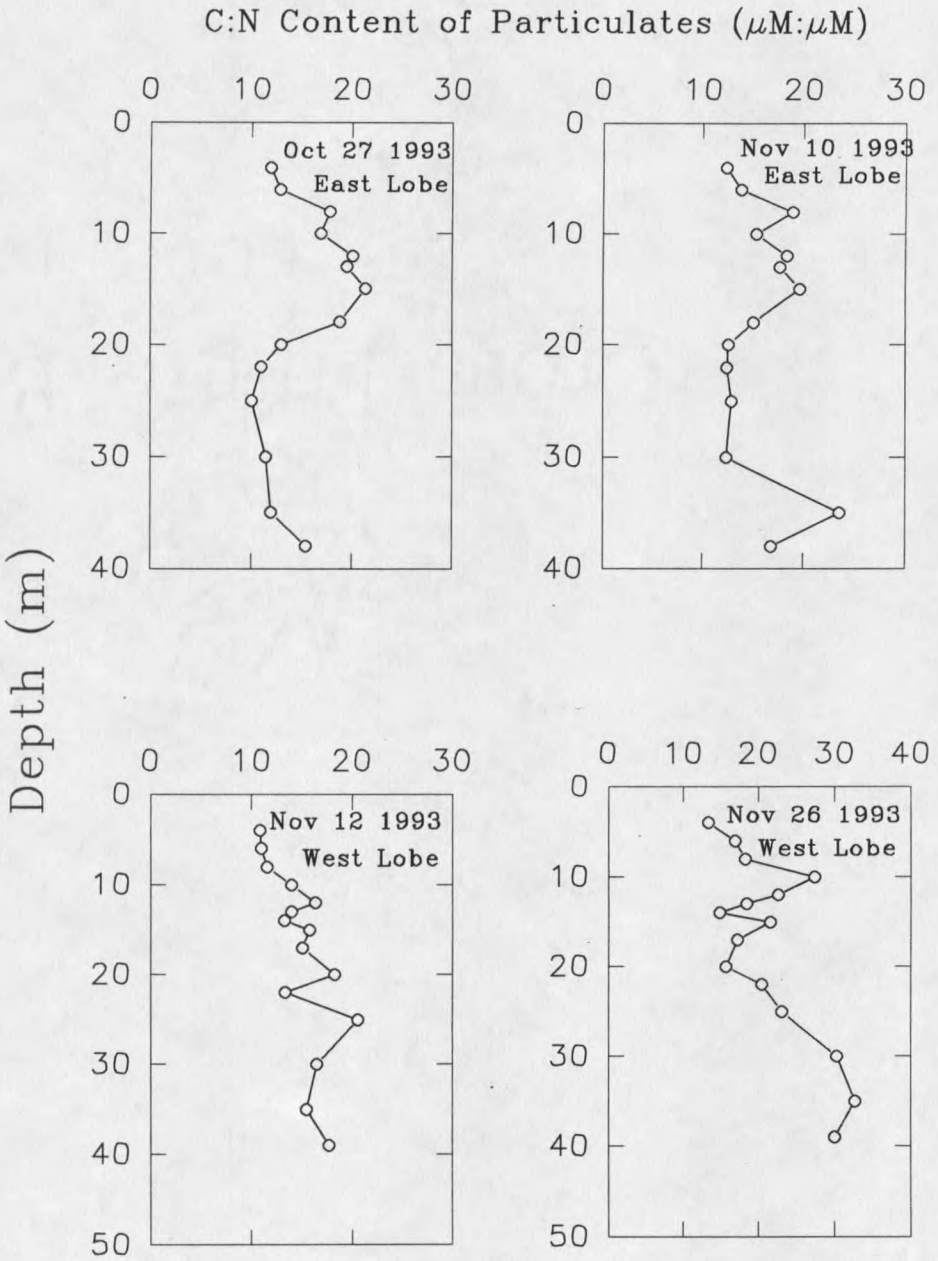

Figure 3. PC:PN ratios (μM:μM) in east and west lobes of Lake Bonney, 1993.

the ratio associated with balanced growth (Redfield 1957). However, the proportion of PC or PN attributable to viable phytoplankton is unclear; measured PC:PN ratios may be significantly influenced by detritus and bacteria.

Irradiance

Profiles of the natural log of irradiance are illustrated in Figure 4. There were two distinct layers of turbidity as measured by light attenuation : the ice-cover and the water column. Extinction coefficients of photosynthetically available radiation (PAR), determined with the following equation , were relatively constant with depth:

$$K_{par} = \ln (I_0 / I_z) / z \qquad (2)$$

where K_{par} = extinction coefficient of PAR (m^{-1})

I_0 = irradiance at an initial depth (μmoles quanta m^{-2} s^{-1})

I_z = irradiance at depth z (μmoles quanta m^{-2} s^{-1})

Extinction coefficients for both lobes during the 1993 season are summarized in Table 2. As the season progressed from austral

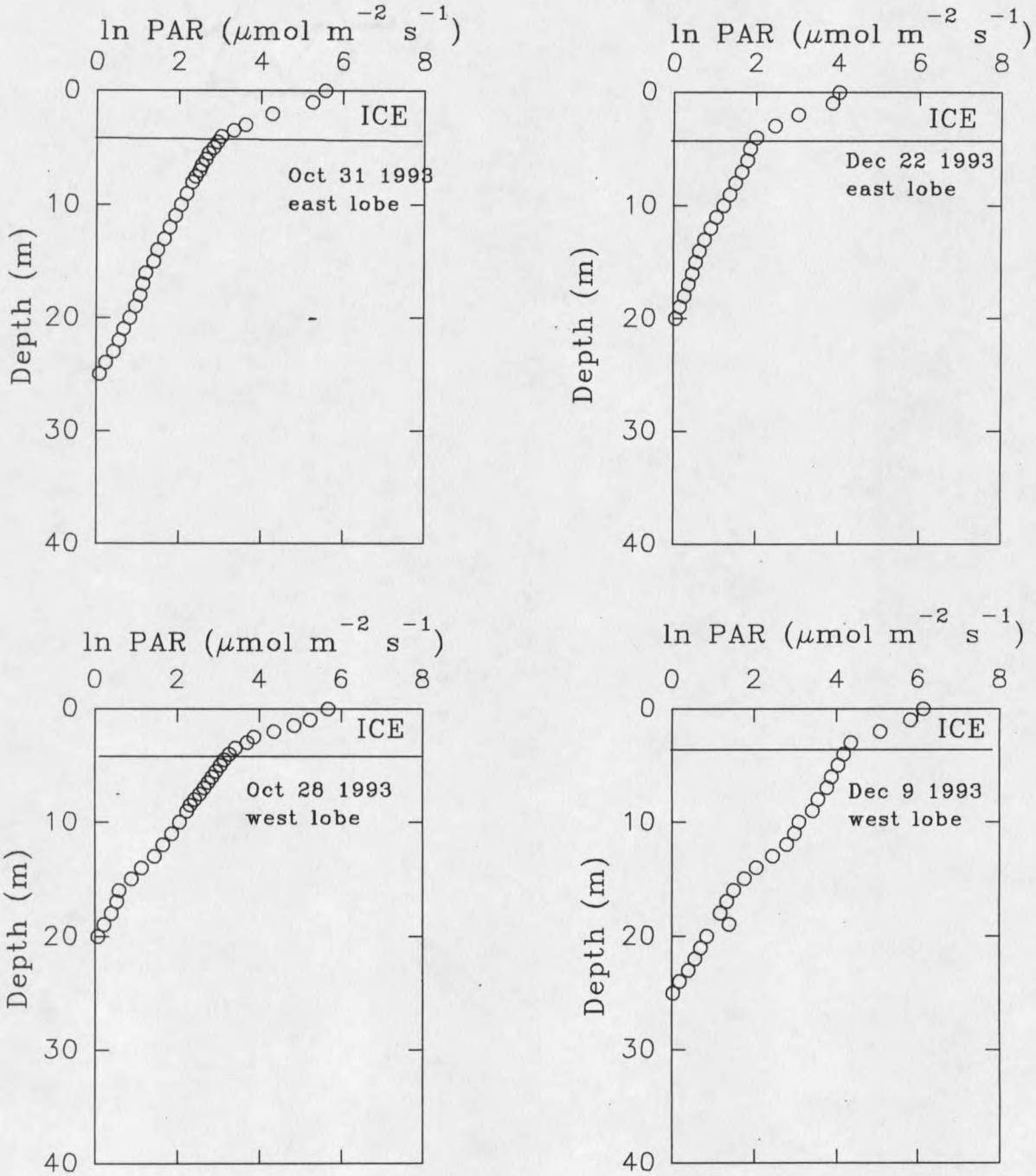

Figure 4. Natural log of irradiance (μmol m^{-2} s^{-1}) in east and west lobes of Lake Bonney, 1993.

spring to austral summer, there was no clear temporal trend in K_{par} in either lobe.

Table 2 . Extinction coefficients (K_{par}) in west and east lobe water columns, 1993 data. Coefficients calculated from light attenuation from 5 m to 25 m, with the exception of Nov 25 west lobe (5 m to 16 m).

EAST LOBE

Date	K_{par} (m^{-1})
Oct 31	0.102
Nov 10	0.134
Nov 25	0.080
Dec 9	0.124
Dec 22	0.125

WEST LOBE

Date	K_{par} (m^{-1})
Oct 28	0.191
Nov 13	0.188
Nov 25	0.158
Dec 9	0.201
Dec 22	0.124

Averages and ranges of calculated in situ daily irradiances at 5 m, 13 m, 17 m, and 25 m in the east lobe are summarized in Table 3. Irradiances were calculated from daily irradiances at 10 m with Equation 2 assuming $K_{par} = 0.1$

Table 3. Averages and ranges (in parentheses) of daily irradiance (μmole m^{-2} s^{-1}) during austral summer, fall, winter and spring, 1993. All irradiances rounded to the nearest whole number.

	5 m	13 m	17 m	25 m
Jan-March	18 (2-30)	8 (1-13)	5 (1-9)	2 (0-4)
April-June	2 (0-16)	1 (0-7)	1 (0-4)	0 (0-2)
July-Sep	3 (0-12)	1 (0-5)	1 (0-4)	0 (0-1)
Oct-Dec	18 (6-42)	8 (3-18)	6 (2-12)	3 (1-6)

The daily progression of calculated in situ irradiances at 4 east lobe depths on Nov 17, 1993 are illustrated in Figure 5. Irradiances were calculated from 10 min interval readings of irradiance at 10 m with Equation 2 assuming $K_{par} = 0.1$.

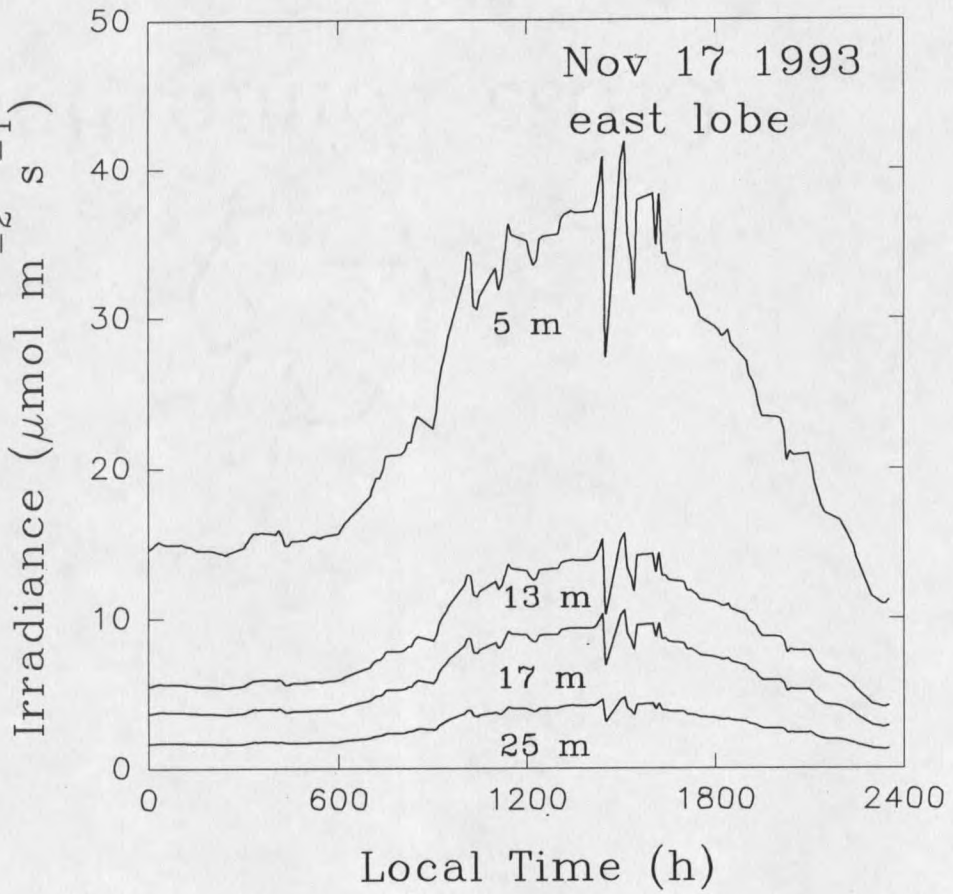

Figure 5. Calculated irradiance at 10 min intervals on Nov 17, 1993 at 5 m, 13 m, 17 m, and 25 m east lobe.

GENERAL OVERVIEW OF ^{15}N EXPERIMENTS

Uptake Measurements

All uptake and regeneration experiments utilized the stable isotope ^{15}N as a label. Uptake rates in uptake experiments (including substrate kinetics, time course, water column, and uptake vs irradiance experiments) were calculated by measurement of the accumulation of ^{15}N into particulates collected on Whatman GF/F glass fiber filters. Uptake rates were calculated using the following equation:

$$V = \frac{(^{15}N_p - 0.442)}{t * ((((^{15}N_a + (0.366 * {}^{14}N * 0.01)) * 100) / (^{14}N + {}^{15}N_a)) - 0.366)} \quad (3)$$

where V = specific uptake rate (h^{-1})

$^{15}N_p$ = ^{15}N atom-percent of particulates after incubation

$^{15}N_a$ = concentration (μM) of ^{15}N labeled nutrient at beginning of incubation.

^{14}N = natural concentration (μM) of nutrient at beginning of incubation.

t = time (h)

0.422 = estimated background ^{15}N atom-percent of particulates.

0.366 = global average background ^{15}N atom-percent

All ^{15}N filters were rinsed with approximately 20 ml DIW immediately after filtration to remove excess inorganic nitrogen. ^{15}N atom-percents of rinsed and unrinsed filters were compared to test the effects of rinsing. The ^{15}N atom-percent of the filters consistently decreased after rinsing, indicating that the net effect of rinsing was removal of non-assimilated nitrogen. However, this test could not determine if rinsing resulted in cell breakage and release of assimilated nitrogen. Tests conducted by Sharp (1993) indicated that rinsing the seston from 17 m east lobe with DIW did not significantly reduce organic carbon content; presumably the same is true of organic nitrogen.

Abiotic adsorption of nitrogen to the filters was monitored with frequent "kill" tests. In these tests, samples were treated with approximately 20 ml formalin for a final concentration of approximately 5% before addition of nutrients. At this concentration, retention of inorganic nitrogen on the filter is limited to abiotic adsorption to the seston and to the filter. Kill experiments were conducted concurrently with most uptake experiments and received identical rinses. In some cases, kill samples were incubated for 24 h; in other cases, kill samples were incubated for 5-10 min. The length of incubation had no discernible effect on the final ^{15}N atom-percent of the filter. Apparently, abiotic adsorption is not a time dependent process, at least within the time frame of these experiments.

Results of 1992 kill experiments indicated methodological problems, presumably because the formalin used was of varying quality (in some experiments, the formalin was highly polymerized). For instance, uptake rates of NH_4^+ in formalin treated samples often

exceeded dark uptake of NH_4^+. Fifty kill experiments conducted over the 1993 season from a single batch of non-polymerized formalin provided more realistic measurements of abiotic adsorption. These experiments indicate no significant difference in adsorption of NH_4^+ and NO_3^-. It should be noted that formalin can quickly react with NH_4^+ to form hexamethylenetetramine and/or formamide (Chiayvareesajja and Boyd 1993). This reaction might affect adsorption of NH_4^+ to filters. If abiotic adsorption of NH_4^+ is significantly greater in non-formalin treated samples compared to formalin treated samples, the abiotic adsorption of NH_4^+ in samples in this study could be significantly underestimated, thus leading to significant overestimation of uptake rates. This is particularly true with samples below 13 m in each lobe where ambient NH_4^+ pools were large, and a small amount of $^{15}NH_4^+$ adhering to the filter or particulate matter could significantly increase estimated uptake rates.

There was no significant correlation ($r^2 = 0.06$, $p > 0.05$) between ^{15}N atom-percent of the filtrate and ^{15}N atom-percent of the seston in kill experiments (Figure 6). The average ($\pm$ standard deviation) ^{15}N atom-percent of all NH_4^+ and NO_3^- kill experiments conducted in 1993 was 0.442% $\pm$ 0.160%. This average was subtracted from all ^{15}N atom-percent readings in all 1993 uptake experiments as an approximate correction for abiotic adhesion and background ^{15}N abundance. In instances where the measured ^{15}N

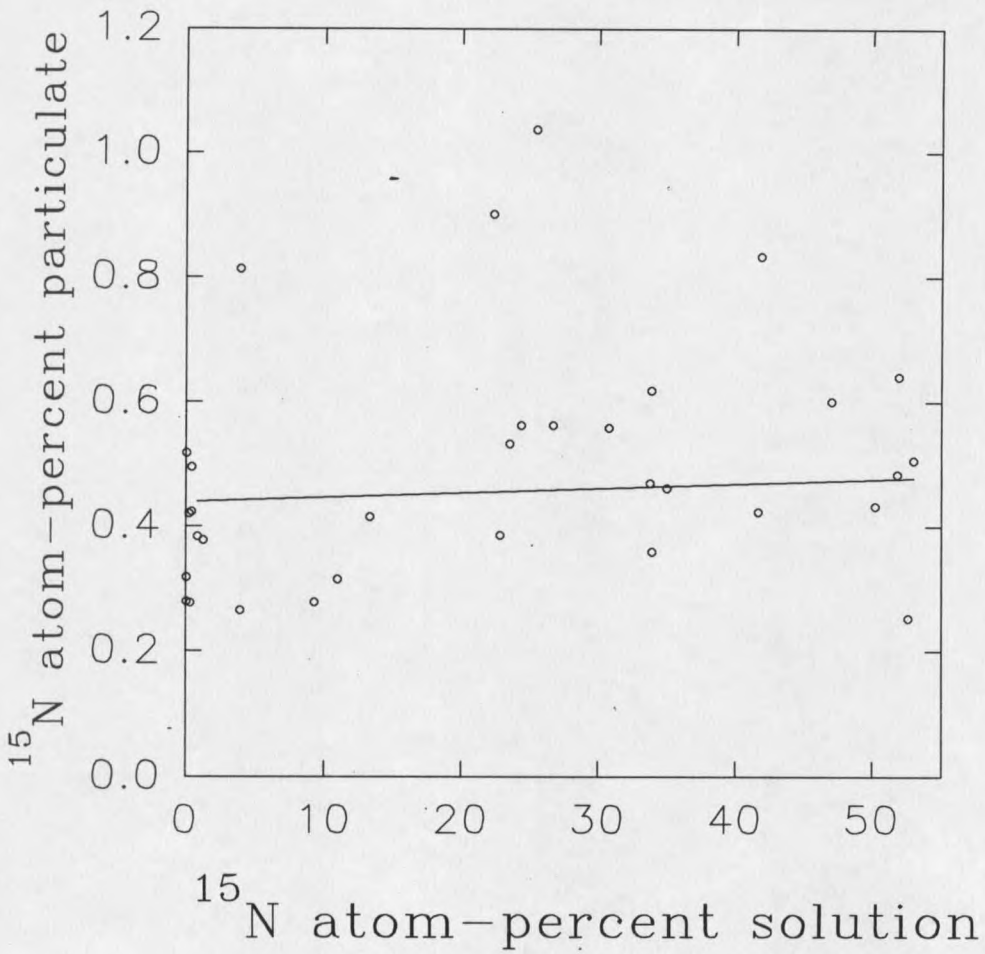

Figure 6. ^{15}N atom percent particulate vs ^{15}N atom percent solution for NO_3^- and NH_4^+ kill experiments from 1993. Line represents linear regression.

atom-percent was below 0.442%, the uptake rate was assumed to be zero. Owing to the uncertainty of kill experiments conducted during 1992, uptake rates from this season were corrected with the global average background ^{15}N atom-percent of 0.366 %. This correction is not significantly different from the correction used for 1993 samples ($p > 0.05$). Note that all p values in this study were determined with a t distribution.

NH_4^+ regeneration rates were calculated by measurement of dilution of $^{15}NH_4^+$. Measurement of dissolved $^{15}NH_4^+$ required extraction of NH_4^+ from solution, a process described below.

^{15}N Determination

^{15}N atom-percent ((^{15}N:^{15}N + ^{14}N)*100) was measured with an atomic emission spectrometer following Dumas combustion of the sample (Timperley and Priscu 1986). Samples achieve maximum discharge with approximately 10 µg N (Timperley and Priscu 1986); the amount of sample analyzed was adjusted accordingly. Calibration of the instrument was periodically monitored with ^{15}N standards. A combined standard curve containing all standard measurements over a two year period show a good fit with a linear least squares regression (r^2= 0.9997, Figure 7), indicating that instrument

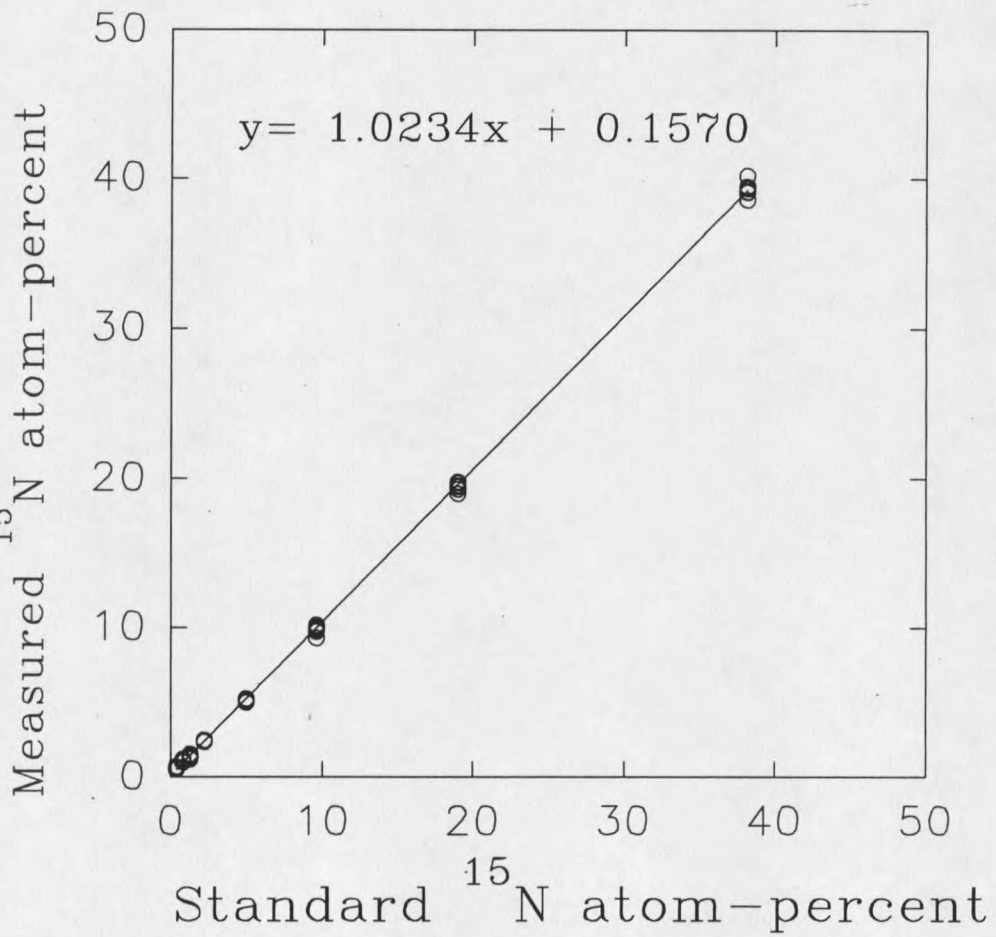

Figure 7. Standard ^{15}N atom percent vs ^{15}N atom percent measured with atomic emission spectrometer. Line represents linear regression. All standard measurements from 1992 and 1993 included.

accuracy did not vary significantly over this period. All sample ^{15}N atom-percent measurements were corrected using this combined standard curve.

PART II

Uptake and Regeneration of Dissolved Inorganic Nitrogen.

INTRODUCTION

It is well established that in nitrogen limited marine systems, phytoplankton are capable of physiological adjustment to enhance utilization of DIN. For instance, many phytoplankton are capable of rapid short term NH_4^+ uptake rates (Priscu 1987, Harrison et al. 1989, Goldman and Glibert 1982). If nitrogen starved phytoplankton are exposed to a pulse of NH_4^+, the short-term (< 1 h) uptake rate of NH_4^+ may be 20 times larger than the growth rate (Harrison et al. 1989). In contrast, uptake of nutrients such as NO_3^-, NO_2^-, and Si often lags for hours after addition, indicating an induction period necessary for activating uptake (Harrison et al. 1989). In general, the trend of uptake rate over time is an indicator of the nutrient history and physiological state of the community (Dugdale 1977, Goldman and Glibert 1982, Priscu 1987, Harrison et al. 1989).

In addition to maximizing time dependent responses, nitrogen deficient phytoplankton are capable of increasing affinity and long term uptake rates for DIN, particularly NH_4^+ (Suttle and Harrison, 1988, McCarthy 1981). Utilization of NH_4^+ supports "regenerated production" (Dugdale and Goering 1967), and the rates of regeneration dictate the sustainable levels of productivity. Indeed, regeneration rates are closely associated with primary productivity even in relatively nutrient rich marine coastal waters (Gardner et al. 1993).

Because productivity in freshwater systems is often assumed a priori to be limited by phosphorus, there have been relatively few investigations of nitrogen regeneration and physiological uptake response in freshwater systems. However, the assumption that all freshwater systems are phosphorus limited is simplistic; a number of freshwater systems have recently been shown to be primarily nitrogen limited (e.g. Dodds et al 1991, Priscu and Priscu 1984).

Several Antarctic lakes in the dry valleys of South Victoria Land were suspected to be primarily nitrogen deficient (Priddle et al. 1985, Priscu 1989, Woolston and Priscu 1993). Evidence for potential nitrogen deficiency included relatively low N:P supply ratios, increased productivity following DIN enrichment, and increased DIN uptake rates following DIN enrichment (Priddle et al. 1985, Woolston and Priscu 1993).

The permanently ice-covered lakes of the dry valleys of South Victoria Land, Antarctica have been hypothesized to be nitrogen deficient, and several features of these lakes facilitate analysis of the processes of nitrogen uptake and regeneration. These features include lack of upper trophic levels, hydraulic stability, and strong chemical gradients.

In many aquatic systems, the process of NH_4^+ regeneration is primarily associated with zooplankton excretion. Koike et al. (1986) estimated that over 95% of the NH_4^+ regenerated in the euphotic zone of Antarctic coastal waters was produced by microzooplankton and net zooplankton. Koike et al. (1986) noted that the role of bacteria and phytoplankton in NH_4^+ regeneration is largely unknown and difficult to discern; both bacteria and phytoplankton can

assimilate as well as produce NH_4^+. Furthermore, bacteria have high nutritional requirements and a high affinity for scarce nutrients (Harrison 1992). These observations have led some researchers to conclude that bacteria may be inefficient remineralizers of nitrogen in most aquatic environments (Harrison 1992).

There is no doubt that bacteria are theoretically capable of significant regeneration. Bacterial production may be supported largely by dissolved organic matter (DOM) leaked from phytoplankton (Angelo 1989), and deamination of DOM regenerates NH_4^+. Furthermore, NH_4^+ is produced as a byproduct of bacterial decomposition of phytoplankton. However, Goldman (1987) noted that bacterial regeneration of NH_4^+ may be extremely inefficient when the C:N ratio of the organic substrate is 10:1 or greater. In such instances, NH_4^+ is presumably retained by the bacteria. Since this ratio is often exceeded in nature, Goldman concluded that bacterial regeneration of NH_4^+ is insignificant in most aquatic systems.

Zooplankton are apparently rare or absent in the perennially ice-covered lakes of the McMurdo dry valleys; NH_4^+ regeneration in these lakes is therefore regulated by bacteria and phytoplankton. Some researchers have suggested that the lack of zooplankton would diminish the extent and efficiency of regeneration. Parker and Simmons (1985) claimed that the simplified food chains in Antarctic aquatic systems minimized the potential for nutrient recycling. Likewise, Matsumoto (1993) claimed that productivity of dry valley lakes is limited by a "paucity of nutrient cycling." However, Priscu et

al. (1989) found that rates of NH_4^+ regeneration either equaled or exceeded rates of NH_4^+ uptake in Lakes Fryxell and Vanda. This observation, in concert with high measured NH_4^+ uptake rates, led them to conclude that regeneration of NH_4^+ supports production in these lakes. The nitrogen dynamics in these lakes were termed "functionally similar" to the dynamics of systems where regeneration is dominated by zooplankton (Priscu et al. 1989). Ellis-Evans (1983) also reported significant nutrient regeneration in an Antarctic lake and speculated that bacterial activity was responsible for the bulk of nutrient cycling.

Although NH_4^+ is considered to be a regenerated nutrient, it can be quickly converted to other forms of DIN. In low productivity systems, nitrification could convert patches of NH_4^+ into patches of NO_3^- and NO_2^- before DIN uptake by phytoplankton. Therefore, NO_3^- and NO_2^- in such systems could be considered to be regenerated nutrients.

This study investigates uptake of DIN and regeneration of NH_4^+ in several distinct, vertically stratified planktonic communities in Lake Bonney. Glacial melt streams are the primary external source of nutrients to Lake Bonney. These streams had intermittent flow during the 1992 season and relatively low flow during the 1993 season (personal observation). Lake Bonney during the 1993 season could therefore be considered to be a closed system with respect to nutrients. Microplankton in a closed, non-turbulent system utilize

nutrients from two sources: steady molecular diffusion and biologically mediated regeneration.

As in Lakes Vanda and Fryxell, NH_4^+ in Lake Bonney is regenerated almost entirely by bacteria and phytoplankton. Strong gradients in nutrient supply and stratification of phytoplankton and bacterial communities in Lake Bonney allow for investigation of the interactions between uptake and regeneration in a variety of environments. Specifically, my study examined nitrogen uptake and regeneration in the communities of the upper trophogenic zone (5 m), the middle trophogenic zone (13 m) and the lower trophogenic zone (17 m east lobe). Regeneration and uptake was also measured at 20 m west lobe and 25 m in each lobe; at these depths, phytoplankton productivity is undetectable (Sharp 1993).

My study was designed to test the following hypotheses:

1. NH_4^+ is the preferred source of DIN for all Lake Bonney planktonic communities.
2. Regeneration of NH_4^+ provides a major nitrogen source for phytoplankton in the upper trophogenic zone.
3. Regenerated NO_3^- and NO_2^- provide a minor source of nitrogen for phytoplankton in the upper trophogenic zone.
4. Nitrogen deficient phytoplankton are capable of rapid short term uptake of NH_4^+, and, to a lesser extent, NO_3^- and NO_2^-.

To test these hypotheses, the following objectives were undertaken:

1. Quantify the ambient and maximal rates of uptake of NH_4^+, NO_3^-, and NO_2^-.

2. Quantify the affinity for NH_4^+, NO_3^-, and NO_2^-.

3. Quantify the ambient rates of regeneration of NH_4^+.

4. Determine the sources of regenerated NH_4^+.

METHODS

Isotope Dilution

Water was collected at each depth in a 5 liter Niskin bottle and gently decanted into 4.2 liter bottles (glass bottles were used for the 1992 season, polycarbonate bottles were used for the 1993 season). The bottles were kept dark in a cooler and quickly returned to the lakeshore laboratory. Aliquots were removed for PN, PC, DIN, and CHL analysis.

The bottles were then inoculated with $^{15}NH_4Cl$ (99 atom-percent). Water from 5 m east lobe, 5 m west lobe, 13 m east lobe, 13 m west lobe, 17 m east lobe, and 20 m west lobe were enriched with 10 μmoles of $^{15}NH_4Cl$ for a final $^{15}NH_4Cl$ concentration of approximately 2.5 μM. Water from 25 m east lobe and 25 m west lobe were inoculated with 15 μmoles of $^{15}NH_4Cl$ for a final $^{15}NH_4Cl$ concentration of approximately 3.75 μM. Bottles were inverted several times after addition of $^{15}NH_4Cl$ to mix the isotope. Within 5 min of addition, 250 ml of each sample was filtered on a precombusted 25 mm Whatman GF/F glass fiber filter at low vacuum (< 0.5 atm). The filter funnel was attached to a vacuum chamber, and the filtrate was collected in an acid-washed 250 ml HDPE bottle. The filter was dried, then frozen, and returned to MSU for analysis of particulate ^{15}N. The filtrate was frozen and returned to Crary

laboratory for NH_4^+ analysis. The remaining filtrate was frozen and returned to MSU for dissolved ^{15}N analysis.

Following initial filtration, the bottles were placed in an incubator. All bottles were wrapped in neutral density screening to simulate in situ irradiance. Irradiance within the incubator was monitored with a Li-Cor 4π quantum sensor attached to a Li-Cor model LI-1000 data logger. 250 ml aliquots were removed approximately every 8 h for filtration as described above.

Size Fractionated Regeneration

Water was collected in a 5 liter Niskin bottle at 5 m in both lobes. Water was gently decanted into 4.2 liter bottles (glass bottles were used for the 1992 season, polycarbonate bottle were used for the 1993 season). The bottles were kept dark in a cooler and quickly returned to the lakeshore laboratory. Aliquots were removed for CHN, DIN, and CHL analysis.

Before addition of 10 μmoles of $^{15}NH_4Cl$ (final addition approximately 2.7 μM), the sample was filtered through a 0.7 μm Gelman filter. Based on previous microscopic examination of Lake Bonney samples (Sharp 1993), this filtration removed phytoplankton and epiphytic bacteria but not free-living bacteria. A 250 ml aliquot was filtered onto a precombusted 25 mm Whatman GF/F glass fiber filter less than five min after inoculation with $^{15}NH_4Cl$. The filtrate was collected in an acid washed 250 ml HDPE bottle. The incubation bottles were incubated in an incubator. Neutral density screening was used to simulate in situ irradiance. Additional 250 ml aliquots

were removed for filtration approximately every 16 h for 3 d. The filters were air dried, frozen, and returned to MSU for ^{15}N analysis.

Substrate Effects on Regeneration

Water was collected with 2 casts of a 9 liter Niskin bottle at 5 m in each lobe. Water was gently decanted into 3, 4.2 liter bottles (glass bottles were used for the 1992 season, polycarbonate bottle were used for the 1993 season). The bottles were kept dark in a cooler and returned to the lakeshore laboratory. Aliquots were removed for CHN, DIN, and CHL analysis.

For each lobe, the bottles were inoculated with varying amounts of $^{15}NH_4Cl$: 5 μmoles (final addition approximately 1.4 μM), 10 μmoles (final addition approximately 2.7 μM), and 15 μmoles (final addition approximately 4 μM). The bottles were inverted several times. Less than five min after inoculation, a 250 ml aliquot from each bottle was filtered through a precombusted 25 mm Whatman GF/F filter. The filtrate was collected in an acid washed 250 ml HDPE bottle. The incubation bottles were incubated in a light and temperature controlled incubator. Neutral density screening was used to simulate in situ irradiance. An additional 250 ml aliquot was removed from each bottle for filtration approximately every 16 h. The filters were air dried, frozen, and returned to MSU for ^{15}N analysis. The filtrate was frozen and returned to MSU for NH_4^+ and ^{15}N analysis.

^{15}N Analysis of Filtrate

NH_4^+ was extracted with activated zeolite (Ionsiv W-85; Union Carbide), a molecular sieve with a high affinity for NH_4^+. Following the methods of Angelo (1989), 0.1 mg activated zeolite (4 h at 200° C) per ml of filtrate was added. After zeolite addition, the sample was mixed thoroughly and then allowed to settle for 30 min. The sample was mixed again and filtered on a precombusted 25 mm Whatman GF/C glass fiber filter at low vacuum (<0.5 atm). The zeolite was trapped by the filter, and the filter was dried at 55° C for 24 h before ^{15}N atom-percent analysis.

The efficiency of NH_4^+ extraction by zeolite varies as a function of salinity. Tests conducted by Angelo (1989) indicated that extraction efficiency in fresh water approached 90%, but extraction efficiency in salinities typical of seawater (6.3 mM NaCl) were less than 50%. At 1 M NaCl, extraction of NH_4^+ was undetectable (Angelo 1989).

The efficiency of NH_4^+ extraction by zeolite is an important parameter. Atomic emission analysis can only be conducted on a limited range of nitrogen levels; it is therefore necessary to be able to control the amount of nitrogen that has been trapped by the zeolite. As previously stated, ^{15}N atom-percent analysis via atomic emission spectrometry is optimized at approximately 10 μg nitrogen.

To test the efficiency of NH_4^+ extraction by zeolite in Lake Bonney samples, NH_4^+ concentration of filtered water was measured before and after zeolite extraction. Water was collected during the 1992 season and stored frozen in 1-gallon collapsible HDPE containers. At MSU, approximately 200 ml was filtered and the filtrate collected in HDPE bottles. After removal of 4 aliquots from each bottle for NH_4^+ analysis, activated zeolite was added to each sample. As previously described, the zeolite suspension was mixed, allowed to settle for 30 min, mixed again, and filtered. A vacuum chamber was used for filtering, and the filtrate was collected directly into an acid washed HDPE bottle. Four aliquots were removed from each bottle for NH_4^+ analysis. Efficiency of NH_4^+ extraction was determined with the following equation (Angelo 1989):

$$\% \text{ efficiency} = 100\ [1 - (([NH_4^+]_{post})([NH_4^+]_{pre})^{-1})] \quad (4)$$

where $[NH_4^+]_{post}$ = concentration of NH_4^+ after zeolite extraction

$[NH_4^+]_{pre}$ = concentration of NH_4^+ before zeolite extraction.

Tests were conducted to determine if distillation increased efficiency of NH_4^+ extraction in saline samples. NaCl and NH_4Cl were added to deionized water to simulate a range of Lake Bonney environments. The samples were brought to pH 10.0 with NaOH and steam distilled in a rotary evaporator (60 RPM, 90° C) for 40 min. NH_4^+ was extracted from the distillate with the zeolite method

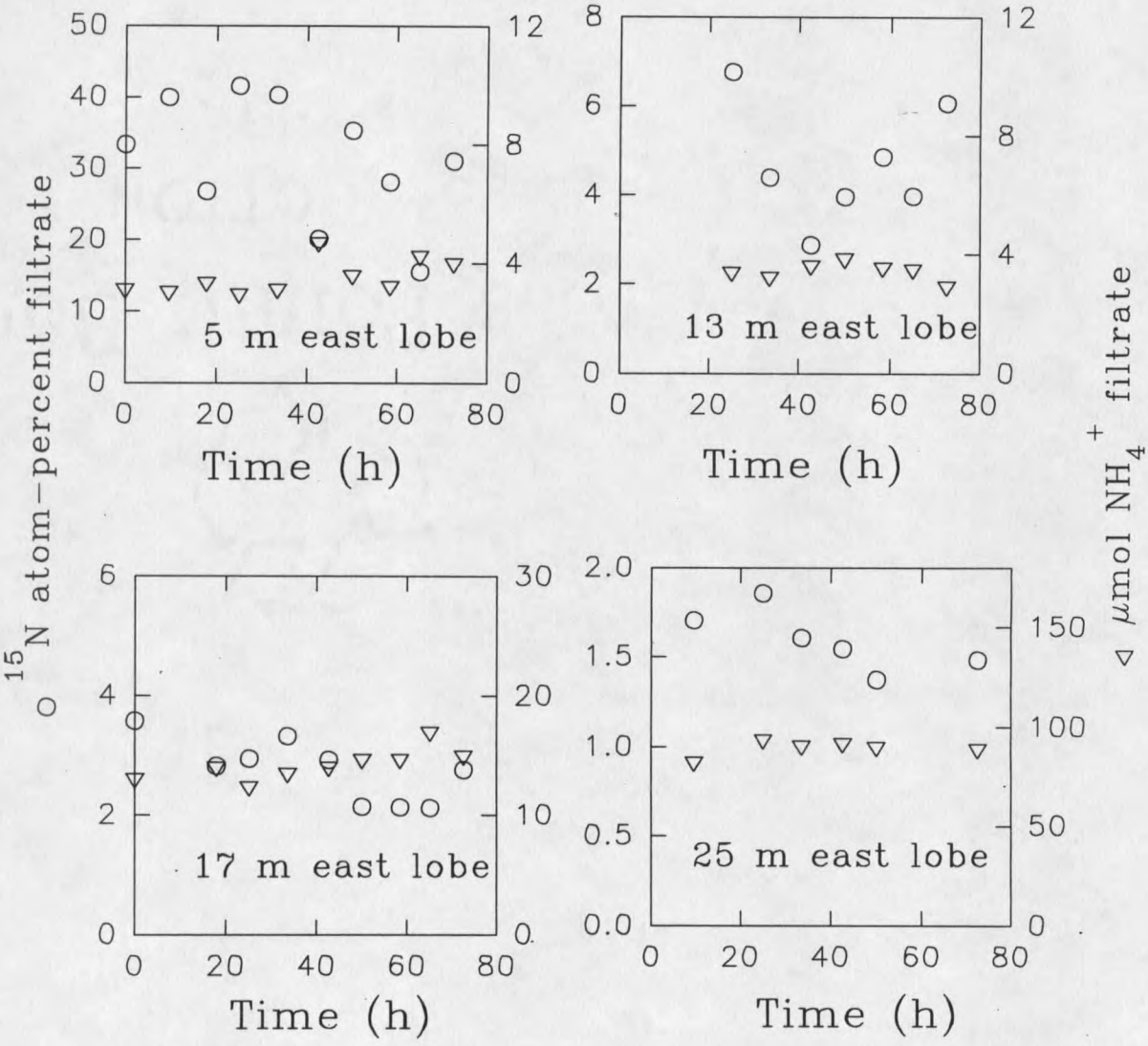

Figure 8. NH_4^+ extraction efficiencies using zeolite with and without a rotary evaporator. Salinity in lake samples was measured with a refractometer.

previously described. Results from zeolite extraction tests, with and without the use of the rotary evaporator, are illustrated in Figure 8. It should be noted that zeolite itself contains nitrogen. Through atomic emission spectrometry, Priscu (unpublished data) determined that each mg of activated zeolite contains 0.03 μg nitrogen. In applications described here, nitrogen in the zeolite will lead to a slight (approximately 2%) underestimation of the ^{15}N-NH_4^+ atom-percent of the filtrate.

Gardner et al. (1993) reported a method utilizing high performance liquid chromatography for analysis of atom-percent of dissolved nitrogen. This method allows for efficient ^{15}N atom-percent detection of small sample volumes (< 5 ml), but was not recommended for situations where the labeled nitrogen added would be large relative to ambient nitrogen. Therefore, this generally promising method is unsuitable for analysis of regeneration in the surface waters of Lake Bonney.

Another potentially complicating factor associated with the use of zeolite is isotope discrimination. Zeolite has a higher affinity for $^{14}NH_4^+$ than for $^{15}NH_4^+$, and the magnitude of this effect increases with increasing salinity. Angelo (1992) estimated that isotopic discrimination ranged from 5% in fresh water to approximately 15% at 6 mM NaCl to 90% at 1 M NaCl.

Isotope discrimination was determined concurrently with some tests of extraction efficiency. After removal of 4 aliquots for NH_4^+ analysis, measured amounts of $^{15}NH_4^+$ were added to each bottle. Before zeolite addition, four additional aliquots were removed for

NH_4^+ analysis. This allowed for precise estimation of the ^{15}N atom-percent enrichment. Activated zeolite was then used to extract NH_4^+ as previously described. Isotope discrimination was determined with the following equation (Angelo 1989):

$$\% \text{ discrimination} = 100 \left[\left({}^{15}N_{fil} - {}^{15}N_{zeo} \right) {}^{15}N_{fil}^{-1} \right] \quad (5)$$

where $^{15}N_{fil}$ = known atom-percent enrichment of the filtrate

$^{15}N_{zeo}$ = measured atom-percent enrichment of the zeolite

Isotope discrimination values at various depths are summarized in Table 4.

Table 4. Percent isotope discrimination for extraction of NH_4^+ with zeolite at varying ^{15}N enrichments.

Depth (m)	Lobe	% discrimination	Initial atom-%-filtrate
5	East	76.8	22.2
5	East	56.7	49.8
13	East	78.4	41.1
13	East	79.0	47.3
17	East	81.0	17.8
25	East	74.5	24.9
5	West	76.3	25.1
20	West	94.8	4.9
20	West	55.7	3.1

It should be noted that regeneration is determined by rate of change of ^{15}N atom-percent, not the ^{15}N atom-percent at any given

time point. Constant rates of isotope discrimination within a given experiment will therefore not affect calculations of regeneration.

Zeolite provides a convenient method for analysis of dissolved $^{15}NH_4^+$. Compared to other published methods of NH_4^+ extraction (e.g., microdiffusion, precipitation with a mercury salt, and extraction into indophenol blue) (Kristiansen and Paasche, 1989), the use of zeolite is relatively simple. It is not time or labor intensive and does not result in the production of hazardous waste. However, there are several issues that must be addressed before experimental application of zeolite.

As Figure 8 illustrates, zeolite extraction efficiencies were low, occasionally even below detection, for the highly saline deep water of Lake Bonney. However, the deep water of Lake Bonney also contains high concentrations of NH_4^+; therefore, the zeolite extracted sufficient nitrogen for ^{15}N analysis despite low efficiency.

Calculation of Regeneration

In all regeneration experiments, regeneration was calculated between each time point with the following equations (Angelo 1989, Laws 1984):

$$r = \frac{\ln ({}^{15}N_{ft}/{}^{15}N_{f0})\ (S_0 - S_t)}{\ln (S_t/S_0)\ t} \quad ; \quad S_t \neq S_0 \qquad (6)$$

$$r = \frac{\ln ({}^{15}N_{f0}/{}^{15}N_{ft})\ S_0}{t} \quad ; \quad S_t = S_0 \qquad (7)$$

where $r = NH_4^+$ regeneration rate ($\mu M\ h^{-1}$)

${}^{15}N_{f0} = {}^{15}N\text{-}NH_4^+$ atom-percent excess of filtrate at start of time step

${}^{15}N_{ft} = {}^{15}N\text{-}NH_4^+$ atom-percent excess of filtrate at end of time step

S_0 = concentration of NH_4^+ (μM) at start of time step

S_t = concentration of NH_4^+ (μM) at end of time step

Standard deviations of regeneration reported in this study are computed from the variance of regeneration rates between each time point.

Production of Ammonium from Serine

Water was collected at each depth in a 5 liter Niskin bottle. Water was gently decanted into 4.2 liter bottles (glass bottles were used for the 1992 season, polycarbonate bottle were used for the 1993 season). The bottles were kept dark in a cooler and returned to the lakeshore laboratory within 30 min. Aliquots were removed for

CHN, DIN, and CHL analysis. Additional water was removed until 2 l remained in each bottle.

Each bottle was inoculated with 2 ml of 1 mM ^{15}N-Serine (99 atom-percent, final addition approximately 1 μM). The bottles were inverted several times. Less than five min after inoculation, a 250 ml aliquot from each bottle was filtered through a precombusted 25 mm Whatman GF/F filter. The filtrate was collected in an acid washed 250 ml HDPE bottle. The incubation bottles were incubated at approximate in situ temperatures and wrapped in neutral density screening to simulate in situ irradiance. An additional 250 ml aliquot was removed from each bottle approximately every 16 h for filtration. The filters were air dried, frozen, and returned to MSU for ^{15}N analysis. The filtrate was also frozen and returned to MSU for NH_4^+ and ^{15}N analysis.

The percentage of serine converted into NH_4^+ was calculated from a modification of Gardner et al.'s (1993) equation:

$$NH_4^+{}_{prod} = 100*((^{15}N_f\ [NH_4^+]) + (^{15}N_p\ [PN]))\ [Ser]^{-1} \quad (8)$$

where $NH_4^+{}_{prod}$ = percentage of serine converted into NH_4^+

$^{15}N_f$ = $^{15}NH_4^+$ atom-percent of filtrate

$[NH_4^+]$ = concentration of NH_4^+ in the filtrate (μM)

$^{15}N_p$ = atom-percent of the seston

[PN] = concentration of particulate nitrogen in the seston (μM)

[Ser] = concentration of ^{15}N-serine added (μM).

Equation 8 is based on the assumption that a negligible amount of labeled serine is assimilated into the seston. However, the ability of phytoplankton and bacterioplankton to assimilate dissolved organic nitrogen (DON) is highly variable; uptake rates can range from undetectable to 50% the rate of NH_4^+ uptake (Bronk and Glibert 1993). More information about DON dynamics in Lake Bonney is needed for complete interpretation of this experiment. The results of Equation 8 should be considered a maximum estimate; the actual percentage of serine converted to NH_4^+ is probably lower.

Substrate Kinetics

Water was collected with a 9 liter Niskin. To reduce gas exchange, water from 13 m and deeper was transferred through a siphon hose to a clean 20 liter carboy. Water from 5 m was added to the carboy through a funnel. In all cases, the carboy was kept out of direct sunlight during the transfer. The carboy was then wrapped in a dark blanket and quickly returned to the lakeshore laboratory.

Water from the carboy was used to fill 10 to 14 liter polycarbonate bottles. Remaining water was analyzed for nutrients, CHN, and CHL. The polycarbonate bottles were enriched with varying levels of $^{15}NH_4Cl$, $Na^{15}NO_3$, or $Na^{15}NO_2$. Nutrient additions are summarized in Table 5.

Samples from the 1992 season were attached to a spreader bar and incubated in situ at the depth of collection. In the 1993 season, samples were incubated in a light and temperature controlled incubator; neutral density screening was used to approximate in situ irradiance. Irradiance within the incubator was monitored with a Li-

Cor 4π quantum sensor attached to a Li-Cor model LI-1000 data logger. Temperature inside the incubator was measured with a thermometer in a water bath.

Table 5. ^{15}N enrichments for substrate kinetics.

	$\mu M\ ^{15}N$		
Location	NH_4^+	NO_3^-	NO_2^-
5 m east lobe	0.1	0.1	0.05
	0.3	0.3	0.1
	0.7	0.7	0.3
	1.0	1.0	0.7
	1.5	1.5	1.0
	1.7	1.7	1.5
	2.0	2.0	1.7
	3.0	3.0	2.0
	7.0	7.0	3.0
	10.0	10.0	7.0
	15.0	15.0	10.0
	20.0	20.0	15.0
13 m east lobe	0.1	2.0	0.05
	0.3	3.0	0.1
	0.7	7.0	0.3
	1.0	10.0	0.7
	1.5	15.0	1.0
	1.7	20.0	1.5
	2.0	30.0	1.7
	3.0	40.0	2.0
	7.0	---	3.0
	10.0	---	7.0
	15.0	---	10.0
	20.0	---	15.0

Table 5, cont. ^{15}N enrichments for substrate kinetics.

Location	μM ^{15}N		
	NH_4^+	NO_3^-	NO_2^-
17 m east lobe	0.1	2.0	0.05
	0.3	3.0	0.1
	0.7	7.0	0.3
	1.0	10.0	0.7
	1.5	15.0	1.0
	1.7	20.0	1.5
	2.0	30.0	1.7
	3.0	40.0	2.0
	7.0	---	3.0
	10.0	---	7.0
	15.0	---	10.0
	20.0	---	15.0
25 m east lobe	10.0	10.0	2.0
	12.0	15.0	3.0
	15.0	20.0	7.0
	17.5	30.0	10.0
	20.0	40.0	15.0
	25.0	50.0	20.0
	30.0	60.0	30.0
	35.0	70.0	40.0
	40.0	---	---
	50.0	---	---
	60.0	---	---
	70.0	---	---

Table 5, cont. ^{15}N enrichments for substrate kinetics.

Location	μM ^{15}N		
	NH_4^+	NO_3^-	NO_2^-
5 m west lobe	0.1	0.05	0.05
	0.3	0.1	0.1
	0.7	0.3	0.3
	1.0	0.7	0.7
	1.5	1.0	1.0
	1.7	1.5	1.5
	2.0	1.7	1.7
	3.0	2.0	2.0
	7.0	3.0	3.0
	10.0	7.0	7.0
	15.0	10.0	10.0
	20.0	15.0	15.0
13 m west lobe	2.0	2.0	0.05
	3.0	3.0	0.1
	7.0	7.0	0.3
	10.0	10.0	0.7
	15.0	15.0	1.0
	20.0	20.0	1.5
	30.0	30.0	1.7
	40.0	40.0	2.0
	---	---	3.0
	---	---	7.0
	---	---	10.0
	---	---	15.0

Table 5, cont. ^{15}N enrichments for substrate kinetics.

Location	μM ^{15}N		
	NH_4^+	NO_3^-	NO_2^-
20 m west lobe	10.0	2.0	0.05
	15.0	3.0	0.1
	20.0	7.0	0.3
	30.0	10.0	0.7
	40.0	15.0	1.0
	50.0	20.0	1.5
	60.0	30.0	1.7
	70.0	40.0	2.0
	---	---	3.0
	---	---	7.0
	---	---	10.0
	---	---	15.0
25 m west lobe	10.0	0.1	0.05
	15.0	0.5	0.1
	20.0	1.0	0.3
	30.0	1.5	0.7
	40.0	3.0	1.0
	50.0	7.0	1.5
	60.0	10.0	1.7
	70.0	15.0	2.0
	---	---	3.0
	---	---	7.0
	---	---	10.0
	---	---	15.0

After approximately 24 h of incubation, samples were filtered onto precombusted 47 mm Whatman GF/F glass fiber filters under

low vacuum (<0.5 atm). After filtration, filters were rinsed with approximately 20 ml DIW to remove inorganic nitrogen. Filters were air dried in aluminum weigh-boats for several days before being transported to Crary laboratory. After arrival at Crary laboratory, filters were frozen (-45° C) before transport to MSU for final analysis.

Where appropriate, calculated uptake rates were fitted to substrate concentration with the Michaelis-Menten equation:

$$V = \frac{V_{max} * S}{K_S + S} \qquad (9)$$

where V = specific rate of uptake (h^{-1})

S = substrate concentration (μM)

V_{max} = rate of uptake at saturating levels of S (h^{-1})

K_S = substrate level at which $V = 0.5\ V_{max}$ (the half saturation constant) (μM).

Uptake rates at any level of S can be obtained from this equation once V_{max} and K_S are known (Murphy 1980, Priscu and Priscu 1984). To aid in construction of the Michaelis-Menten curve, it was assumed that V=O when S=0. This datum point was included in all data sets described by Michaelis-Menten kinetics. When uptake rates did not conform to Michaelis-Menten kinetics, trends were estimated with a linear least squares regression.

Regeneration can significantly affect measurements of substrate kinetics. Models constructed by Garside (1984) indicate that isotope dilution of tracer amounts (10% ambient) of ^{15}N

additions in an oligotrophic system can lead to 50% or greater underestimation of uptake after 12 h of incubation. Conversely, identical rates of isotope dilution of large ^{15}N enrichments (10 x ambient) do not significantly affect calculation of uptake. Clearly, the differential effects of regeneration could produce apparent, yet artificial, substrate effects on uptake.

NH_4^+ uptake rates were corrected for changes in NH_4^+ specific activity from $^{14}NH_4^+$ regeneration with the following equation (Angelo 1989):

$$0 \approx \frac{-1 + (1- k)^{1-\left(\frac{r}{V_C}\right)}}{\frac{kr}{V_C} - k} - \frac{V_C}{V} \qquad (10)$$

where $k = V\ t\ S^{-1}$

$r = NH_4^+$ regeneration rate ($\mu M\ h^{-1}$)

V_C = specific uptake rate corrected for isotope dilution (h^{-1})

Other parameters are as in Equation 9. V_C was adjusted until the equation approximated 0 ($\pm$ 0.005). The function is very sensitive to changes in V_C; to achieve the desired result (0 $\pm$ 0.005), it was necessary to estimate V_C to at least 5, and often 6, decimal places. Note that predicted uptake rates described by mathematical

functions, not measured uptake rates, were adjusted for regeneration.

Water Column Uptake

Water samples were collected at selected depths throughout the water column with a 5 liter Niskin bottle. One liter from each depth was transferred to a 1 liter HDPE bottle for analysis of background nutrients, PC and PN. Remaining water was used to fill 2, 1 liter polycarbonate bottles. The polycarbonate bottles were kept in a sealed cooler before inoculation. Each bottle was inoculated with a measured volume of 10 mM solution of either $^{15}NH_4Cl$, $Na^{15}NO_3$, or $Na^{15}NO_2$.

^{15}N additions and depths sampled are summarized in Table 6. Nutrient enrichments were designed to saturate uptake of nitrogen (i.e., designed to measure V_{max}) (Woolston and Priscu 1993). After inoculation, the bottles were inverted several times and suspended at the depth of collection. Incubation began between 2200 and 0000 local time, during which time the lake did not receive direct sunlight. Bottles were removed from the lake after 24 h of incubation.

Samples were filtered on precombusted 47 mm Whatman GF/F glass fiber filters under low vacuum (<0.5 atm). After filtration, filters were rinsed with approximately 20 ml DIW to remove inorganic nitrogen. Filters were dried in aluminum weigh-boats for several d before being transported to Crary laboratory. After arrival at Crary laboratory, filters were frozen (-45° C) before transport to MSU for final analysis.

Table 6. ^{15}N Enrichments for water column experiment.

	μM ^{15}N		
EAST LOBE Depth	NH_4^+	NO_3^-	NO_2^-
5	1.0	1.0	0.2
10	1.0	2.0	0.2
13	1.0	5.0	0.2
17	15.0	6.0	0.2
22	15.0	50.0	30.0
25	50.0	50.0	30.0
30	50.0	50.0	30.0
35	50.0	50.0	30.0
WEST LOBE			
5	0.5	7.0	1.0
13	5.0	15.0	1.0
15	30.0	15.0	1.0
17	30.0	15.0	1.0
20	30.0	15.0	1.0
25	30.0	0.2	1.0
30	30.0	0.5	1.0
35	30.0	15.0	1.0

Time Course

Water was collected in a 9 liter Niskin bottle and immediately decanted into incubation bottles. All 1992 samples and west lobe 1993 samples were incubated in 4 liter bottles (glass in 1992,

polycarbonate in 1993). East lobe 1993 samples were incubated in 1 liter polycarbonate bottles. During the 1993 season, a 500 ml aliquot from each depth was removed for analysis of CHN and DIN. For NO_3^- uptake, bottles were enriched with saturating amounts of either $Na^{15}NO_2$ or $Na^{15}NO_3$ (i.e. $V=V_{max}$)(Woolston and Priscu, 1993). For NH_4^+ uptake, one bottle from each depth was enriched with saturating amounts of $^{15}NH_4Cl$ (Woolston and Priscu, 1993). An additional bottle from each depth was enriched with a subsaturating amount of $^{15}NH_4Cl$ ($\approx$ 10% ambient). After inoculation, the bottles were inverted several times. Less than five min after inoculation, an aliquot (500 ml during 1992, 250 ml during 1993) from each bottle was filtered through a precombusted Whatman GF/F filter (47 mm for 1992, 25 mm for 1993). The incubation bottles were incubated in an incubator. Neutral density screening was used to simulate <u>in situ</u> irradiance. Additional aliquots were removed from each bottle for filtration approximately every 4 h. The filters were air dried, frozen, and returned to MSU for ^{15}N analysis.

RESULTS

Isotope dilution

Changes in $^{15}N\text{-}NH_4^+$ atom-percent and NH_4^+ concentration of the filtrate over time are illustrated in Figure 9. Due to widely scattered $^{15}N\text{-}NH_4^+$ atom-percent measurements, calculated regeneration rates varied greatly between time points in each experiment and could not be adequately modeled with either a linear or exponential function. Therefore, regeneration was calculated between each time point, and the regeneration rates were averaged (see Morrisey and Fisher 1988). Average regeneration rates and standard deviations are summarized in Table 7. Despite large coefficients of variance, a general trend is evident: regeneration rates increase with depth in each lobe.

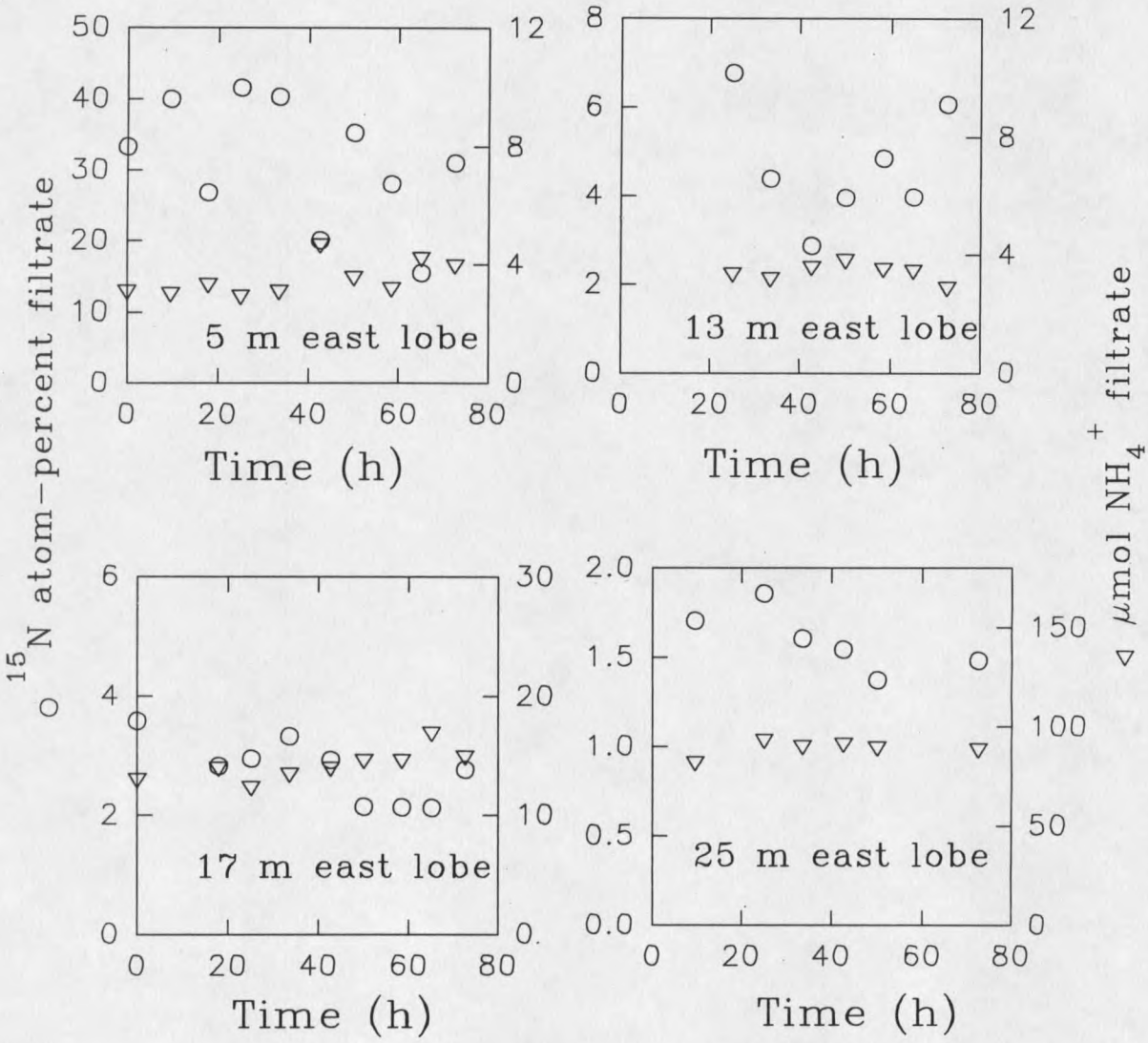

Figure 9. NH_4^+ (μM) and ^{15}N atom-percent in the filtrate for isotope dilution experiments, east lobe 1992.

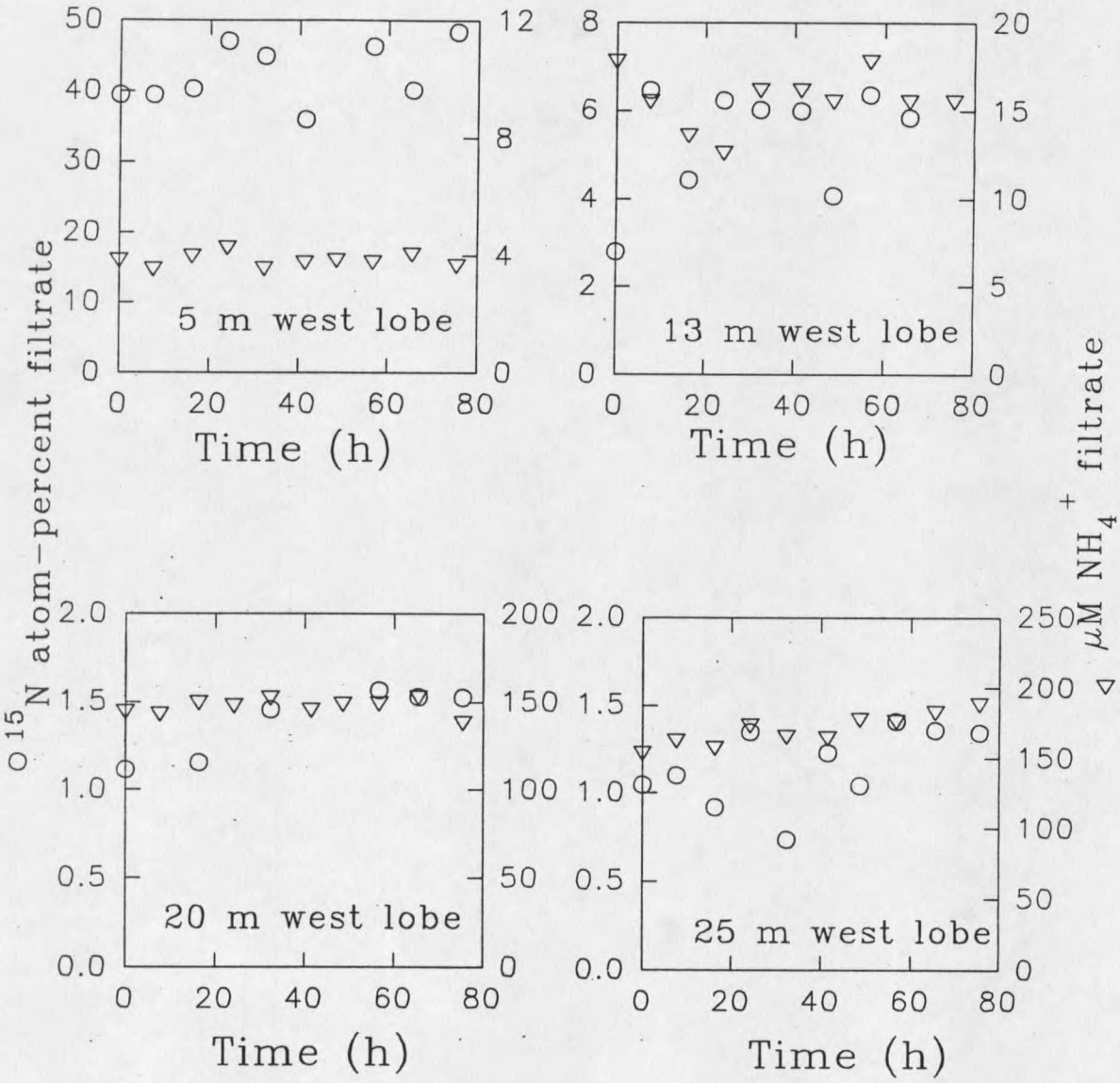

Figure 9, cont. NH_4^+ (μM) and ^{15}N atom-percent in the filtrate for isotope dilution experiments, west lobe 1992.

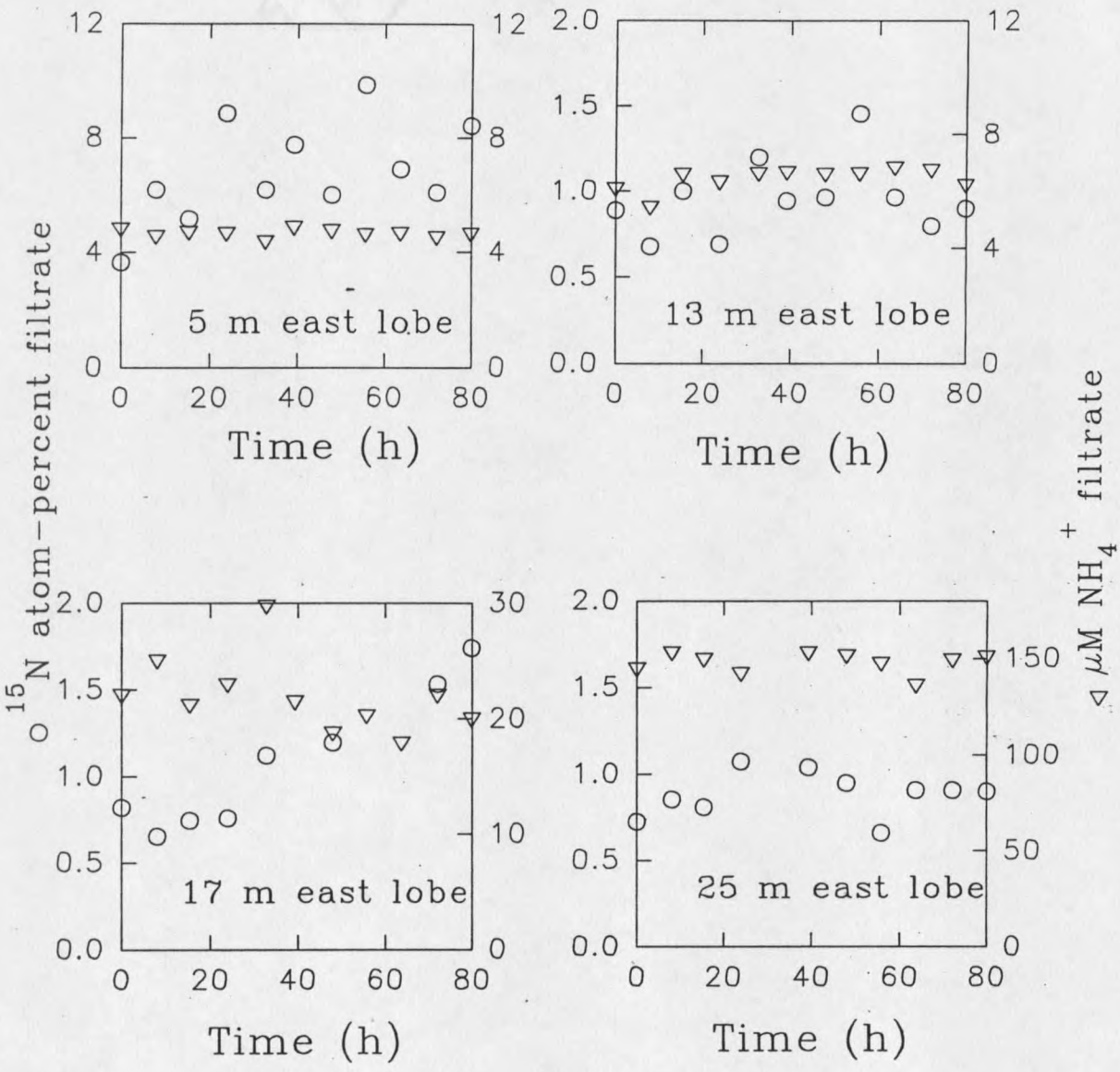

Figure 9, cont. NH_4^+ (μM) and ^{15}N atom-percent in the filtrate for isotope dilution experiments, east lobe 1993.

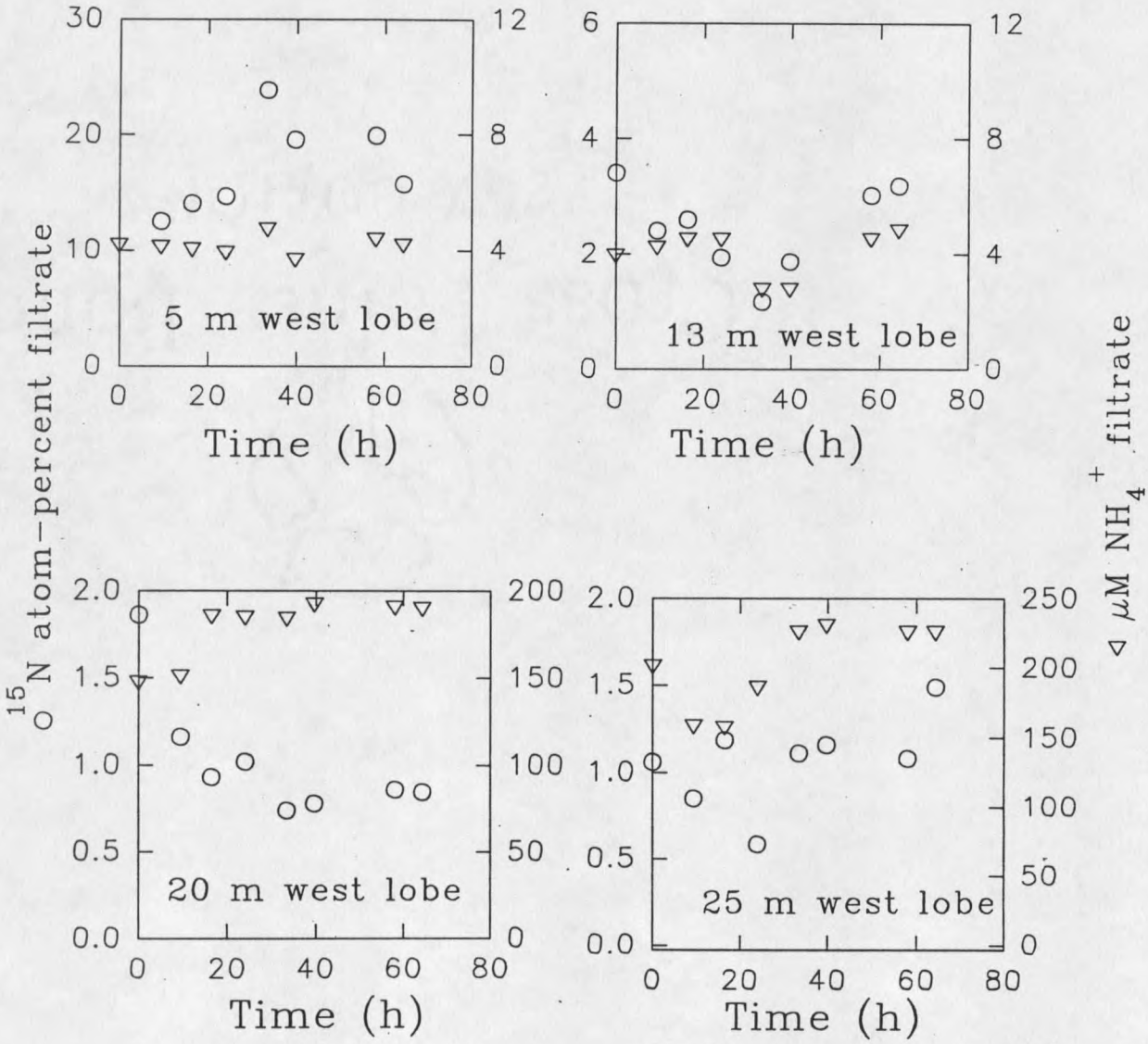

Figure 9, cont. NH_4^+ (μM) and ^{15}N atom-percent in the filtrate for isotope dilution experiments, west lobe 1993.

Table 7. Average rates of NH_4^+ regeneration ($\mu M\ h^{-1}$) ($\pm$ 1 standard deviation) in isotope dilution experiments after 24 and 80 h of incubation.

Depth (m)	Lobe	r_{24}	r_{80}
1992 experiments			
5	East	-0.03 $\pm$ 0.18	-0.01 $\pm$ 0.26
13	East	-0.04 $\pm$ 0.20	-0.03 $\pm$ 0.16
17	East	-0.02 $\pm$ 0.21	0.05 $\pm$ 0.40
25	East	1.25 $\pm$ 1.07	0.73 $\pm$ 1.24
5	West	-0.02 $\pm$ 0.05	-0.01 $\pm$ 0.10
13	West	0.06 $\pm$ 0.66	0.01 $\pm$ 0.72
25	West	4.32 $\pm$ 13.83	-1.17 $\pm$ 10.09
1993 experiments			
5	East	-0.18 $\pm$ 0.26	-0.06 $\pm$ 0.23
13	East	0.10 $\pm$ 0.60	0.01 $\pm$ 0.47
17	East	0.13 $\pm$ 1.12	-0.35 $\pm$ 1.16
25	East	-3.96 $\pm$ 5.36	-0.85 $\pm$ 7.58
5	West	-0.11 $\pm$ 0.11	-0.02 $\pm$ 0.14
13	West	0.11 $\pm$ 0.15	0.04 $\pm$ 0.17
20	West	5.72 $\pm$ 6.06	2.76 $\pm$ 6.64
25	West	7.56 $\pm$ 17.67	2.13 $\pm$ 13.77

Size Fractionation

Average regeneration rates from size fractionation experiments after 24 and 48 h of incubation are summarized in Table 8. As with

isotope dilution experiments, the standard deviations of regeneration estimates are large relative to the average regeneration rates, making it difficult to determine a trend.

Table 8. Average regeneration rates ($\mu M\ h^{-1}$, $\pm$ standard deviation) of size fractionated samples after 24 and 48 h of incubation.

Depth	Lobe	r_{24}	r_{48}
5	east	-0.12 $\pm$ 0.08	-0.02 $\pm$ 0.19
5	west	-0.25 $\pm$ 0.39	-0.07 $\pm$ 0.31

Substrate Effect on Regeneration

As with the previous experiments, ^{15}N atom-percent measurements for substrate effects on isotope dilution experiments were widely scattered. These data (Table 9) indicate that NH_4^+ regeneration increases with increasing NH_4^+ concentration, particularly in the east lobe; however, large coefficients of variance of regeneration estimates prevent definitive interpretation.

Table 9. NH_4^+ regeneration rates (± 1 standard deviation) from substrate effects on regeneration experiments. Regeneration values averaged from 80 h incubation.

ID	r
5 East low enrichment	-0.02 ± 0.14
5 East medium enrichment	0.03 ± 0.10
5 East high enrichment	0.02 ± 0.06
5 West low enrichment	0.00 ± 0.07
5 West medium enrichment	-0.03 ± 0.24
5 West high enrichment	-0.02 ± 0.07

Ammonium production from serine

Changes in ^{15}N-NH_4^+ atom-percent and NH_4^+ concentration of the filtrate are illustrated in Figure 10. The average percentages of ^{15}N-serine converted to NH_4^+ from several time points during a 50 h incubation are summarized in Table 10. The potential for NH_4^+ production from serine generally increased with depth. Since ambient serine concentrations also increase with depth from 5 m to 25 m in the east lobe (from 0.04 to 0.14 μM, respectively) (Priscu, unpublished data), it can be assumed that the amount of NH_4^+ produced from deamination of serine increases with depth.

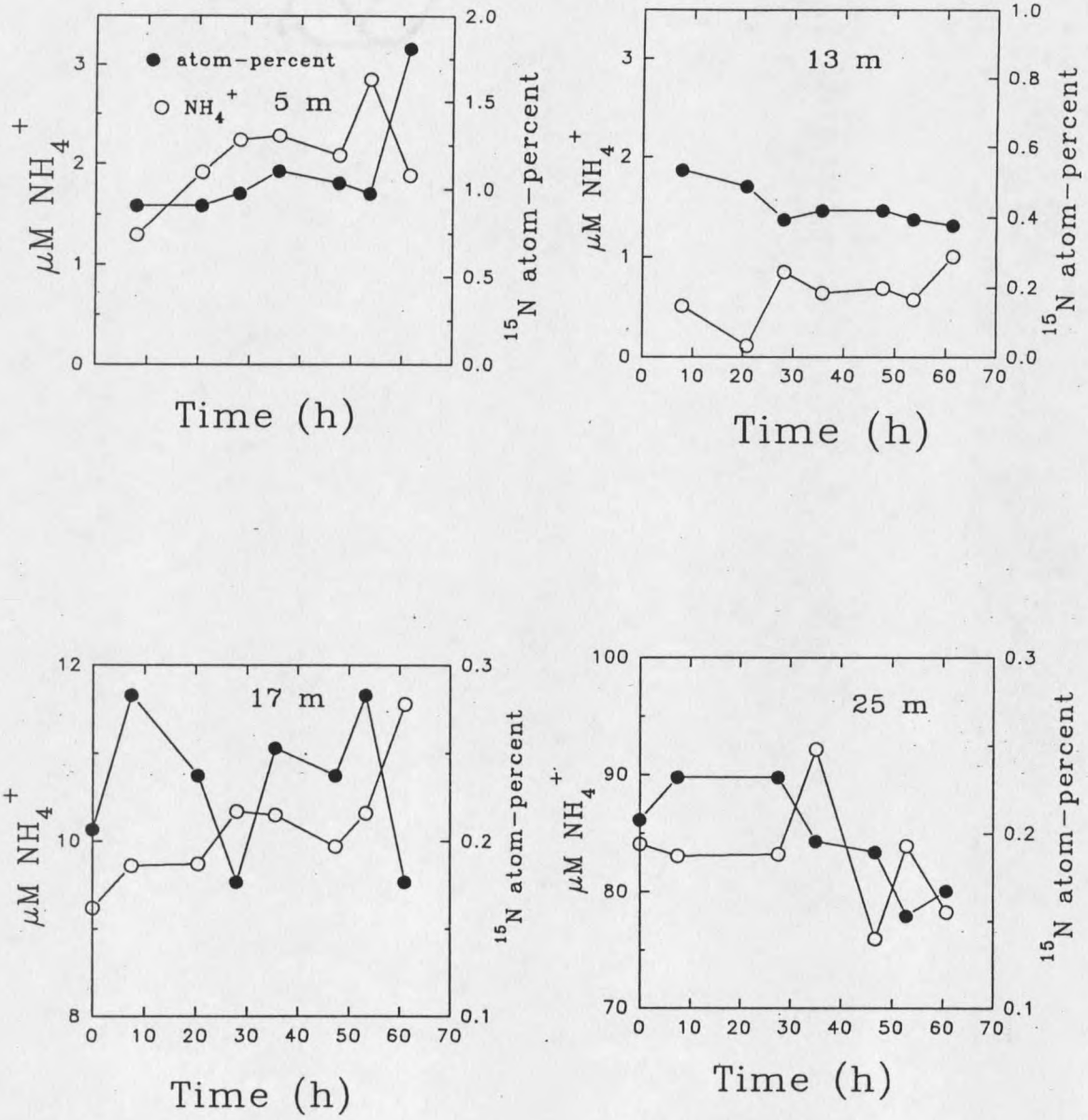

Figure 10. NH_4^+ (μM) and ^{15}N-NH_4^+ atom-percent in the filtrate for serine experiments, 1992.

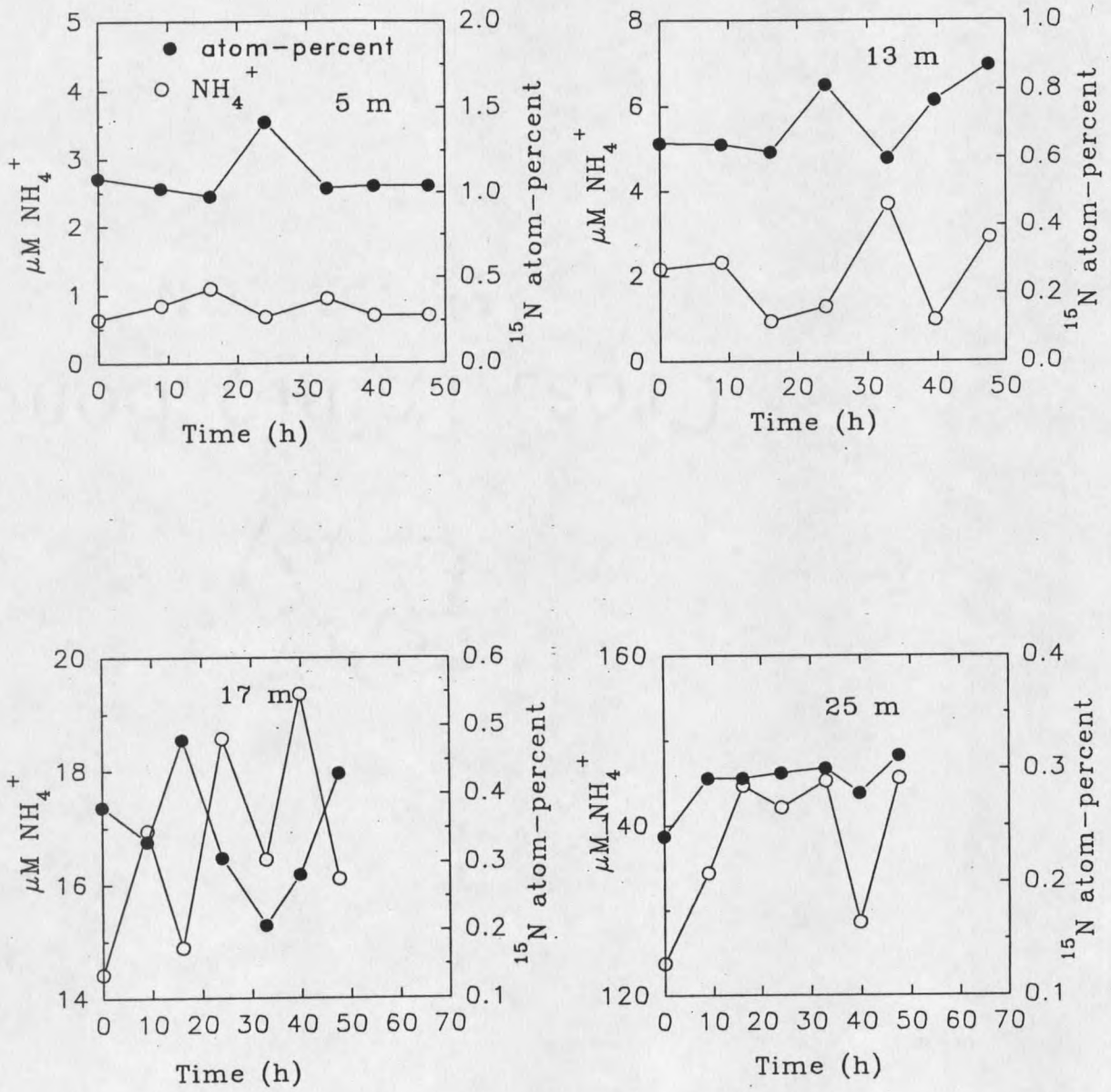

Figure 10, cont. NH_4^+ (μM) and ^{15}N-NH_4^+ atom-percent in the filtrate for serine experiments, 1993.

Table 10. Average percentage of added ^{15}N-serine converted to $^{15}NH_4^+$ ($\pm$ 1 standard deviation) after 50 h of incubation.

East Lobe Depth (m)	% serine converted 1992	1993
5	2.37 $\pm$ 0.63	1.11 $\pm$ 0.29
13	0.48 $\pm$ 0.08	1.88 $\pm$ 0.70
17	2.34 $\pm$ 0.21	6.33 $\pm$ 2.04
25	16.23 $\pm$ 3.40	41.38 (one reading)

Substrate kinetics

Michaelis-Menten kinetics could be used to model NH_4^+ uptake at 5 and 13 m in both lobes (Figure 11). NH_4^+ uptake at deeper depths did not conform to Michaelis-Menten kinetics; a linear least squares regression was used to describe uptake trends at these depths. Michaelis-Menten parameters, V_{max} and K_s, are summarized in Table 11. Parameters were determined using Marquardt's algorithm. Reported standard deviations of Michaelis-Menten parameters are equivalent to the standard error of the estimate.

Large coefficients of variance of isotope dilution measurements (> 100%) prevent precise regeneration corrections of NH_4^+ uptake. However, there is no question that some regeneration does occur and that regeneration does, to some extent, affect measurements of

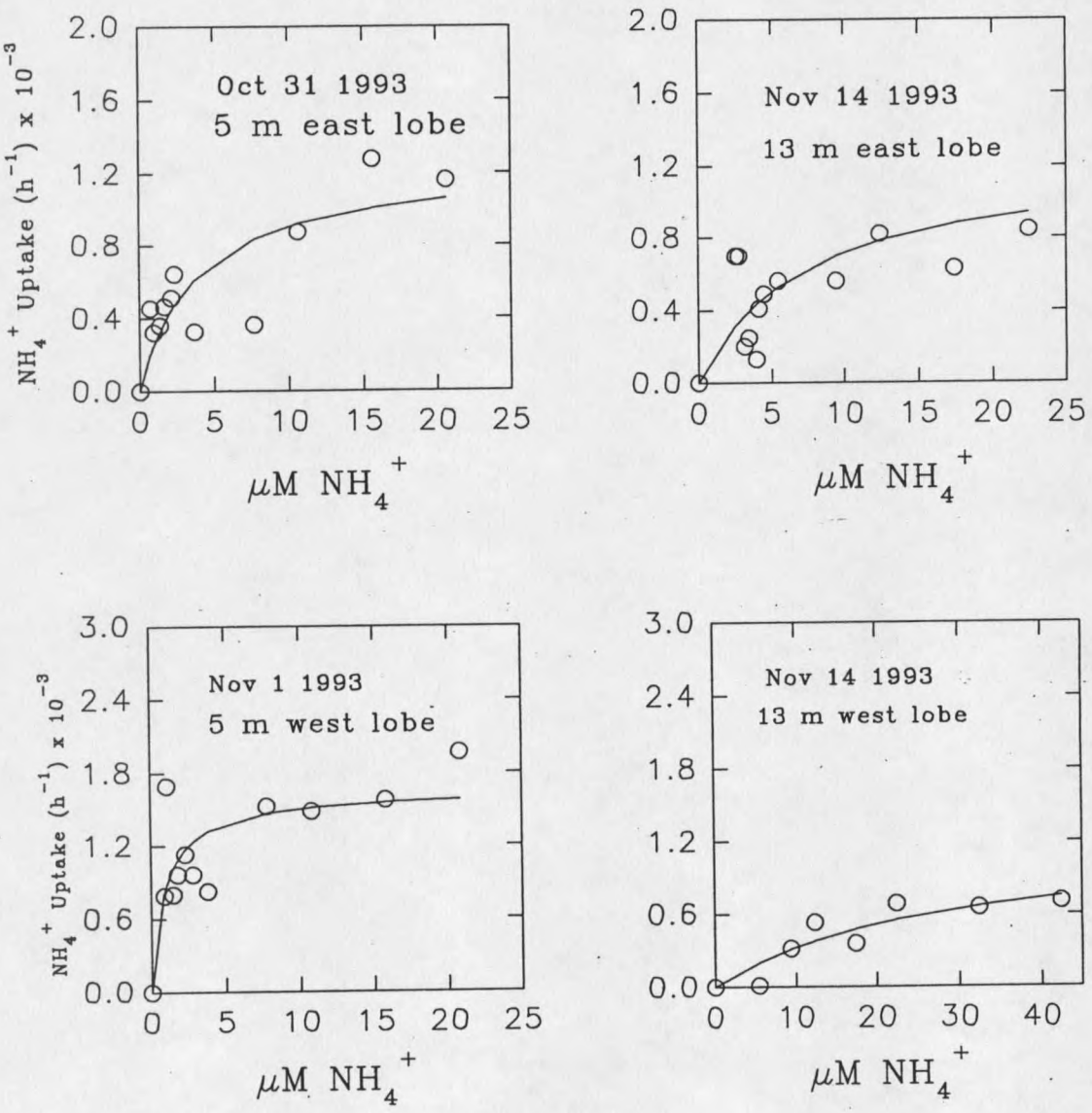

Figure 11 . Substrate kinetics of NH_4^+ uptake. Data fitted with Michaelis-Menten equation using Marquardt's algorithm.

substrate kinetics of NH_4^+ uptake. Positive regeneration rates were needed to assess the potential influence of regeneration on NH_4^+ substrate kinetics. The 24 h average from 1993 experiments was used to estimate in situ NH_4^+ regeneration rates, and the 24 h average plus one standard deviation was used as an estimate of the highest in situ NH_4^+ regeneration rates allowed by the data. In instances where the average regeneration rate was negative (e.g. 5 m west lobe), only the average plus one standard deviation was used for adjustment of uptake rates. Parameters from corrected curves, along with uncorrected parameters, are summarized in Table 11.

To reiterate, theoretical uptake rates along the Michaelis-Menten curve, not the original uptake measurements, were corrected for regeneration. Because they were derived from idealized curves, the standard deviations of "corrected" parameters do not indicate the degree of fit of the original data to the Michaelis-Menten curve. Standard deviations of corrected parameters have two causes. Firstly, because correction for regeneration has a relatively large effect on low enrichment samples compared to high enrichment samples, regeneration correction changes the shape of the curve; the corrected curve can no longer be precisely described by Michaelis-Menten kinetics. Secondly, because Equation 10 never equaled 0, there was a small (< 5%) degree of error in estimation of corrected uptake rates .

Uptake of NO_3^- and NO_2^- never approximated Michaelis-Menten kinetics, and the slopes of linear least square regressions were never significantly different from zero, with the exception of 5

m east lobe. These results indicate that ambient uptake of NO_3^- and NO_2^- is generally substrate saturated.

Table 11 . Original and corrected parameters of Michaelis-Menten Curves. All uptake values multiplied by 10^4. ID labels include depth, lobe (E or W), and nutrient analyzed.

r	V_{max} x 10^4	K_s
1993 Experiments		
$5ENH_4^+$		
0.00	12.57 ± 2.54	3.89 ± 2.09
0.08	11.69 ± 0.02	1.77 ± 0.11
$5WNH_4^+$		
0.00	16.49 ± 2.36	0.95 ± 0.58
0.001	16.49 ± 2.36	0.95 ± 0.58
$13ENH_4^+$		
0.00	12.53 ± 2.84	7.47 ± 3.63
0.10	11.53 ± 0.13	4.17 ± 0.13
0.70	12.98 ± 0.00	0.64 ± 0.01
$13WNH_4^+$		
0.00	13.60 ± 0.66	33.80 ± 2.83
0.11	12.72 ± 0.75	24.76 ± 2.86
0.26	13.16 ± 1.51	21.44 ± 5.102

V_{max} and K_S values for NH_4^+ uptake at 5 m in each lobe were not significantly changed by correction for isotope dilution; corrected values were within one standard deviation of original estimates. In the case of 5 m west lobe, Equation 10 approximated zero when V_C=V; therefore, no adjustment was necessary. At 5 m in each lobe, the original, uncorrected Michaelis-Menten parameters were used for estimation of ambient uptake rates. At 13 m in each lobe, V_{max} of NH_4^+ uptake was not significantly changed by correction for regeneration; however, K_S significantly decreased after correction for regeneration. Therefore, the Michaelis-Menten parameters obtained after correction with average regeneration rates were used to estimate ambient uptake rates at 13 m in each lobe.

Of experiments that were described by a least squares linear regression (see Table 12), most slopes were not significantly different than 0 ($p > 0.05$). The average V of these experiments is presented as an estimate of V_{max}. A few experiments were described by slopes significantly different than 0 ($p < 0.05$); V_{max} in these experiments is estimated to be the highest y value of the regression within the experimental range of substrate concentrations. Despite the apparently significant response to increasing substrate concentrations in some experiments, extrapolation of regressions to estimate uptake at ambient nutrient concentration is unwarranted. The y-axis intercept of these regressions is not reported because it is ecologically undefined; there can be no uptake when the substrate concentration equals zero, and uptake cannot be negative. However, all of these regressions have y-axis intercepts that are significantly

different from zero. The fact that portions of these regressions are meaningless indicates that extrapolations are not justified. In summary, only NH_4^+ uptake at 5 and 13 m in both lobes varied significantly with substrate concentration (i.e. at ambient concentrations, $V < V_{max}$).

Table 12. Average uptake (V, h^{-1}) and slopes (h^{-1} μM^{-1}) of linear regressions of substrate kinetics experiments ($\pm$ 1 standard deviation), 1993 experiments. All values multiplied by 10^4. * - indicates a value not significantly different from 0 ($p > 0.05$). ND indicates uptake was not detectable. ID labels include depth, lobe (E or W, and nutrient analyzed.

r	Slope	V_{max}
17ENH_4^+	*-0.16	5.47
0.13	-0.19	6.02
1.25	-0.44	9.94
20WNH_4^+	*0.03	3.37
5.72	0.04	4.50
11.77	0.04	5.60
25ENH_4^+	*0.03	2.97
1.40	0.03	3.33
25WNH_4^+	0.08	3.60
13.41	1.20	5.67

Table 12, cont. Estimated V_{max} (h^{-1}) and slopes (h^{-1} μM^{-1}) of linear regressions of substrate kinetics experiments ($\pm$ 1 standard deviation), 1993 experiments. * - indicates a value not significantly different than 0 ($p > 0.05$). All values multiplied by 10^4.

ID	Slope	V_{max}
$5ENO_3^-$	ND	ND
$5WNO_3^-$	ND	ND
$13ENO_3^-$	-7.67	*27.14
$13WNO_3^-$	*-0.05	*0.02
$17ENO_3^-$	*0.02	*0.00
$20WNO_3^-$	ND	ND
$25ENO_3^-$	*-0.01	*1.70
$25WNO_3^-$	ND	ND
$5ENO_2^-$	0.08	0.45
$5WNO_2^-$	*0.01	*0.08
$13ENO_2^-$	*0.00	*0.10

At $17ENH_4^+$ and $20WNH_4^+$, the average uptake rate was not significantly increased by correction with the average regeneration rate. However, $17ENH_4^+$ and $20WNH_4^+$ uptake was significantly increased when the average regeneration rate plus one standard deviation was inserted into Equation 10. Average uptake of NH_4^+ was not significantly affected by regeneration at 25 m in either lobe.

Since average regeneration rates did not significantly affect average uptake rates, NH_4^+ uptake experiments at 17 m east lobe, 20 m west lobe, 25 m east lobe, and 25 m west lobe do not require correction for regeneration. It should be noted, however, that if regeneration rates during a given experiment are larger than the reported 24 h average, NH_4^+ uptake could be significantly underestimated.

Compared to 5 m east lobe, K_S for NH_4^+ uptake was relatively low at 5 m west lobe, indicating relatively high affinity for NH_4^+ at 5 m in the west lobe. K_S values for NH_4^+ uptake increased with depth from 5 to 13 m in each lobe. This is particularly evident in the west lobe where K_S for NH_4^+ uptake was more than 30 times greater at 13 m than at 5 m. However, the trend towards increasing K_S with increasing depth is diminished when uptake rates are corrected for regeneration. For instance, if regeneration of NH_4^+ at 13 m in the east lobe was occurring at a rate of 0.70 $\mu M\ h^{-1}$ (the measured 24 h average plus one standard deviation), the K_S for NH_4^+ uptake at this depth would be lower than the K_S at 5 m in the east lobe.

Nutrient gradients (See Part I, Results, Nutrients) were used to estimate the rate of upward diffusion of nutrients into selected 1 m thick layers. Diffusive flux (DF) was determined with the following equation:

$$DF = g * K_Z * \frac{1}{1\ m} * \frac{1\ m^3}{10^3\ liter} \qquad (11)$$

where DF = rate of upwards diffusive flux ($\mu M\ h^{-1}$)

g= nutrient gradient($\mu mole\ m^{-4}$)

K_Z = molecular diffusion coefficient ($5.4 \times 10^{-6}\ m^2\ h^{-1}$)

1 m = thickness of the layer

$1/10^3$ = conversion of m^3 to liters.

Upward diffusion rates of NH_4^+ and NO_3^- into 1 m thick layers with midpoints at 5 and 13 m in each lobe are summarized in Table 13 . In situ uptake rates of each nutrient are included for comparison. NH_4^+ uptake rates were calculated with Michaelis-Menten parameters. To obtain V (h^{-1}), S was assumed to equal the concentration of NH_4^+ at the bottom of the layer. Because NH_4^+ was not measured at 5 m during routine data collection, the average of NH_4^+ concentrations at 4 m and 6 m was used in calculation of V. Multiplying V by the particulate nitrogen at 5 and 13 m produced the uptake parameter ρ ($\mu M\ h^{-1}$). Average NO_3^- uptake rates from substrate kinetics experiments were used for estimates of NO_3^- uptake. (V - DF) indicates the rate at which the nutrient must be supplied from sources other than upward diffusive flux (i.e., regeneration) to support in situ uptake.

Table 13. 1993 upward diffusive flux (DF, $\mu M\ h^{-1}$) and uptake rates (ρ, $\mu M\ h^{-1}$) of DIN at 5 and 13 m in each lobe. ID labels include depth, nutrient, and lobe (either E or W).

ID	Date	DF x 10^{-5}	ρ x 10^{-5}	(ρ-DF) x 10^{-5}
5ENH_4^+	10 Nov	0	55.7	55.7
5ENH_4^+	7 Dec	0	102.7	102.7
5WNH_4^+	12 Nov	0	354.6	354.6
5WNH_4^+	9 Dec	0.0	175.0	175.0
13ENH_4^+	10 Nov	1.4	17.2	15.8
13ENH_4^+	7 Dec	0.2	19.7	19.5
13WNH_4^+	12 Nov	19.6	69.9	50.3
13WNH_4^+	9 Dec	97.7	11.9	-22.0
5ENO_3^-	10 Nov	0.2	0.0	-0.2
5ENO_3^-	7 Dec	0.6	0.0	-0.6
5WNO_3^-	12 Nov	0.0	0.0	0.0
5WNO_3^-	9 Dec	0.0	0.0	0.0
13ENO_3^-	10 Nov	0.0	0.8	0.8
13ENO_3^-	7 Dec	5.9	4.9	-0.9
13WNO_3^-	12 Nov	3.2	5.3	2.1
13WNO_3^-	9 Dec	5.3	5.0	-0.3

Water Column Uptake

Water column profiles of DIN uptake (see Figure 12) show several trends. With the exception of a single high value for NO_3^- uptake at 35 m east lobe (1993), NH_4^+ is the preferentially transported form of DIN at all depths. Maximum NH_4^+ uptake occurs consistently within the first chemocline (between 17 and 10 m). In contrast, the highest uptake rates of NO_3^- occurred at 5 m, with the exception of the east lobe 1993 experiment. NO_2^- uptake rates were low and often below detection.

Data for NO_3^- uptake east lobe (1992) and NO_2^- uptake west lobe (1992) are unavailable; the sealed tubes from these experiments inexplicably burst during Dumas combustion of the filters.

Time Course

Time course results are illustrated in Figure 13. Rapid initial uptake was always apparent for NH_4^+ uptake at 5 m in each lobe and 13 m east lobe. In both 1992 and 1993, rapid short term NH_4^+ uptake at 17 m east lobe was not evident after 5 min but was evident after 4 h. If these uptake responses are typical, estimates of maximum uptake rates of NH_4^+ based on 24 h incubations would be significantly underestimated at 5 m in each lobe, 17 m east lobe, and 13 m east lobe.

Uptake of NO_3^- displays a downward trend over time at 5 m in the west lobe and at 13 m in each lobe. However, these trends

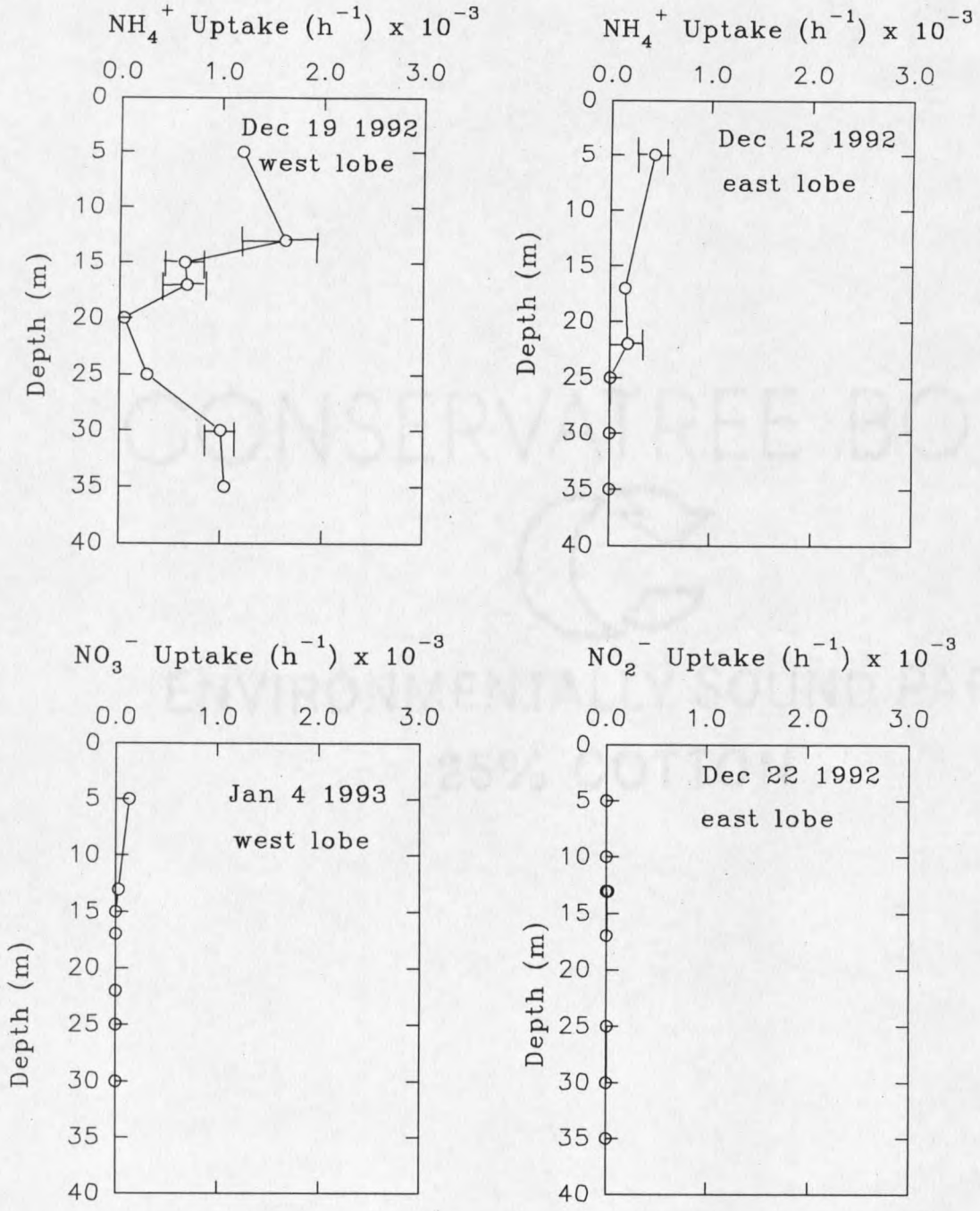

Figure 12 . Uptake of dissolved inorganic nitrogen throughout the water column, 1992 season. Error bars indicate range of uptake measurements at each depth. Note some depths are described by a single datum point.

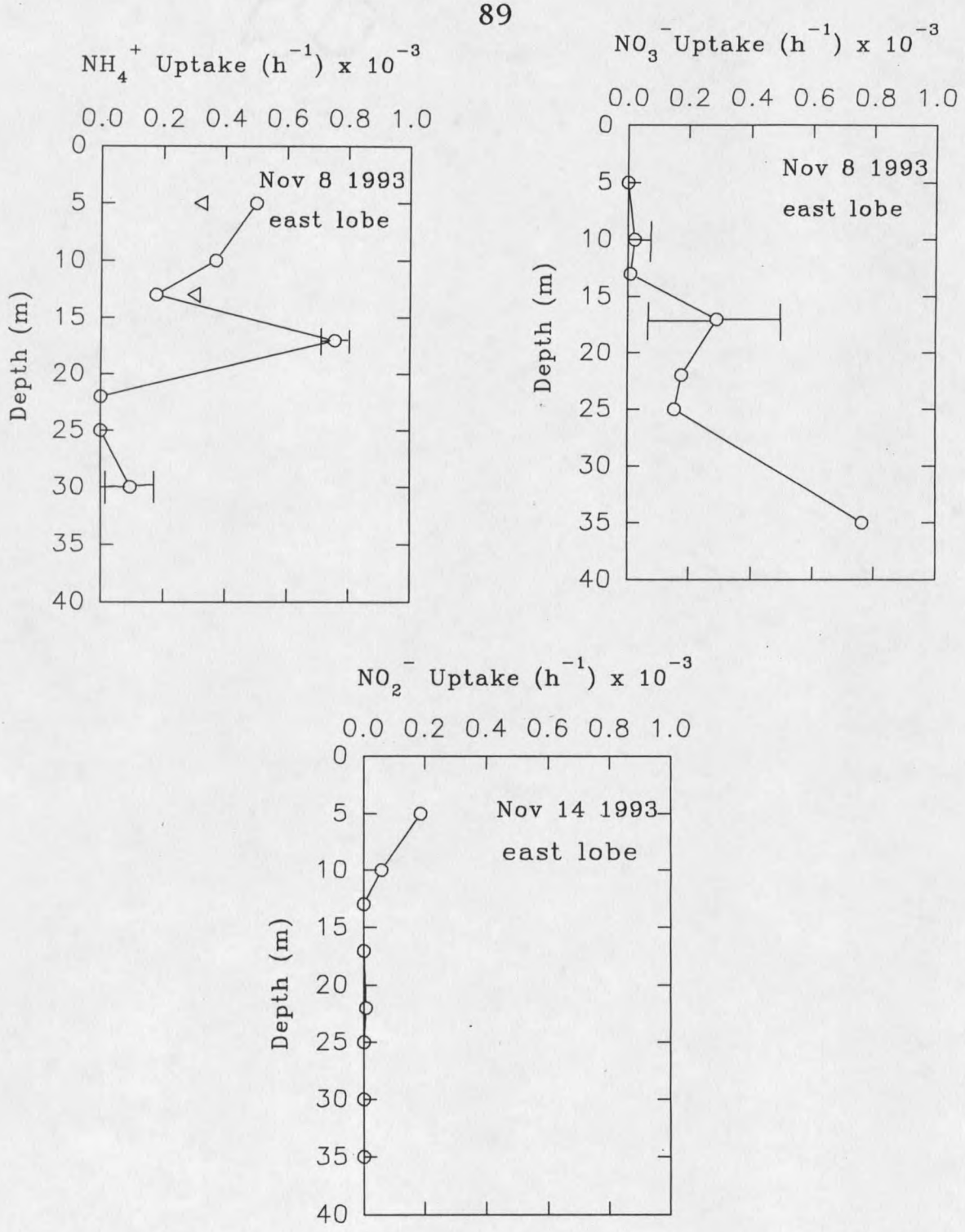

Figure 12, cont. Uptake of dissolved inorganic nitrogen throughout the water column, 1993 season. Error bars indicate range of uptake measurements at each depth.Note some depths are described by a single datum point.

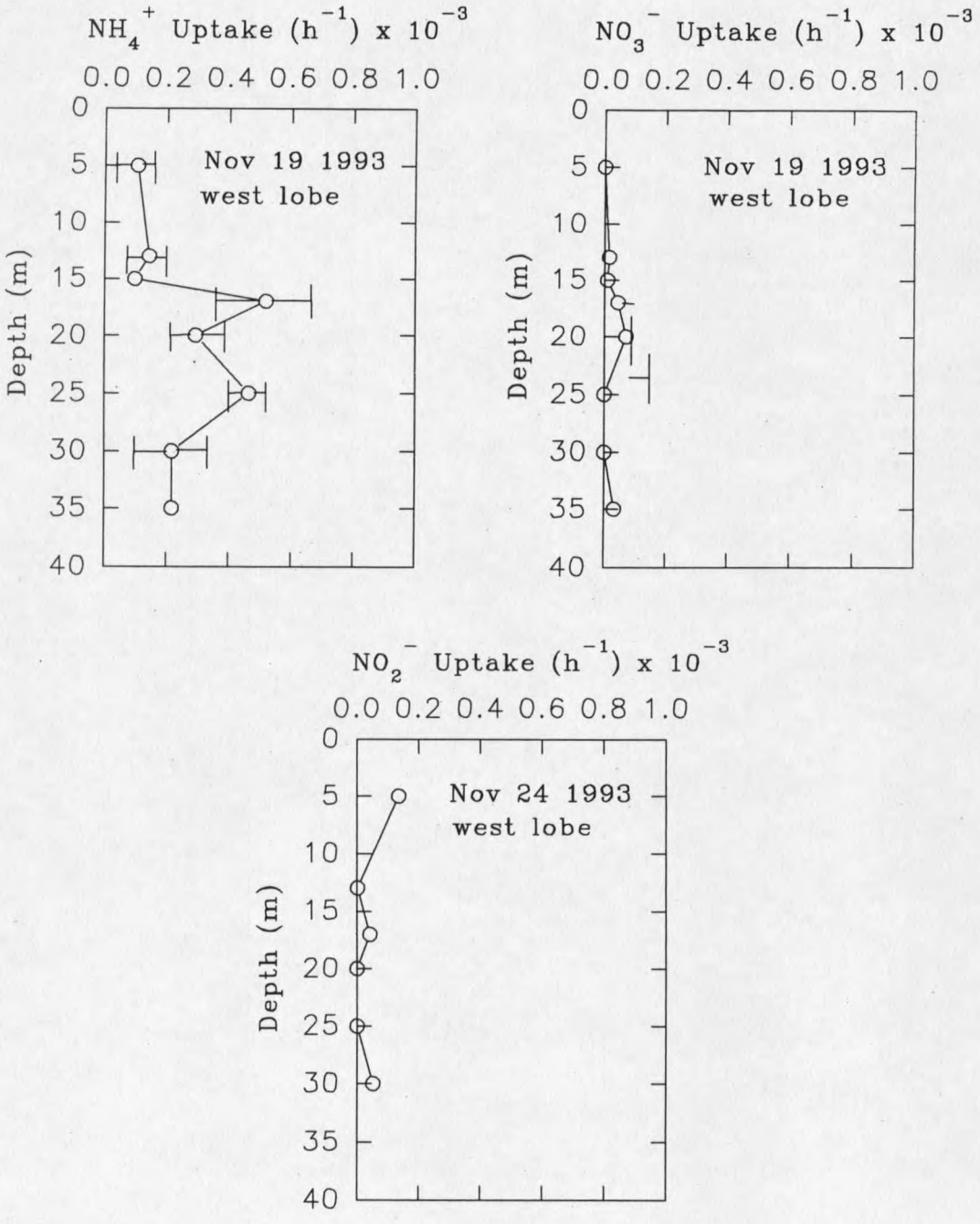

Figure 12 , cont. Uptake of dissolved inorganic nitrogen throughout the water column, 1993 season. Error bars indicate range of uptake measurements at each depth. Note some depths are described by a single datum point.

should be interpreted with caution. In each of the 5 m west lobe and 13 m west lobe time course experiments, uptake rates quickly declined from maximal to below detection. In each case, the ^{15}N atom-percent of particulates actually decreased during the incubation, a phenomenon which has no simple biochemical explanation. As for 13 m east lobe, the decline in the rate of NO_3^- uptake over time appears to be significant. NO_3^- uptake at 5 m east lobe displayed a slow response to nutrient addition as uptake increased with time.

Goldman and Glibert (1982) note that substrate depletion can lead to underestimates of V_{max} measured over a time period greater than surge, particularly if the pool size is small and initial uptake is rapid. Because time course experiments suggested initial rapid uptake of NH_4^+ at 5 m in both lobes, where $[NH_4^+]$ is generally less than 1 μM, there appeared to be potential for substrate depletion. Disregarding 5 min uptake rates, the maximum NH_4^+ uptake rate sustainable on an hourly scale at 5 m was 0.003 μM h^{-1}, measured after 5 h of incubation (east lobe time course, see below). If this rate remained constant, it would take approximately 85 h to exhaust an initial NH_4^+ pool of 0.50 μM. Because the uptake experiments reported here were incubated for approximately 24 h and all initial NH_4^+ pools exceed 0.50 μM, uptake rates reported here were not corrected for substrate depletion.

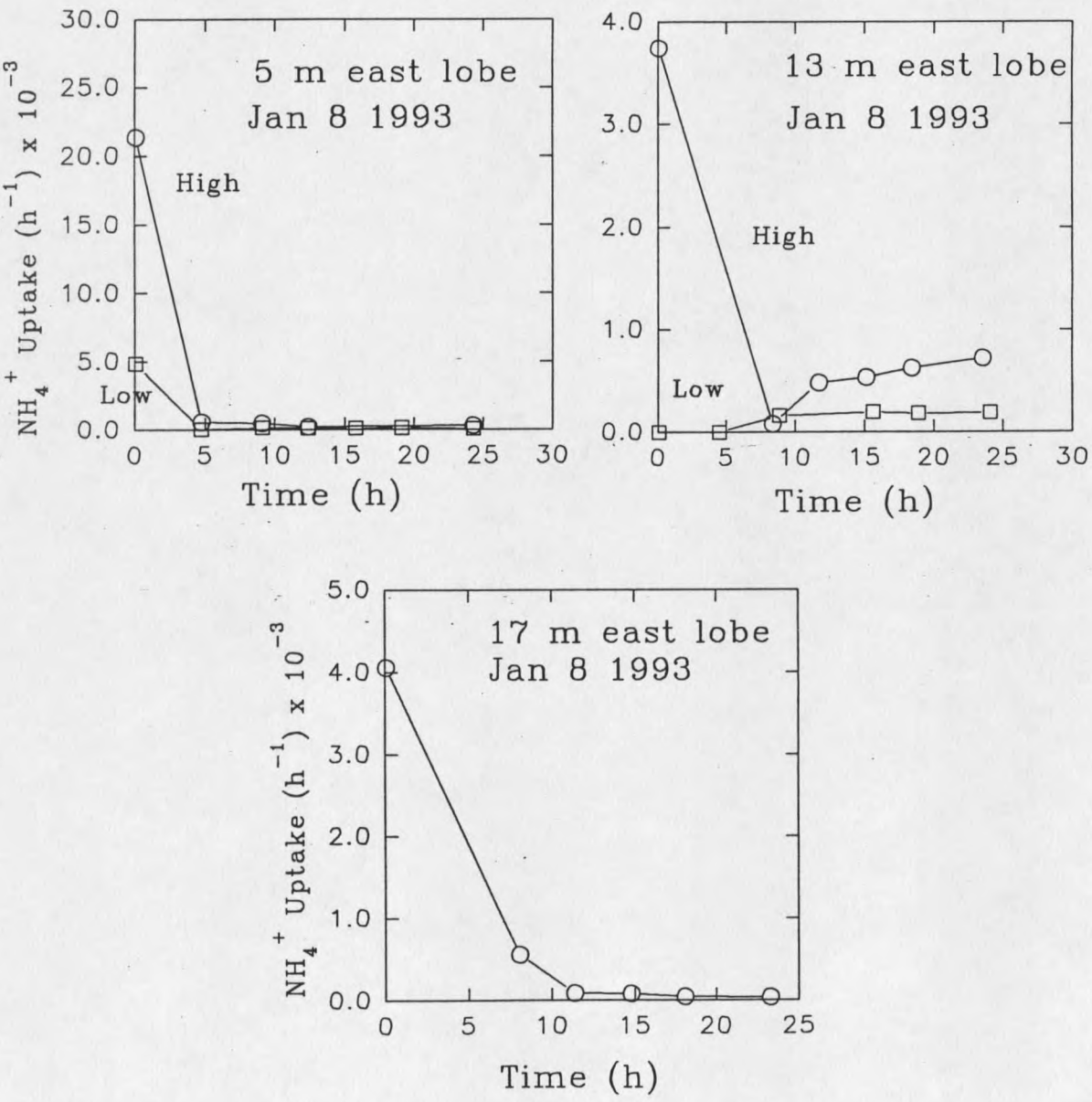

Figure 13. Time course of NH_4^+ uptake (h^{-1}), 1992 season.

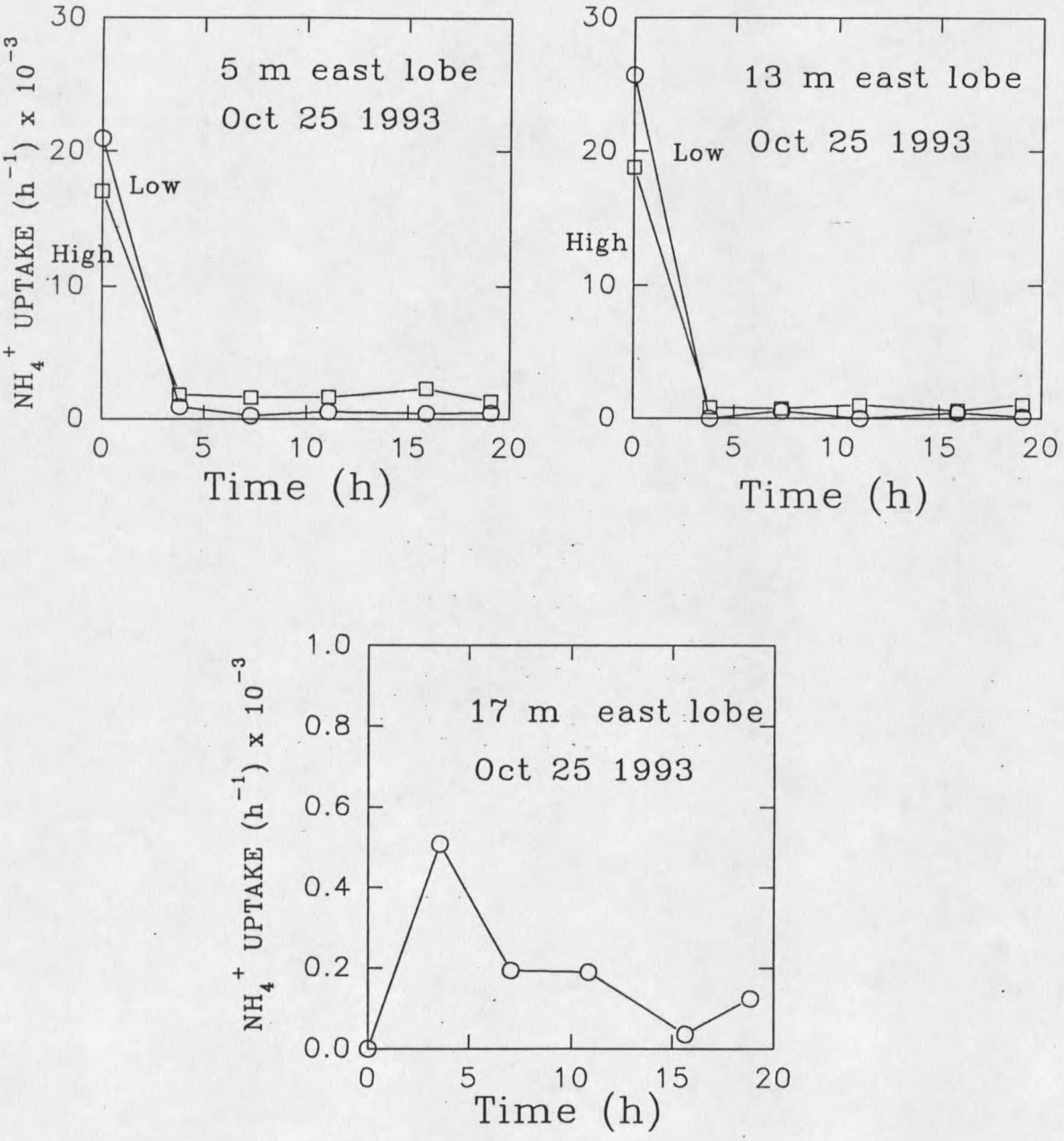

Figure 13, cont . Time course of NH_4^+ uptake, east lobe 1993. Uptake at 5 m and 13 m was measured for both high and low enrichments.

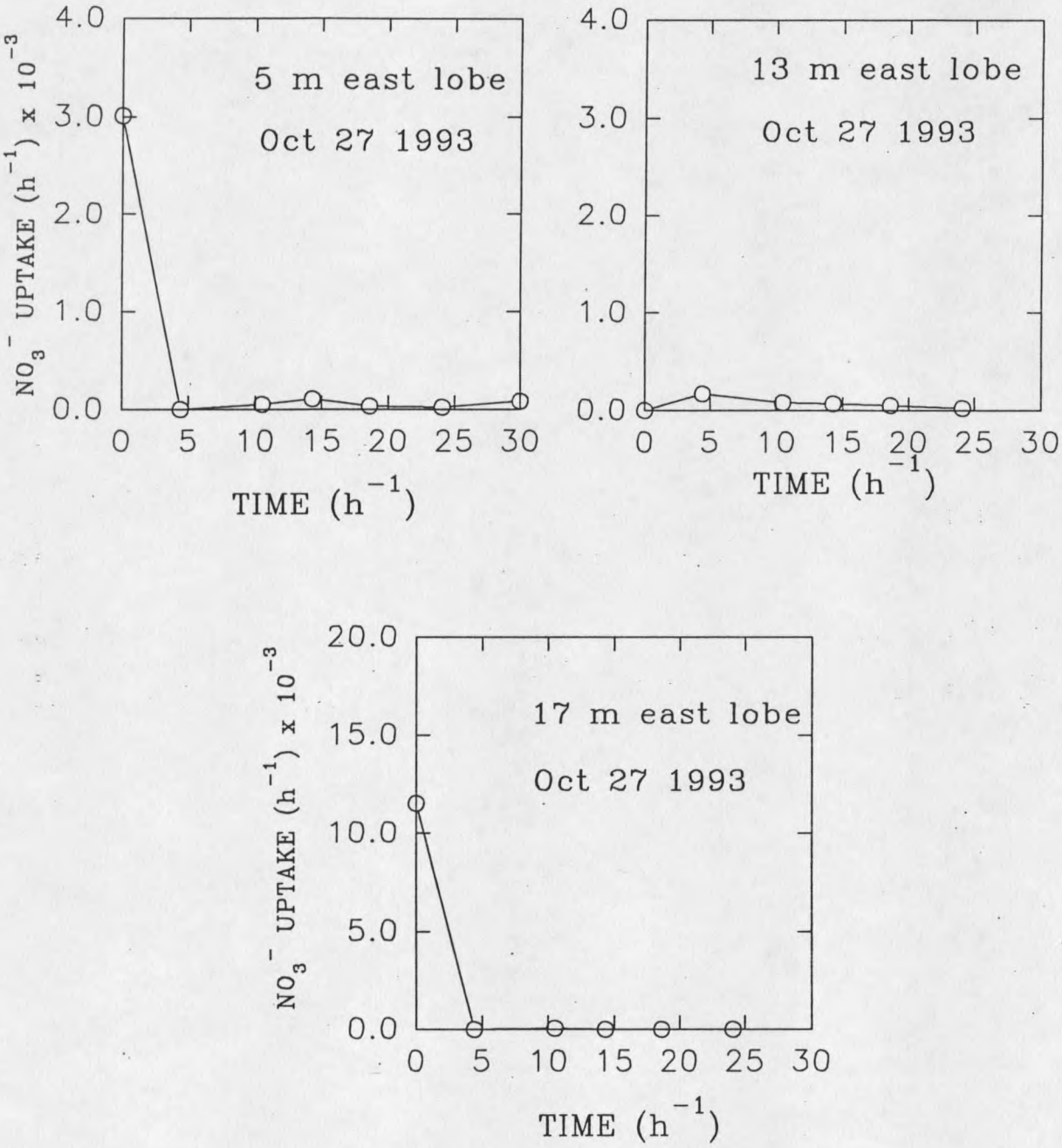

Figure 13, continued . Time course of NO_3^- uptake, east lobe 1993.

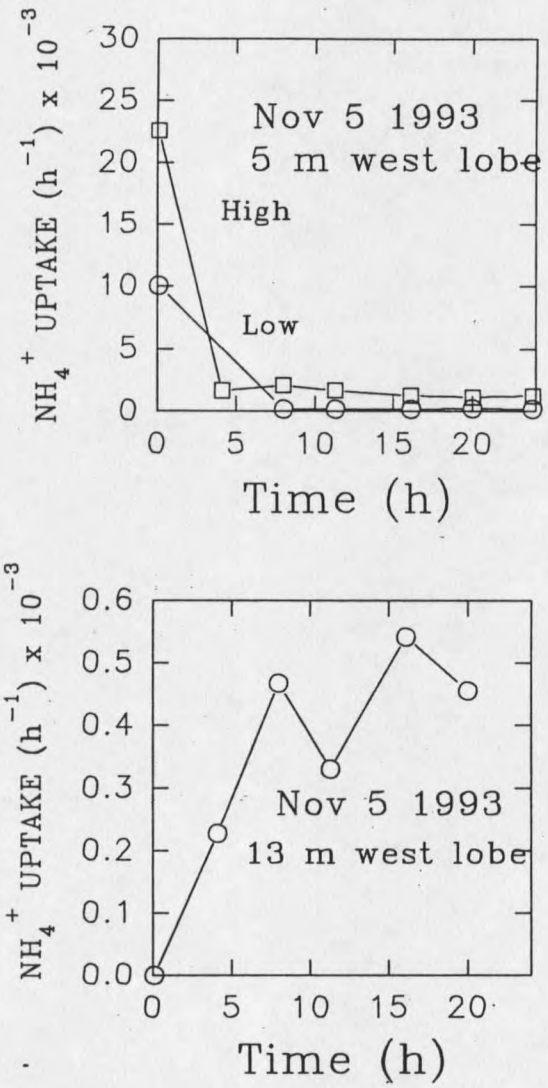

Figure 13, cont . Time course of NH_4^+ uptake, west lobe 1993. Uptake at 5 m and was measured for both high and low enrichments.

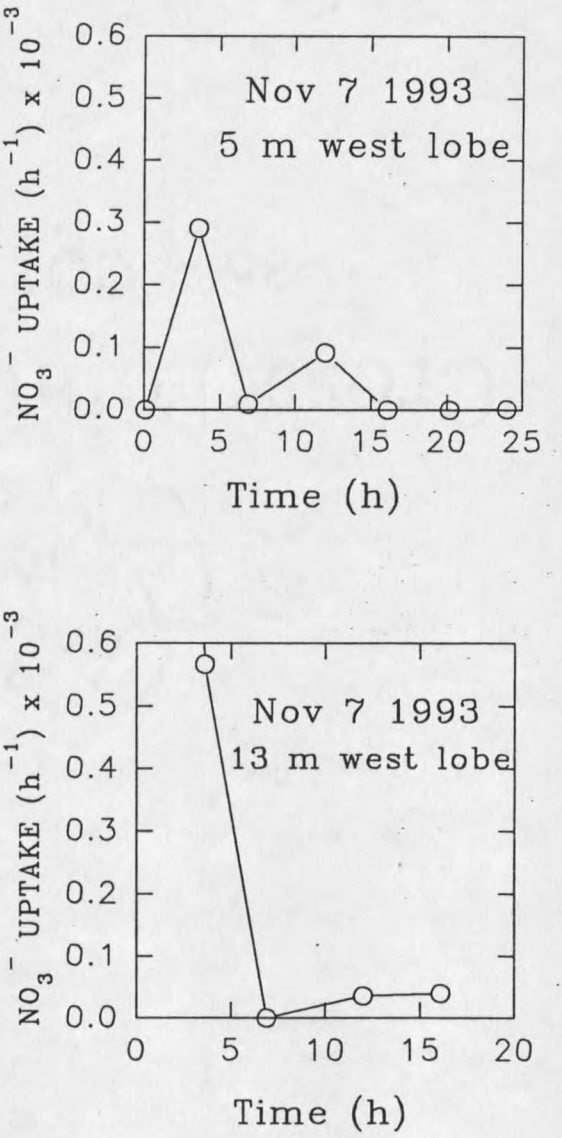

Figure 13, cont . Time course of NO_3^- uptake, west lobe 1993.

DISCUSSION

Regeneration

Results from most regeneration experiments were inconsistent. However, there was a general trend for increasing regeneration with depth. Regeneration appeared to be lowest at 5 m west lobe. Interestingly, the planktonic community at this depth displayed the lowest K_S and the highest V_{max} for NH_4^+ uptake (see Part II, Results, Substrate Kinetics).

Increases in regeneration with depth could result from a number of factors including decreased C:N ratios of organic substrates and increased bacterial activity with depth. Unfortunately, the dynamics of of DON and DOC in Lake Bonney have yet to be fully examined.

Results from the NH_4^+ production from serine experiment indicate that free amino acids below 13 m in the east lobe were quickly consumed, presumably as a carbon source, which results in the production of NH_4^+. The fact that removal of phytoplankton had no discernible effect on regeneration provides further evidence that bacterial deamination of DON is the primary source of regenerated NH_4^+. Further investigation of the dynamics of DON and DOC is needed before the exact role of dissolved organic substrates on regeneration can be determined.

Substrate Kinetics

Substrate kinetics data show that uptake of DIN in a majority of experiments was substrate saturated. Only uptake of NH_4^+ in the upper (5 m in each lobe) and middle (13 m in each lobe) trophogenic zones was significantly enhanced by increased concentrations of substrate. Laboratory-studies with cultured microalgae in steady state growth indicate that the uptake of NO_3^- is often saturated by NO_3^- concentrations of approximately 5 μM (Dugdale and Wilkerson 1992). This is consistent with the finding that NO_3^- uptake in Lake Bonney, where surface NO_3^- concentrations typically exceed 5 μM, cannot be described by Michaelis-Menten kinetics.

V_{max} of DIN uptake is an indicator of the physiological state of the cell; it increases with increasing nitrogen deficiency, given constant temperature and light (Goldman and Glibert 1982, Harrison et al. 1989). V_{max} was highest at 5 m west lobe. V_{max} of NH_4^+ uptake did not change from 5 m east lobe to 13 m east lobe, indicating that, despite its proximity to the chemocline, the planktonic community at 13 m east lobe was not significantly more nitrogen replete than the 5 m east lobe community. This apparent contradiction can be partially explained by the relatively low affinity (large K_s) for NH_4^+ at 13 m in the east lobe, which is discussed in detail below.

V_{max} as reported in this study does not represent the absolute maximum physiological potential for DIN uptake. Time course of DIN uptake experiments showed that, in most cases, specific uptake of DIN at 24 h is lower than specific uptake at 5 min. Also, because inorganic nitrogen uptake can be limited in a low irradiance environment (see Priscu 1989, Priscu et al. 1991, Part III of this report), V_{max} at in situ irradiance may not represent the absolute maximum uptake rate.

K_S is an indicator of the nutritional status of a phytoplankton community. Phytoplankton in nitrogen replete environments often exhibit higher K_S values than identical species growing in nitrogen depleted environments (Owens and Esaias, 1976). Since K_S is independent of CHL concentration and detritus, it is a sensitive measure of a community's response to DIN (Murphy, 1980). K_S for NH_4^+ uptake is lowest at 5 m west lobe. As previously mentioned, the lowest measured NH_4^+ regeneration rates and the highest V_{max} of NH_4^+ uptake also occurred at this depth. Elevated NH_4^+ uptake rates in response to increases in NH_4^+ concentration and high affinity for NH_4^+ may therefore allow phytoplankton at 5 m west lobe to compensate for lower NH_4^+ supply rates.

The decrease in affinity for NH_4^+ with depth in each lobe has two potential explanations. Firstly, the high concentrations of chloride salts (particularly NaCl) in the deep waters of Lake Bonney could decrease the stability of glutamine synthetase, an enzyme

necessary for assimilation of inorganic nitrogen. The interference of salinity with glutamine synthetase has been shown to decrease the affinity for NH_4^+ in halophytic angiosperms (Ahmad et al. 1982). Secondly, high ambient concentrations of DIN could allow phytoplankton in the deep waters of Lake Bonney to satisfy nutritional requirements without high affinity for nutrients. Owing to increases in regeneration rates with depth and upward diffusive flux of nutrients from the chemocline, the supply rates of DIN are greater at 13 m than at 5 m.

Comparisons of diffusion rates and in situ uptake rates (Table 13) indicate that regeneration is the predominant source of NH_4^+ at 5 m and 13 m east lobe and occasionally at 13 m west lobe. In contrast, "regenerated" NO_3^-, produced by nitrification, is apparently not a significant source of DIN at these depths.

Water Column Uptake

Uptake of NH_4^+ exceeded uptake of NO_2^- throughout the water column of each lobe during each season. Uptake of NH_4^+ also generally exceeded NO_3^- uptake, although higher rates of NO_3^- uptake were measured at 25 and 35 m in the east lobe (1993). In planktonic communities with actively photosynthesizing phytoplankton (5 m to 17 m in each lobe), the ratio of measured NH_4^+ uptake (h^{-1}) to total DIN uptake (h^{-1}) ranged from 0.50 (5 m west lobe 1993) to 0.80 (5 m east lobe). At the time of the 1993 west lobe NH_4^+ uptake experiment, NH_4^+ was undetectable at 5 and

13 m; therefore in situ NH_4^+ uptake rates at these depths might have been insignificant. In contrast, in situ uptake of NH_4^+ at 5 and 13 m east lobe 1993 (where NH_4^+ concentration was 0.68 and 1.49 μM respectively) exceeded the combined uptake of NO_2^- and NO_3^-. Although Lake Bonney phytoplankton preferentially utilized NH_4^+ when it was available, periodic scarcity of NH_4^+ increased the relative importance of NO_3^- and NO_2^- uptake in the shallow waters of the west lobe.

The results of this study are consistent with previous studies that indicated a strong preference for NH_4^+ by phytoplankton in perennially ice-covered Antarctic lakes (Priscu et al. 1989) and in off-shore Antarctic waters (Koike et al. 1986). Uptake rates of NH_4^+ provide indirect evidence that regeneration in large part supports phytoplankton protein synthesis, and ultimately productivity, throughout the trophogenic zone (Goldman and Glibert 1982, Priscu et al. 1989).

Phytoplankton protein synthesis at 5 m west lobe, where NH_4^+ regeneration is apparently lowest, may occasionally be primarily supported by NO_2^- and NO_3^- uptake. It is unclear if the relatively low supply rates of NH_4^+ translate into lower growth rates for this community. Levasseur et al. (1993) estimated that marine algal assimilation of nitrogen and carbon at subsaturating irradiance

requires 50% more energy when the algae are grown on NO_3^- compared to NH_4^+. Interestingly, despite the unfavorable energetics of NO_3^- assimilation, growth rates of marine algae at subsaturating irradiance were similar when grown on NO_3^- or NH_4^+ (Levasseur 1993). Although some investigators report declining growth rates associated with NO_3^- assimilation (Rhee and Lederman, 1983), phytoplankton are apparently capable of growing on NO_3^- through physiological adaptations other than reduction of growth rates. In conclusion, phytoplankton growth rates in the shallow waters of the west lobe are not necessarily impaired by the low in situ uptake rates of NH_4^+.

There were generally two peaks of NH_4^+ uptake in the water column of each lobe: at 5 m and within the first chemocline (between 10 and 17 m). Peaks of NH_4^+ uptake within the chemocline correspond roughly with peaks of CHL and primary productivity (see Figure 2).

In contrast, maximum levels of NO_3^- and NO_2^- uptake occurred at 5 m (with the exception of east lobe NO_3^- uptake 1993), where NH_4^+ concentration was lowest. It is well established that the uptake of NO_3^-, the nutrient most associated with "new" production, is partially regulated by the abundance of NH_4^+. However, the simultaneous uptake of different forms of nitrogen pose difficulties

in calculation of uptake. Collos (1987) suggests that reports of NH_4^+ repression of NO_3^- uptake may be artefacts of calculation. Specifically, in experiments where more than one nitrogenous substrate is present and particulate nitrogen changes significantly over the course of incubation, dilution of the particulate ^{15}N label could result from the uptake of unlabeled nitrogen (Collos 1987). The true relationship between NH_4^+ and NO_3^- uptake could best be observed in experiments where particulate nitrogen does not change significantly during the incubation.

Data from the 1993 water column uptake indicate combined uptake of NH_4^+, NO_3^-, NO_2^- at 5 m in the east lobe (≈ 0.0025 μM h^{-1}) would lead to a 0.07% increase in PN (3.77 μM at time zero) over a 24 h incubation. For comparison, the increase in PN at 17 m for the same experiment would be approximately 0.20%. Therefore, calculation of DIN uptake should not be affected by changes in PN over 24 h of incubation.

Time Course

Some researchers have suggested that the potential for short term elevated ("surge") uptake rates of NH_4^+ by phytoplankton could be a physiological adaptation to micropatches of NH_4^+ produced by zooplankton excretion (McCarthy and Goldman 1979, Glibert and Goldman 1981, Goldman and Glibert 1982, Priscu 1987). Although the distribution of nutrients in Lake Bonney on a microscale basis has not been determined, the absence of zooplankton should

decrease the potential for significant micropatches. Rapid short term uptake of NH_4^+ in Lake Bonney could therefore indicate that this strategy may have competitive value beyond utilization of micropatches (See Currie 1984). Rapid initial uptake of NO_3^-, a nutrient which is often assumed to not persist in micropatches (Dortch et al. 1982, Priscu et al. 1989), provides further evidence that rapid uptake of DIN may not necessarily be a response to a patchy environment.

An alternative explanation is that hydraulic stability allows for persistence of micropatches of DIN produced by microbial excretion. Although nutrient micropatches are generally associated with zooplankton excretion and not bacterial remineralization (McCarthy and Goldman 1979, Goldman and Glibert 1982, Suttle and Harrison 1988), NH_4^+ regenerated by microplankton in Lake Bonney might persist longer than in other, more turbulent, systems (see Currie 1984, Goldman and Glibert 1982, Priscu et al. 1989). These patches could, in turn, be converted to NO_2^- and NO_3^- by nitrification (Priscu et al. 1989). If microscale patches of DIN do persist in Lake Bonney, short term rapid uptake of DIN by the planktonic community may be an adaptation for utilization of micropatches.

Rapid NH_4^+ uptake is generally associated with systems where productivity is limited by nitrogen (Glibert and Goldman 1981, Dortch et al. 1982, Suttle and Harrison 1988), but some phytoplankton are capable of rapid NH_4^+ uptake even when growth rates are near maximal (Goldman and Glibert 1982). Short-term

rapid uptake of NO_3^- is also an ambiguous indicator of nitrogen deficiency (Priscu 1987). Dortch et al. (1982) reported that several species of marine phytoplankton exhibited a decrease in NO_3^- uptake capability following nitrogen deprivation. Conversely, Priscu (1987) measured rapid short term NO_3^- uptake by phytoplankton in a nitrogen deficient lake.

Uptake of NO_3^- in Lake Bonney was extremely rapid after 5 min at 5 m east lobe, 17 m east lobe and 13 m west lobe relative to rates at 1 h. The ^{15}N atom-percent of particulates after 5 min for 17 m east lobe NO_3^- uptake is within 1 standard deviation of the estimate for abiotic ^{15}N atom-percent enrichment of the filter; elevated NO_3^- uptake rates at time zero at 17 m east lobe may therefore be an experimental artefact. Further investigation of persistence of micropatches of NO_3^- in a non-turbulent environment is necessary to interpret the ecological significance of rapid short term uptake of NO_3^-.

CONCLUSION AND AREAS OF FURTHER RESEARCH

The planktonic communities of Lake Bonney show a strong preference for NH_4^+ as a source of inorganic nitrogen. It can therefore be concluded that regeneration of NH_4^+ primarily fuels productivity, a conclusion supported by comparisons of NH_4^+ diffusion rates and uptake rates in the trophogenic zone. In areas where regeneration is low, e.g. 5 m west lobe, phytoplankton have adapted/acclimated to maximize utilization of NH_4^+ through high affinity for NH_4^+, surge uptake of NH_4^+ pulses, and high sustained NH_4^+ uptake rates.

It is evident that NH_4^+ regeneration is an important ecological process in Lake Bonney, but the rates and sources have not been adequately determined by this study. Any further investigation into nitrogen transformations in the water column of Lake Bonney should include an attempt to clarify this issue.

Although uptake rates of NH_4^+ were not substrate saturated in the shallow water of either lobe, uptake kinetics of NO_3^- and NO_2^- indicate that productivity is generally not nitrogen limited. The apparent contradiction between this conclusion and particulate C:N ratios, which indicate nitrogen deficiency, may be partially explained through analysis of the control of irradiance on nutrient uptake (See Part III). It is possible that the uptake response of inorganic carbon

and inorganic nitrogen to irradiance could create diel and seasonal periodicity of C:N uptake ratios even when DIN is available in saturating amounts.

Several previous researchers had concluded that productivity and phytoplankton growth during the latter part of the summer was limited by nutrient supply in Lake Bonney (Lizotte and Priscu 1993, Sharp 1993). If phytoplankton are indeed not limited by nitrogen supply, it is likely that phosphorus limits productivity. Transformations of phosphorus should be studied for a thorough understanding of controls of productivity in Lake Bonney.

PART III

Irradiance Requirements of DIN Uptake

INTRODUCTION

Phytoplankton cellular growth rate, and thus primary productivity, is a function of light, temperature, and nutrient supply (Hecky and Kilham 1988). These parameters are not entirely separable. For example, phytoplankton nitrogen assimilation into protein in nitrogen replete systems utilizes the energy and the carbon skeletons produced by photosynthesis and has been shown to be directly correlated with irradiance (Turpin 1991, Kanda et al. 1989). Uptake of inorganic nitrogen (in this study, "uptake" refers to transport plus assimilation) is also often strongly correlated with irradiance, although the mechanism for this remains unclear (Priscu 1989, Cochlan et al. 1990).

The ratio of uptake rates of carbon and nitrogen have frequently been used as a measure of nutritional status of phytoplankton (DiTullio and Laws 1986, Priscu et al 1989). However, nitrogen uptake and carbon uptake can be differentially dependent on irradiance. Photosynthesis is a first order process with respect to irradiance; nitrogen uptake is a second order process that utilizes photochemical energy as well as energy derived from intermediary metabolism (Priscu et al. 1991, Miyazaki et al. 1987). As a result, nitrogen uptake versus irradiance (NI) curves can differ significantly from photosynthesis versus irradiance (PI) curves in the same communities. Specifically, inorganic nitrogen uptake in the dark can be significant, whereas biosynthetic dark inorganic carbon uptake is negligible for most phytoplankton. Priscu et al. (1991) demonstrated that dissimilarities in the response of carbon and

nitrogen uptake to irradiance can lead to strong diel periodicity of carbon and nitrogen uptake ratios (see also Miyazaki et al. 1987). Thus, the effects of irradiance on nitrogen uptake must be known for determination of cellular carbon and nitrogen uptake ratios, and, ultimately, phytoplankton growth.

Although nitrogen and carbon uptake do not respond identically to irradiance, the response of each process to irradiance can provide an indication of adaptation (long-term evolutionary response) and acclimation (short-term physiological response) to ambient irradiance. Both NI and PI curves provide insight into aspects of phytoplankton physiology including the efficiency of light utilization, the ability to quickly maximize uptake under optimal conditions, and the ability to sustain uptake under limiting conditions.

Controversy currently exists regarding the general effect of irradiance on nitrogen uptake in polar aquatic systems. Previous studies indicate nitrogen uptake is generally less dependent on irradiance in polar systems than in temperate or tropical systems (Smith and Harrison 1991). However, Muggli and Smith (1993) noted that irradiance was the most important factor regulating NH_4^+ uptake in the Greenland Sea. In addition, Kanda et al. (1989) reported a strong relationship between photosynthesis and NO_3^- uptake in Auk Bay, Alaska. Apparently, the degree to which nitrogen uptake depends on irradiance is a function of nutrient supplies, the stability of the light field, and the physiological state of the phytoplankton (Smith and Harrison 1991, Kanda et al. 1989).

This study investigates inorganic nitrogen uptake in response to irradiance by planktonic communities in stable, low light environments. It consists of measurements of eight different planktonic communities (sampled at 4 depths in each lobe of Lake Bonney) over two years. Lake Bonney's gradients of nutrients, irradiance, and phytoplankton will allow for comparison of NI responses in markedly different environments. Variability of NI curves will clarify the interactive roles of nutrient supply and irradiance in regulation of inorganic nitrogen uptake.

Hypotheses

This study investigated the following hypotheses:

1. Nitrogen uptake is extremely "shade adapted," i.e., quickly saturated with respect to irradiance, particularly in nitrogen depleted surface waters.
2. Photoinhibition of nitrogen uptake, when present, occurs at an irradiance substantially above ambient levels.
3. Compared to NH_4^+ uptake, NO_3^- uptake is more dependent on irradiance, i.e. uptake of NO_3^- in the dark will be a lower proportion of maximum uptake.

METHODS

Experimental Procedure

As with substrate kinetics, water for the nitrogen uptake versus irradiance experiments was collected in a 20 liter carboy and transferred to a series of 1 liter polycarbonate bottles. Additional water was reserved for analysis of DIN, CHN, and CHL. The procedure for collection and measurement of DIN, CHN, and CHL was identical to methods described in Part I. Each bottle in a given experiment received identical enrichments of $^{15}NH_4Cl$, $Na^{15}NO_3$, or $Na^{15}NO_2$. Uptake of NO_2^- was not analyzed at 20 m west lobe, 25 m east lobe, or 25 m west lobe during the 1992 season. Uptake of NO_3^- was not analyzed at 25 m west lobe during the 1992 season.

After inoculation, bottles were placed in sleeves of neutral density screening. Variations in thickness and color (either black, gray or white) of the sleeves created a range of light transmittance. Three bottles for each experiment were not placed in sleeves: one clear bottle, one dark bottle (wrapped in aluminum foil and several layers of duct tape), and the kill bottle.

The bottles were incubated in a temperature and light controlled incubator for approximately 24 h. Calibration tests with a Li-Cor 4π quantum sensor attached to a Li-Cor model LI-1000 data logger indicated that irradiance was not consistent throughout the incubator. Irradiance measurements have been adjusted for each bottle's position within the incubator. Irradiance throughout the

incubation period was recorded by a Li-Cor 4π quantum sensor attached to a Li-Cor model LI-1000 data logger. Wide fluctuations in irradiance occurred during 1992 incubations owing to fluctuations in our field generator's power output. For 1992 experiments, nitrogen uptake is compared to the average irradiance during the incubation. An uninterrupted power supply unit eliminated this problem for 1993 experiments.

Samples were incubated for approximately 24 h then filtered onto precombusted 47 mm Whatman GF/F glass fiber filters under low vacuum (<0.6 atm). After filtration, filters were rinsed with approximately 20 ml DIW to remove inorganic nitrogen. Filters were air dried in aluminum weigh-boats for several days before being transported to Crary Laboratory where filters were frozen (-45° C) before transport to MSU for final analysis.

Calculations

Uptake rates were calculated as described in Part I. Data which approximated a hyperbolic curve with no evidence of photoinhibition were fitted to the following equation (Platt et al. 1980) :

$$V = V_m * \tanh\left(\frac{\alpha I}{V_m}\right) + D \quad (12)$$

Where V= specific uptake rate (h^{-1})

α = the slope of the light limited portion of the curve (h^{-1} (μmole^{-1} m^{-2} s^{-1})$^{-1}$)

I = irradiance (μmole m^{-2} s^{-1})

D = specific uptake of the substrate (h^{-1}) when I=0

V_m = rate of light saturated uptake (h^{-1}). Note that D, when present, must be added to this term.

Data that exhibited a hyperbolic increase coupled with photoinhibition were modeled with the following Equation (Platt et al. 1980):

$$V = V_S (1 - e^{-a}) e^{-b} + D \quad (13)$$

where V_S = maximum DIN uptake (h^{-1}) in the absence of photoinhibition.

$a = \frac{\alpha I}{V_S}$

$b = \frac{\beta I}{V_S}$, where β = the slope of the photoinhibited portion of the curve

V_m of experiments described by Equation 13 was determined with the following equation:-

$$V_m = V_S * (\alpha/\alpha + \beta) * (\beta/\alpha + \beta)^{\beta/\alpha} \quad (14)$$

Where all terms are defined as in Equations 12 and 13.

Linear fits were applied to data that did not conform to either

Equation 12 or 13. V_m of linear models was determined to be the maximum y-value of the regression within the experimental range of irradiance.

RESULTS

NH_4^+ uptake at 5 m and 13 m in both lobes could almost always be described by either Equations 12 or 13 (Figure 14). The exception was the 5 m east lobe sample (1992) where uptake increased approximately linearly with irradiance. NH_4^+ and NO_3^- uptake at 17 m east lobe (1993) could be modeled by Equation 12. NO_2^- uptake at 5 m east lobe (1993) and 5 m west lobe (1993) could also be approximated by Equation 12, but the data are relatively scattered, possibly due to the error inherent in measurement of such low uptake rates. All other data sets were fit linearly with a least squares regression. Reported standard deviations for all data sets are equivalent to the standard error of the estimate as determined by Marquardt's algorithm.

Results from both seasons indicate a strong positive correlation between NO_3^- and NO_2^- uptake and irradiance at 5 m in both lobes. Weaker positive correlations between NO_2^- uptake and irradiance occurred at 17 m east lobe (both seasons). NO_3^- uptake was weakly correlated with irradiance at 13 m east lobe (1992), 13 m west lobe (both seasons), and 20 m west lobe (1992). Slopes of linear regressions of NH_4^+ uptake were significantly positive at 5 m east lobe (1992), 20 m west lobe (1992) and 25 m west lobe (1993). Only one experiment could be modeled with a significantly negative slope : NH_4^+ 25 m east lobe (1993).

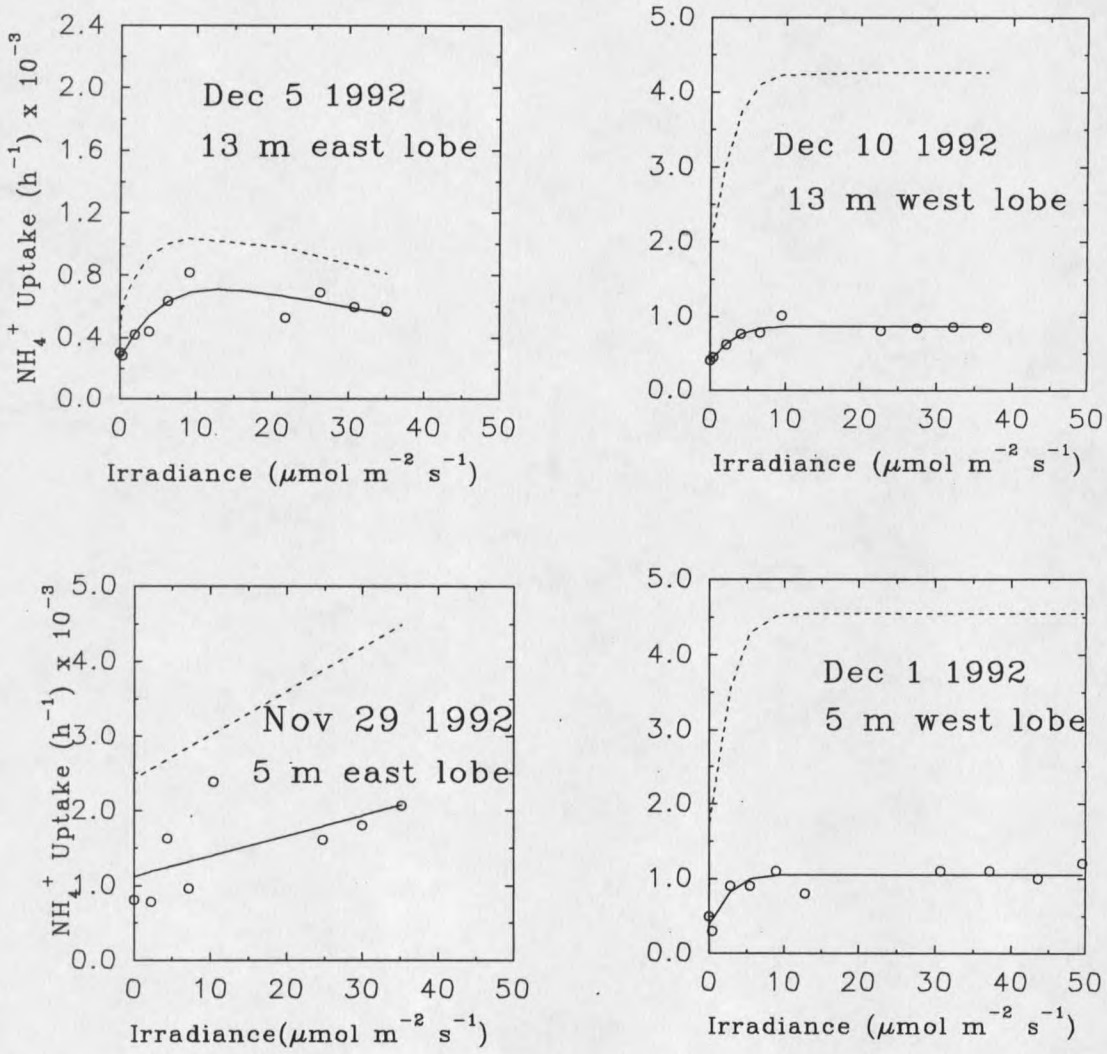

Figure 14 . NH_4^+ uptake vs irradiance,1992. Data from 13 m east lobe fitted with inhibition equation and data from 5 m east lobe fitted with linear regression. Others fitted with hyperbolic tangent equation. Dashed line describes function normalized for chlorophyll a concentration (μM N μg CHL l^{-1} h^{-1}).

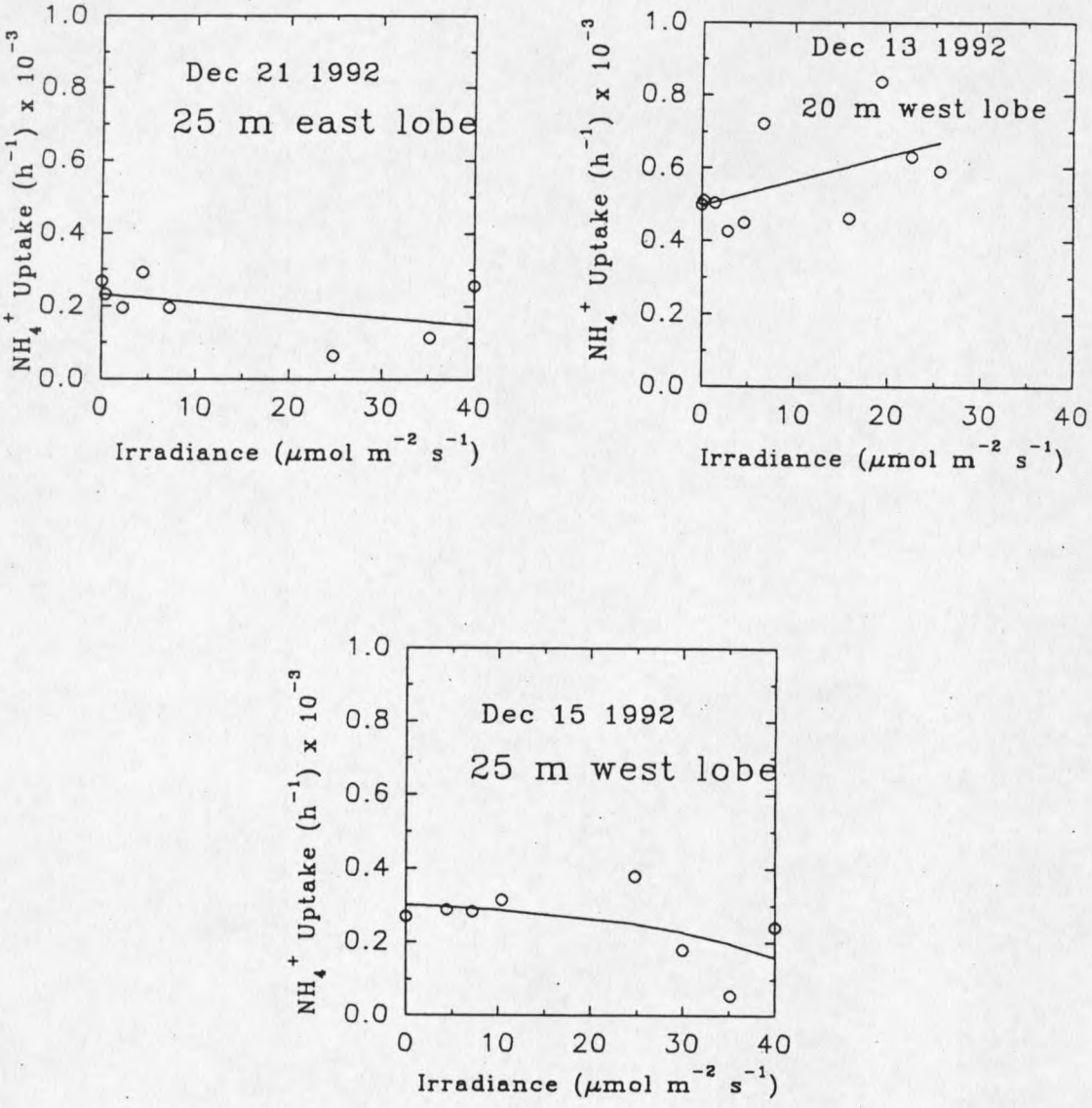

Figure 14, cont . NH_4^+ uptake vs irradiance, 1992. Data from 25 m west lobe fitted with inhibition equation, others fitted with linear regression.

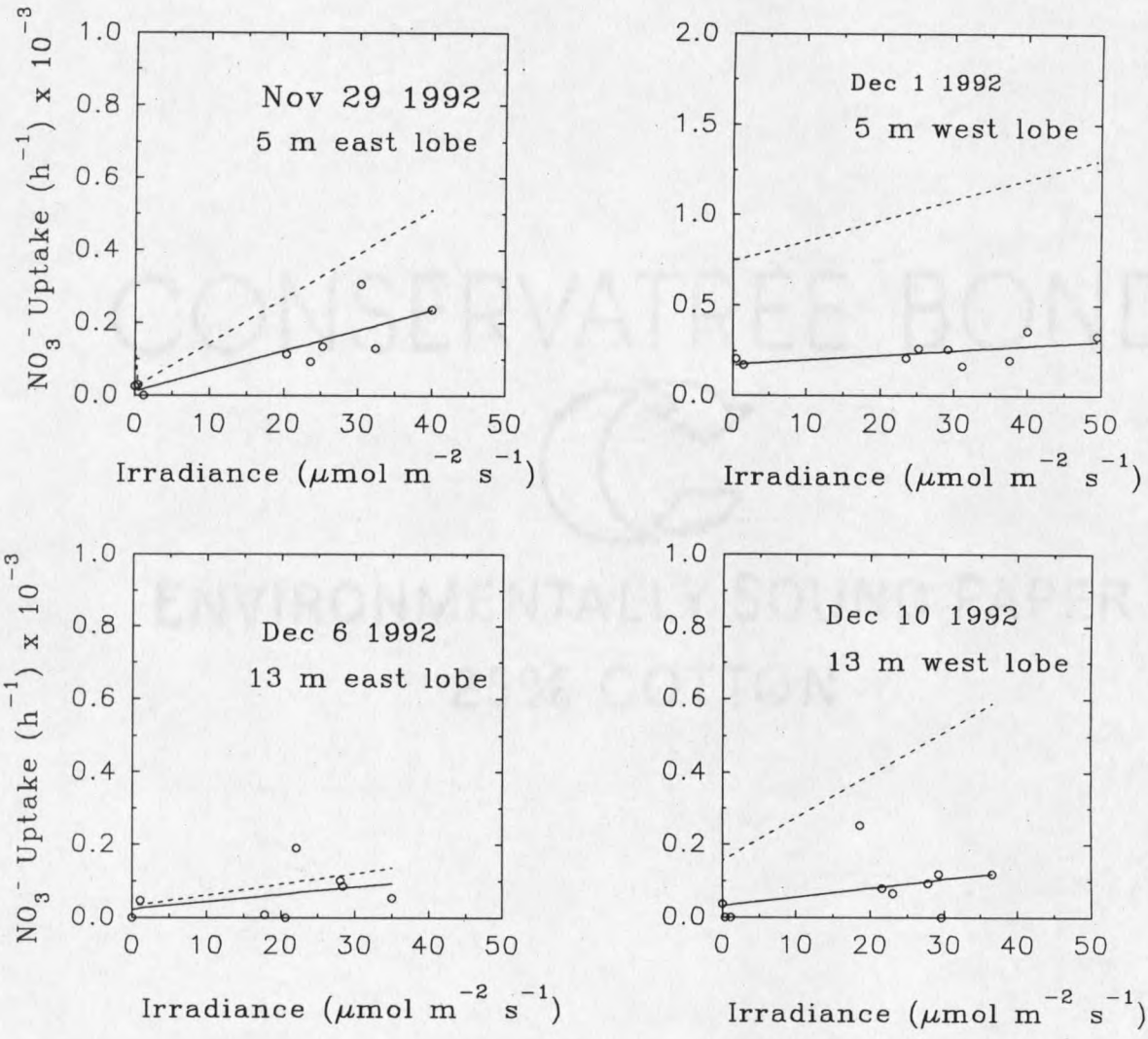

Figure 14, cont. NO_3^- uptake vs irradiance, 1992. Data fitted with linear regression. Dashed line describes function normalized for chlorophyll a concentration ($\mu M\ N\ \mu g\ CHL\ l^{-1}\ h^{-1}$).

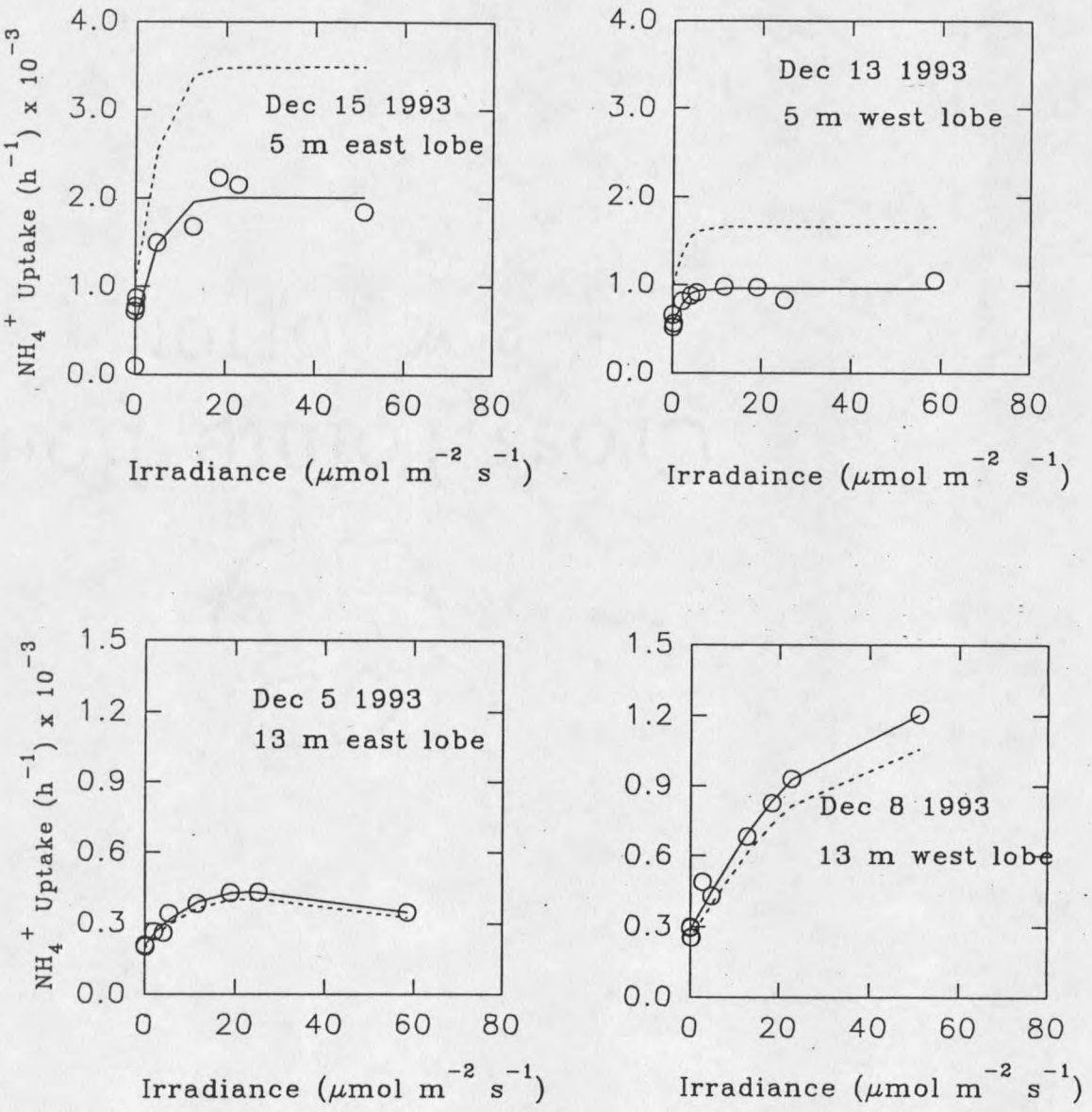

Figure 14, cont . NH_4^+ uptake vs irradiance, 1993. Data from 13 m east lobe fitted with inhibition equation; others fitted with hyperbolic tangent equation. Dashed line describes function normalized for chlorophyll a concentration (μM N μg CHL l^{-1} h^{-1}).

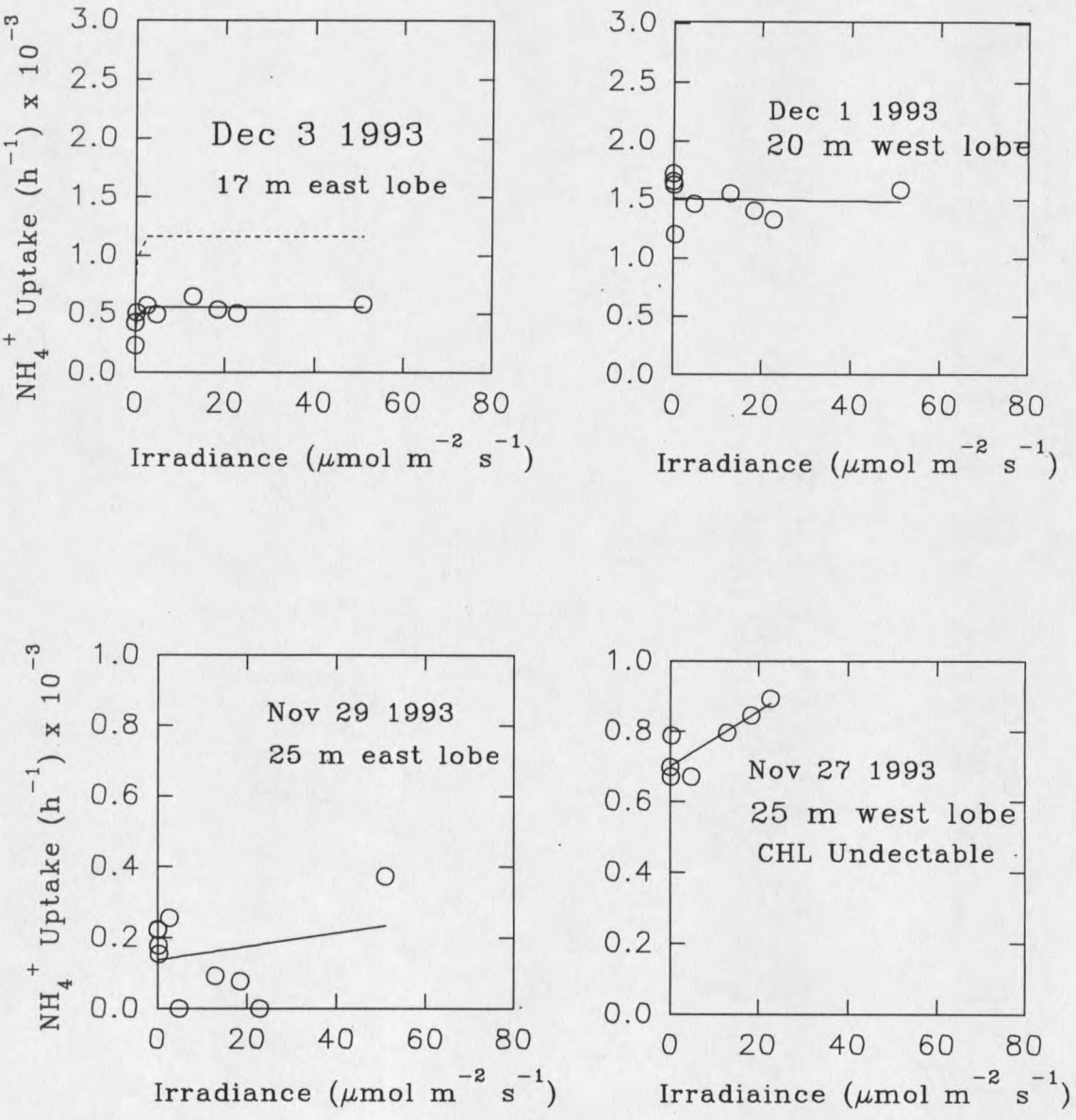

Figure 14, cont. NH_4^+ uptake vs irradiance, 1993. Data from 17 m east lobe fitted with hyperbolic tangent equations. Others fitted with linear regressions. Dashed line describes function normalized for chlorophyll a concentration (μM N μg CHL l^{-1} h^{-1}).

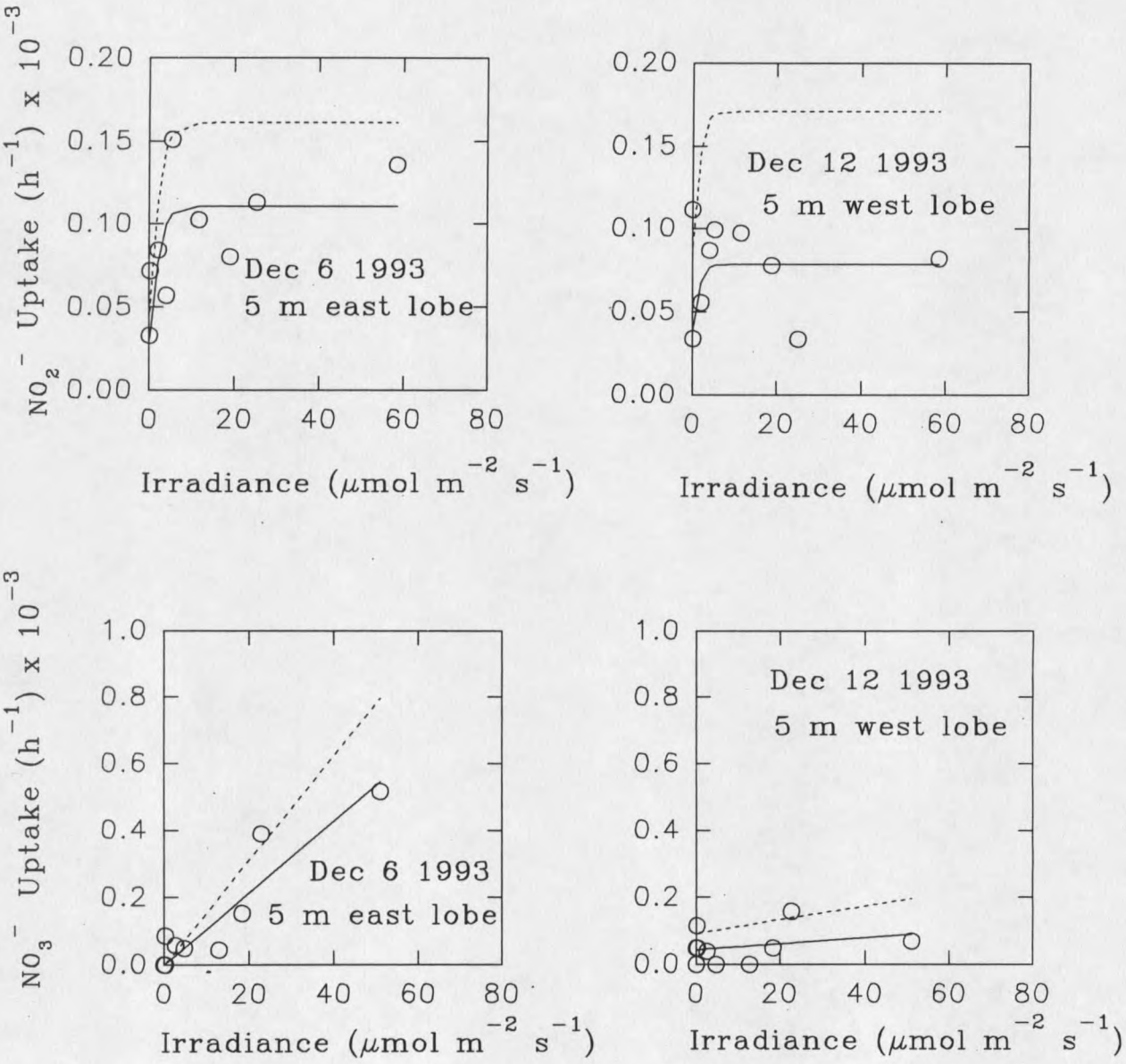

Figure 14, cont . NO_3^- and NO_2^- uptake vs irradiance, 1993. Data for NO_2^- uptake at 5 m in each lobe fitted with hyperbolic tangent equation. Others fitted with linear regression. Dashed line describes function normalized for chlorophyll a concentration (μM N μg CHL l^{-1} h^{-1}).

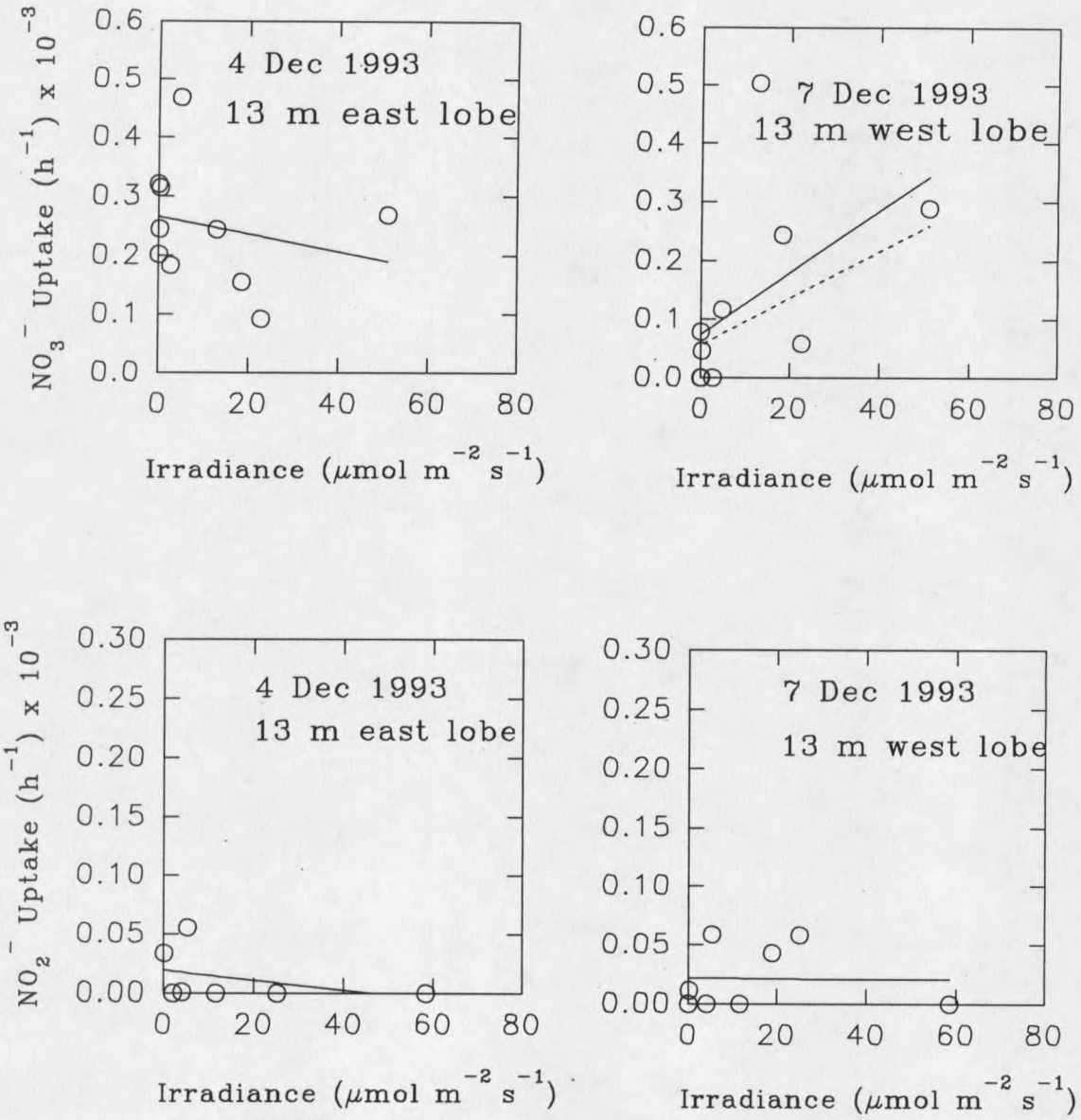

Figure 14, cont . NO_3^- and NO_2^- uptake vs irradiance, 1993. Data fitted with linear regression. Dashed line describes function normalized for chlorophyll a concentration (μM N μg CHL l^{-1} h^{-1}).

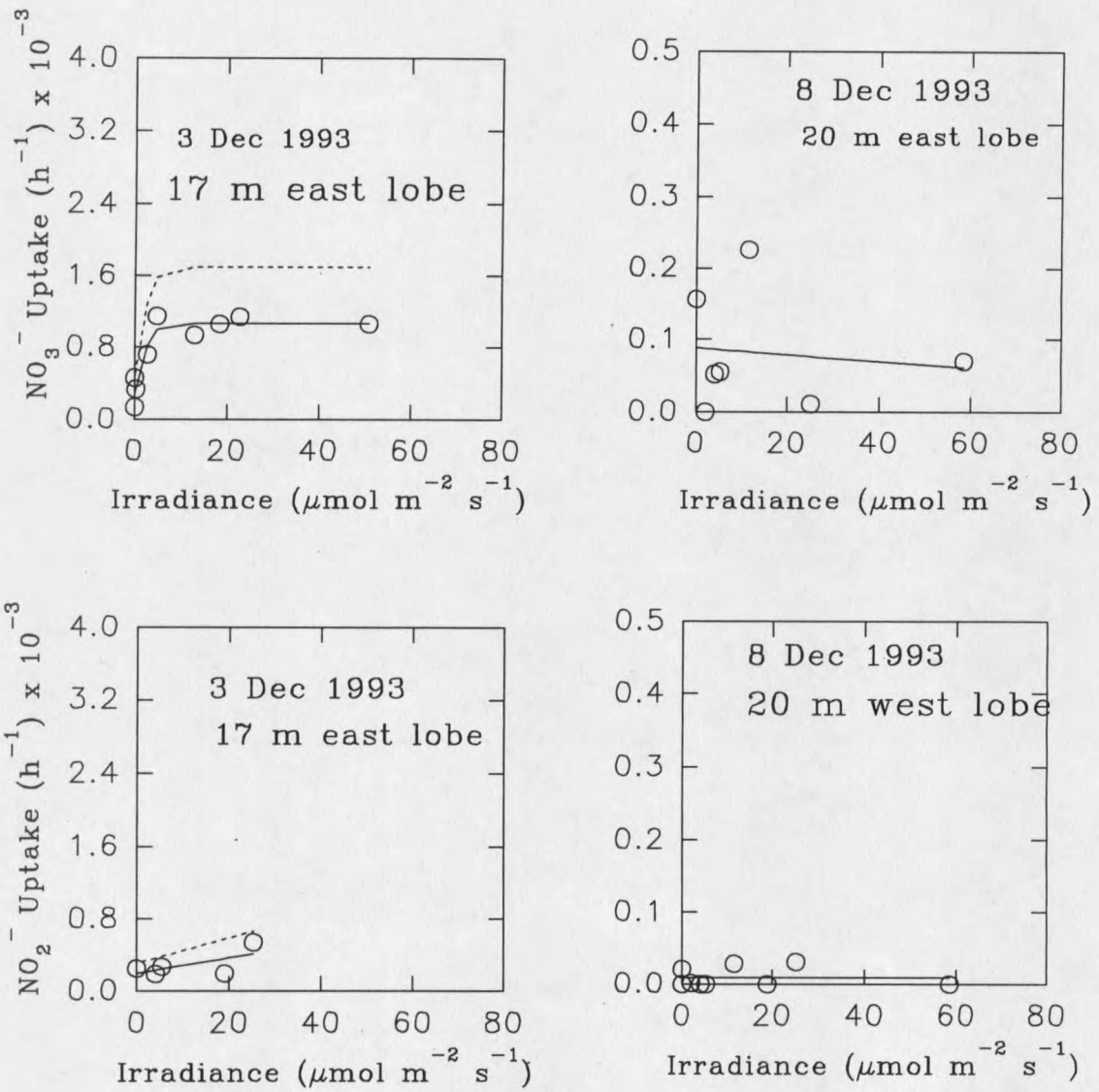

Figure 14,cont . NO_3^- and NO_2^- uptake vs irradiance, 1993. Data fitted with linear regression. Dashed line describes function normalized for chlorophyll a concentration (μM N μg CHL l^{-1} h^{-1}).

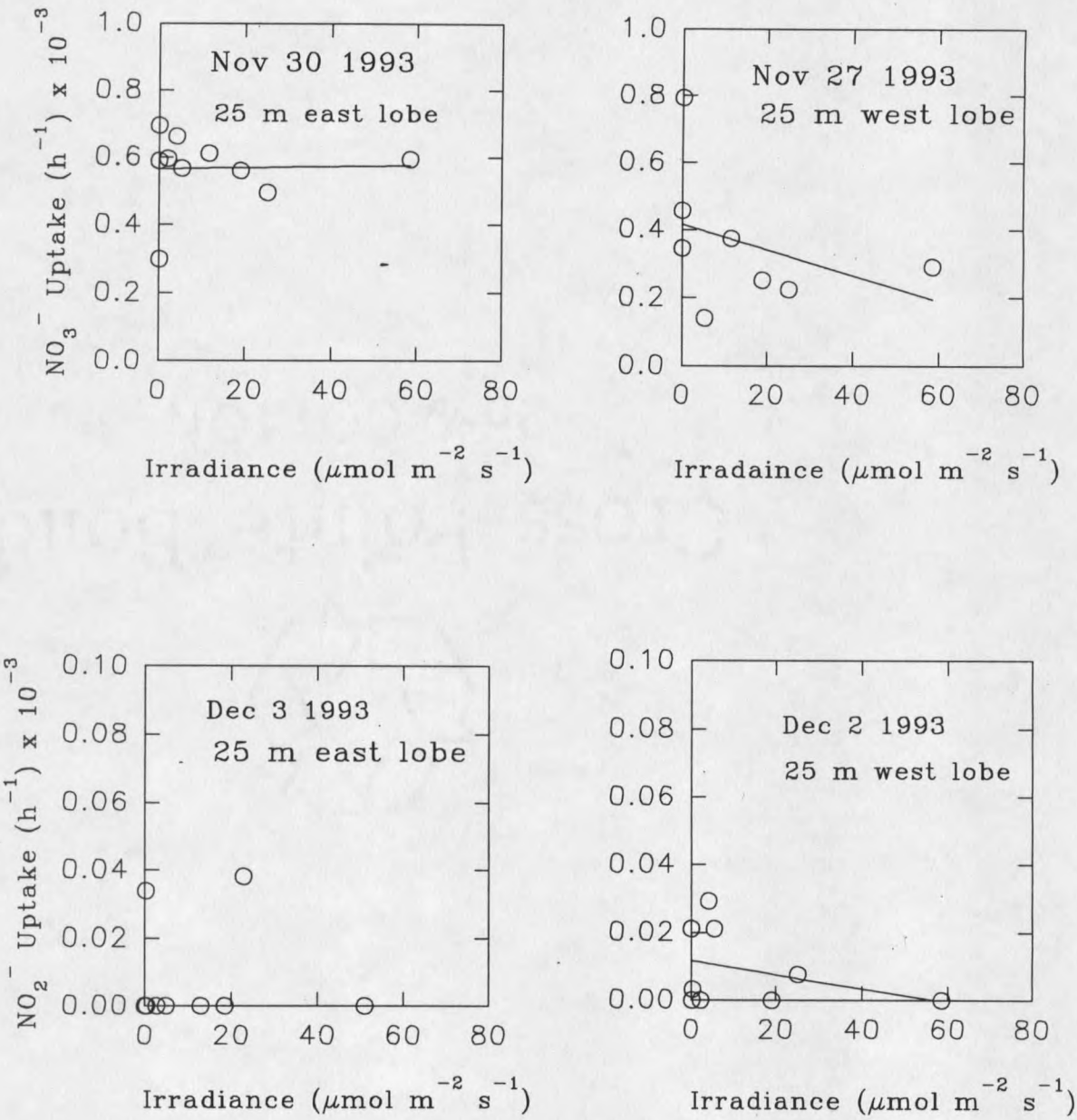

Figure 14, cont . NO_3^- and NO_2^- uptake vs irradiance, 1993. Data fitted with linear regression.

The slopes of linear regressions, as well as α values from hyperbolic curves, were generally highest for NH_4^+ uptake. Linear regression slopes and α values tended to decrease with depth. The ratio of dark uptake to maximum uptake ($D:V_m$) indicates a distinction between phytoplankton communities.

I_k values of samples described by hyperbolic equations were generally similar, ranging from 2.72 to 3.95, with three significant exceptions. 5 m east lobe (1993) and 13 m west lobe (1993) displayed relatively high I_k values (6.59 and 29.42, respectively). I_k at 17 m east lobe (1993) was relatively low (0.44). It should be noted that α in this experiment was not significantly different from zero ($p > 0.05$); I_k at this depth could therefore be much larger.

Photoinhibition was apparent for 13 m east lobe NH_4^+ uptake during both seasons and both data sets were fitted to Equation 13; however, β was not significantly different from zero ($p > 0.05$) in either case, indicating that photoinhibition was not significant. Uptake of NH_4^+ was negatively correlated with irradiance at 25 m east lobe (1992), but the slope was not significantly different from zero ($p > 0.05$). It appears that uptake of inorganic nitrogen by Lake Bonney phytoplankton is not subject to significant photoinhibition at the irradiances used in these experiments which encompass those found in the lake.

Note that NI graphs depict measured uptake rates, theoretical uptake functions, and, in some cases, functions normalized to the concentration of CHL at time zero in the sample. Experiments in which nitrogen uptake was not significantly correlated with

irradiance were not normalized to CHL. Parameters of the NI curves, specific to nitrogen, are summarized in Table 14 .

Table 14 . Nitrogen uptake vs irradiance parameters for hyperbolic curves. All values (except I_k and D:V_m) multiplied by 10^4. *- indicates a value not significantly different from 0 ($p > 0.05$). ID labels include depth, lobe (E or W) and nutrient analyzed.

ID	V_m	α	D	I_K	D: V_m x 100
1993 Experiments					
$5ENH_4^+$	20.05 ± 2.56	2.16 ± 1.08	5.80 ± 1.40	*9.28 ± 6.13	29 ± 8
$5ENO_2^-$	1.11 ± 0.33	*0.26 ± 0.24	*0.30 ± 0.21	*4.27 ± 3.68	*27 ± 21
$5WNH_4^+$	9.50 ± 0.76	1.12 ± 0.46	5.70 ± 0.50	8.48 ± 3.85	60 ± 7
$5WNO_2^-$	*0.78 ± 0.40	*0.18 ± 0.51	*0.37 ± 0.26	*4.33 ± 6.65	*47 ± 41
$13ENH_4^+$	* 9.81 ± 32.55	0.25 ± 0.05	2.05 ± 0.13	*47.44 ± 132.52	*17 ± 47
$13WNH_4^+$	12.60 ± 0.73	0.33 ± 0.03	2.89 ± 0.02	38.18 ± 3.23	23 ± 1
$17ENH_4^+$	5.58 ± 0.11	*5.12 ± 5.81	3.30 ± 0.07	1.08 ± 0.03	59 ± 2
$17ENO_3^-$	10.65 ± 1.02	2.47 ± 0.09	2.73 ± 0.08	4.31 ± 0.44	26 ± 3
1992 Experiments					
$5WNH_4^+$	*10.43 ± 11.90	*1.86 ± 1.03	3.82 ± 1.14	*5.61 ± 7.26	*37 ± 43
$13ENH_4^+$	8.28 ± 0.88	1.00 ± 0.52	2.62 ± 0.69	*10.90 ± 7.57	24 ± 7
$13WNH_4^+$	8.67 ± 0.74	1.16 ± 0.38	4.09 ± 0.49	7.47 ± 3.22	47 ± 7

Table 15 . Slope (h^{-1} μmole quanta^{-1} m^2 s), dark uptake (y-intercept, h^{-1}), and maximum uptake (h^{-1}) for uptake vs irradiance experiments described by linear regression. All values multiplied by 10^4. *- indicates a value not significantly different from 0 ($p > 0.05$). ID labels include depth, lobe (E or W), and nutrient.

ID	SLOPE	D	V_m	D:V_m x 100
1993 Experiments				
5ENO_3^-	0.11 ± 0.02	*-0.02 ± 0.30	5.45	*0 ± 6
5WNO_3^-	*0.01 ± 0.01	0.41 ± 0.20	0.93	44 ± 22
13ENO_3^-	*-0.02 ± 0.02	2.66 ± 0.42	2.66	100 ± 16
13ENO_2^-	*0.00 ± 0.01	0.19 ± 0.11	0.19	100 ± 58
13WNO_3^-	*0.05 ± 0.03	*0.74 ± 0.58	3.43	*22 ± 17
13WNO_2^-	*0.00 ± 0.01	*0.15 ± 0.19	0.15	*100 ± 127
17ENO_2^-	0.09 ± 0.06	1.96 ± 0.85	4.18	47 ± 20
20WNH_4^+	*-0.01 ± 0.04	15.11 ± 0.76	15.11	100 ± 5
20WNO_3^-	*0.00 ± 0.02	*0.66 ± 0.55	0.66	100 ± 83
20WNO_2^-	*0.00 ± 0.01	0.02 ± 0.01	0.02	100 ± 50
25ENH_4^+	*0.02 ± 0.03	1.36 ± 0.47	2.38	57 ± 20
25ENO_3^-	*0.00 ± 0.02	5.65 ± 0.45	5.65	100 ± 8
25ENO_2^-	*0.00 ± 0.00	0.07 ± 0.07	0.07	*100 ± 100
25WNH_4^+	0.08 ± 0.02	7.00 ± 0.27	8.80	80 ± 3
25WNO_3^-	*-0.04 ± 0.04	4.16 ± 0.91	4.16	100 ± 22
25WNO_2^-	*0.00 ± 0.00	0.12 ± 0.05	0.12	100 ± 42

Table 15, cont. Slope (h^{-1} μmole quanta^{-1} m^2 s), dark uptake (y-intercept, h^{-1}), and maximum uptake (h^{-1}) for uptake vs irradiance experiments described by linear regression. All values multiplied by 10^4. *- indicates a value not significantly different than 0 ($p > 0.05$). ID labels include depth, lobe (E or W) and nutrient.

ID	SLOPE	D	V_m	D : V_m
1992 Experiments				
5ENH_4^+	0.27 ± 0.14	11.19 ± 0.26	21.99	0.51
5ENO_3^-	0.06 ± 0.01	*0.11 ± 0.32	2.35	0.05
5ENO_2^-	0.02 ± 0.01	0.31 ± 0.14	1.24	0.25
5WNO_3^-	0.03 ± 0.01	1.70 ± 0.38	3.19	0.53
5WNO_2^-	0.01 ± 0.00	*0.03 ± 0.11	0.56	0.05
13ENO_3^-	0.02 ± 0.02	*0.22 ± 0.43	0.92	0.24
13ENO_2^-	*0.01 ± 0.02	0.53 ± 0.22	0.93	0.57
13WNO_3^-	0.02 ± 0.02	*0.32 ± 0.42	1.05	0.30
13WNO_2^-	*0.00 ± 0.02	0.96 ± 0.34	0.96	1.0
17ENH_4^+	*-0.01 ± 0.02	2.68 ± 0.36	2.68	1.0
17ENO_3^-	*0.00 ± 0.38	3.23 ± 0.74	3.23	1.0
17ENO_2^-	0.02 ± 0.01	*0.25 ± 0.93	1.22	0.20
20WNH_4^+	0.07 ± 0.04	4.96 ± 0.56	6.69	0.74
20WNO_3^-	0.09 ± 0.05	5.35 ± 0.76	8.05	0.66
25ENH_4^+	-0.02 ± 0.02	2.33 ± 0.37	2.33	1.0
25ENO_3^-	*0.02 ± 0.03	3.53 ± 0.74	4.33	0.82

In some situations, correction for regeneration could significantly affect measured relationships between NH_4^+ uptake and irradiance. It was previously determined that a regeneration rate of 0.08 μM NH_4^+ h^{-1} at 5 m east lobe could lead to a 10% underestimation of uptake at substrate concentrations used for uptake vs irradiance experiment (see Part II, Results,Substrate Kinetics). A similar result is found if a regeneration rate of 0.10 μM NH_4^+ h^{-1} is assumed for 13 m east lobe. Unfortunately, attempts to measure regeneration rates in Lake Bonney did not produce definitive results; it is therefore impossible to precisely correct uptake rates for regeneration. To understand the potential effect of regeneration on measurements reported here, functions from selected experiments were adjusted using regeneration rates estimated from 1993 isotope dilution experiments (see part II). Specifically, the functions describing NH_4^+ uptake rates at 5 and 13 m east lobe and 13 m west lobe(1993 data) were corrected for regeneration using Equation 10. Parameters from corrected curves are summarized in Table 16 .

Corrections for regeneration increased V_1, α, and D; however, the corrected parameters were not significantly different than the original estimates.

Table 16. Uptake parameters for selected 1993 uptake vs irradiance experiments corrected for regeneration. All parameters, except for r (rate of regeneration, μmoles NH_4^+ h^{-1}), multiplied by 10^4.

ID	r	V_1	α	D
$5ENH_4^+$	0.08	15.79	2.40	6.46
$13ENH_4^+$	0.10	9.01	0.29	2.31
$13WNH_4^+$	0.26	10.18	0.35	3.02

The minimum irradiance for saturated uptake was graphically estimated for all experiments described by Equations 13 and 14. This threshold irradiance (I_t) was compared to a representative diel range of in situ irradiance (Nov 17 1993, see Part I, Results, Irradiance). Nov 17 was considered representative of the sampling period because the average irradiance on that date approximated the average daily irradiances from Oct-Dec 1993. Table 17 summarizes I_t and the percentage of Nov 17 1993 when $I<I_t$.

Table 17. Threshold irradiance (I_t, μmol m^{-2} s^{-1}) and estimated percentage of time irradiance was subsaturating during Nov 17, 1993.

ID	I_t	% of day $I<I_t$
1992 experiments		
$5WNH_4^+$	9	0
$13ENH_4^+$	11	83
$13WNH_4^+$	6	38
1993 experiments		
$5ENH_4^+$	15	33
$5WNH_4^+$	7	0
$5ENO_2^-$	10	0
$5WNO_2^-$	4	0
$13ENH_4^+$	21	100
$13WNH_4^+$	50	100
$17ENH_4^+$	4	38

DISCUSSION

Following the methods of MacIsaac and Dugdale (1972), several investigators have applied Michaelis-Menten kinetics to describe the effect of irradiance on nitrogen uptake (Nelson and Conway 1979, Whalen and Alexander 1984, Cochlan et al. 1990). This approach has been popular largely because the parameters of the Michaelis-Menten Equation, V_{max} and K_s, provide useful insight into algal physiology and the ecology of the system. The magnitude of K_s, the half-saturation constant, can be used to gauge the efficiency of phytoplankton light harvesting and nitrogen assimilation. Analogous to the kinetics of substrate concentration, a small K_s would indicate "affinity specialists" (Kilham and Hecky 1988), whereas a large V_{max} would indicate "velocity specialists" (Kilham and Hecky, 1988). In substrate kinetics, affinity specialists are more common in environments where substrate concentration is low and relatively stable (Kilham and Hecky, 1988). A low K_s for a nitrogen uptake vs irradiance (NI) curve would thus be expected in a stable, low-light system.

Despite its frequent application, the Michaelis-Menten Equation is inadequate for describing the dependence of nitrogen uptake on irradiance in most systems. It assumes that there is no dark uptake of nitrogen and no photoinhibition (Priscu 1989). In many environments, particularly those characterized by low nitrogen and low light, these assumptions are likely to be invalid. Priscu (1989) modified the photosynthesis equations of Platt et al. (1980) to

describe the effects of irradiance on nitrogen uptake. The photosynthesis equations are able to describe systems with significant dark uptake and photoinhibition.

Since photosynthesis and nitrogen uptake may not be directly coupled, the parameters of the photosynthesis equations (e.g. α, β, P_m, and I_k) must be reinterpreted when applied to nitrogen uptake (Priscu 1989). When describing photosynthesis, α, the slope of the light limited portion of the curve, is a measure of light harvesting ability and the efficiency of energy conversion (Henley 1993). When describing nitrogen uptake, α is more ambiguous; light is more strongly coupled to protein synthesis than to uptake (Turpin 1991). V_m, the rate of uptake at optimal irradiance; I_k, a standard index of photoadaptation that marks the irradiance at which extrapolations of α and V_m intersect; and β, the slope of the photoinhibited portion of the curve are also made ambiguous by the often indirect physiological relationship between irradiance and nitrogen uptake (Priscu 1989).

While not directly equivalent to the results of photosynthesis vs irradiance (PI) curves, the parameters of NI curves can be used to describe nitrogen uptake in terms of phytoplankton physiological response to irradiance. A large α and low I_k indicate a high affinity for light and an efficient, if indirect, transfer of photosynthetic energy to nitrogen uptake. The Platt equations for photosynthesis have been successfully fitted to NI curves for several lakes and polar marine environments (Priscu 1989, Dodds and Priscu 1989, Smith and Harrison 1991).

The relationship between inorganic nitrogen uptake and irradiance varies with the source and abundance of inorganic nitrogen as well as the level of phytoplankton adaptation and acclimation to ambient irradiance and nutrient concentrations. Previous researchers have noted that NO_3^- uptake is generally strongly dependent upon irradiance. Phytoplankton typically exhibit negligible dark uptake of NO_3^-; conversely, dark NH_4^+ uptake, particularly in nitrogen depleted waters, may range from 40% to 100% of maximum NH_4^+ uptake (MacIsaac and Dugdale 1972, Nelson and Conway 1979, Koike et al. 1986, Smith and Harrison 1991). Because NO_3^- must be reduced to NH_4^+ before assimilation, the dependency of NO_3^- uptake on irradiance may be a result of a requirement of photosynthetic energy for the production of reductants (Miyazaki et al. 1987).

Because the dependence of inorganic nitrogen uptake on irradiance has been shown to increase with increasing nutrient supply (Turpin 1991), it is important to note that experiments reported here were brought to saturating levels of DIN. Consequently, the in situ dependence on irradiance may be less than reported here, particularly in the nitrogen depleted shallow waters. However, since light and nutrients can potentially co-limit nutrient uptake (Healy 1985), it is difficult to make an accurate substrate correction for uptake. Before any estimate of NI relationships at ambient nutrient concentrations can be made, the degree to which light compensates for nutrient supply, and vice versa, must be

determined. Once again, this issue could be clarified through greater understanding of the mechanism for nitrogen uptake.

Dark uptake of both NO_3^- and NH_4^+ tends to become increasingly significant in nitrogen depleted systems (Cochlan et al. 1990). In a nitrogen depleted environment, starch reserves provide the carbon and energy for amino acid synthesis and nitrogen assimilation. In such cases, nitrogen assimilation, and, to a smaller extent, nitrogen transport, is relatively independent of irradiance (Turpin 1991). The ratio of dark uptake to maximum uptake ($D:V_m$) for a particular form of DIN may therefore be used to assess the nutritional status of the community.

At 5 m in each lobe of Lake Bonney, $D:V_m$ is similar for NH_4^+, NO_3^-, and NO_2^-. Furthermore, $D:V_m$ tends to increase with depth for all nutrients. Indeed, in most deep populations (below 13 m), dark inorganic nitrogen uptake was essentially equivalent to the maximum level of uptake.

This final result indicates that $D:V_m$ should only cautiously be used as an indicator for nutrient deficiency. It is apparent that plankton from the deep waters of Lake Bonney are not limited by the availability of inorganic nitrogen; ambient DIN concentrations are extremely high and ambient uptake velocity has been shown to equal V_{max} (see Part II, Results, Substrate Kinetics). The lack of correlation between irradiance and nitrogen uptake in the deep layers implies that bacteria, not phytoplankton, are the main consumers of inorganic nitrogen at these depths. As previously discussed, nitrogen assimilation by algae may not be directly fueled

by irradiance; it is primarily driven by the energy and carbon skeletons of photosynthesis (Turpin 1991). Since photosynthetic capacity does not exist at 25 m in both lobes and 20 m in the west lobe (Sharp 1993, Priscu unpublished data) it is not surprising that nitrogen uptake is not stimulated by increased irradiance at these depths.

D:V_m was consistently higher for all inorganic nitrogenous nutrients at 5 m west lobe compared to 5 m east lobe. This trend, in concert with lower I_k values at 5 m in the west lobe, indicates that, compared to 5 m east lobe, DIN uptake at 5 m in the west lobe is less likely to be limited by irradiance.

Data from 1993 and 1992 show that dark uptake of NO_3^- and NO_2^- at 5 m, particularly in the west lobe, is a significant portion of total NO_3^- and NO_2^- uptake. Low α and I_k values at 5 m in both lobes provides further evidence that NO_3^- and NO_2^- uptake at these depths is not strongly regulated by irradiance. This observation contradicts many previous reports that suggest uptake of NO_3^- and NO_2^- is strongly dependent on irradiance (MacIsaac and Dugdale 1972, Nelson and Conway 1979, Koike et al. 1986, Smith and Harrison 1991). Two potential explanations for this contradiction follow.

(1) Due to chronic nitrogen depletion, phytoplankton at 5 m in each lobe rely on reserves of reduced carbon, not recent photosynthate, to provide the energy and carbon skeletons for assimilation of inorganic nitrogen. Phytoplankton typically adjust NO_3^- and NO_2^- uptake to

correspond with the potential for assimilation. For instance, if the resources for nitrogen assimilation into protein are not available, transport of NO_3^- and NO_2^- tends to be limited (Turpin 1991). Therefore, uptake of NO_3^- and NO_2^- in a system where nitrogen assimilation is controlled by starch reserves would tend to be relatively independent of short term changes in irradiance.
(2) The stability of the light regime in Lake Bonney has allowed phytoplankton at 5 m in each lobe to enhance uptake of NO_3^- and NO_2^- at low levels of irradiance.

The second of these two explanations appears more plausible. Uptake of NO_3^- and NO_2^- was substrate saturated throughout the water column in both lobes (See Part II, Substrate Kinetics, Results), indicating that microbial communities were not nitrogen starved. Furthermore, the degree of nitrogen limitation necessary for depletion of reserves of reduced carbon typically limits uptake of inorganic carbon. Primary productivity measurements indicate that the 5 m communities were actively photosynthesizing at relatively high rates (see Figure 2) during the period of NI experiments.

The second explanation is supported by comparisons of I_k and I_t values with ambient irradiances. I_k values at 5 m in both lobes are consistently lower than values reported for phytoplankton in other systems, indicating extreme adaptation to low irradiances. In fact, the I_k values reported here are much lower than I_k values reported for near surface waters of other perennially ice-covered Antarctic lakes with similar irradiance regimes (Priscu 1989). I_t, the

minimum uptake saturating irradiance, for NO_3^- and NO_2^- uptake at 5 m in both lobes exceeded the minimum irradiance at these depths during the period of collection (approximately 12 μmol m^{-2} s^{-1}, see Figure 5). Presumably owing to phytoplankton adaption/acclimation to low irradiances, uptake of NO_3^- and NO_2^- at these depths is not limited by irradiance during late austral spring and austral summer.

$D:V_m$ values for NH_4^+ uptake at 5 m in each lobe were consistent with $D:V_m$ values reported for other lake and marine environments (MacIsaac and Dugdale 1972, Nelson and Conway 1979, Koike et al. 1986, Smith and Harrison 1991). However, I_k values for NH_4^+ uptake were extremely low at these depths relative to previously reported values for other systems. The highest 5 m I_k for NH_4^+ uptake (9.28, east lobe 1993) is well below I_k values reported for communities in other permanently ice-covered Antarctic lakes and Antarctic sea ice (Priscu 1989, Priscu et al. 1991).

Despite a low I_k relative to other systems, NH_4^+ uptake at 5 m east lobe was light limited approximately 33 % of each day during the 1993 collection period (see Table 19). Furthermore, NH_4^+ uptake at 5 m east lobe during the 1992 season was always irradiance limited during the period of collection (see Figure 5). Compared to 5 m west lobe, the microbial community at 5 m east lobe was apparently less adapted/acclimated for NH_4^+ uptake at low irradiances.

The increase of I_K of DIN uptake with depth, as well as relatively high I_K values at 5 m east lobe compared to 5 m west lobe, parallel trends of K_S of DIN uptake (See Part II, Results, Substrate Kinetics). Both I_K and K_S increase with increasing DIN supply rates. As with K_S, the increase in I_K indicates a relative decline in affinity, in this case for irradiance. However, the mechanisms for a decline in DIN uptake affinity for irradiance are unclear (Priscu 1989).

Although photoinhibition was never significant, there was some indication that NH_4^+ uptake was susceptible to photoinhibition, particularly at depths below 5 m. This is consistent with Priscu et al.'s (1991) finding that the irradiance necessary for photoinhibition in a stable community (e.g., ice algae) is a function of ambient irradiances. Specifically, nitrogen uptake by phytoplankton growing at low irradiances was photoinhibited at relatively low irradiances (Priscu et al. 1991).

Lake Bonney phytoplankton showed no indication of photoinhibition of NO_3^- or NO_2^- uptake at any depth. Previous studies have shown NO_3^- and NH_4^+ uptake rates to be differentially susceptible to photoinhibition, although studies in polar systems have produced conflicting results. Muggli and Smith (1993) reported that NH_4^+ uptake in the Greenland Sea was photoinhibited at lower irradiances than was NO_3^- uptake. However, Smith and Harrison (1991) reported the opposite effect at several sites in the Arctic and Antarctic. Priscu (1989), studying permanently ice-covered Antarctic lakes, found that NH_4^+ uptake was photoinhibited at

relatively low irradiances. Interestingly, β was relatively large for NO_3^- uptake. All of these researchers reported initiation of photoinhibition at irradiances much higher than the maximum irradiances in this current study. Clearly, the mechanism of photoinhibition of nitrogen uptake must be better understood for interpretation of these conflicting results.

NI parameters from the current study were compared to previously reported PI parameters to investigate the role of irradiance in determining C:N uptake ratios. Lizotte and Priscu report PI parameters at two east lobe depths that correspond to the current study: 5 m and 17 m. Nitrogen uptake parameters normalized to CHL at 17 m are an order of magnitude higher than similar parameters at 13 m, thus suggesting nitrogen uptake at 17 m is not entirely due to phytoplankton. Therefore, the 5 m east lobe data are more suitable for investigation of uptake response by phytoplankton. A range of irradiances were inserted into Equation 12, and the resulting NI parameters were compared to PI parameters. The C:N ratio of daily integrated uptake (μM:μM) was 18.35, well above the ratio (6.6) that is assumed to be typical of balanced growth (Redfield 1958). However, C:N uptake ratios indicate balanced growth at approximately 10 μmole quanta m^{-2} s^{-1}. The response of C:N uptake ratios to irradiance is illustrated in Figure 24.

The response of C:N uptake ratios to irradiance illustrates the role of NI relationships in regulating balanced growth. In early spring, when irradiances are low and regenerated nutrients have accumulated (Sharp 1993), C:N uptake ratios can be expected to be

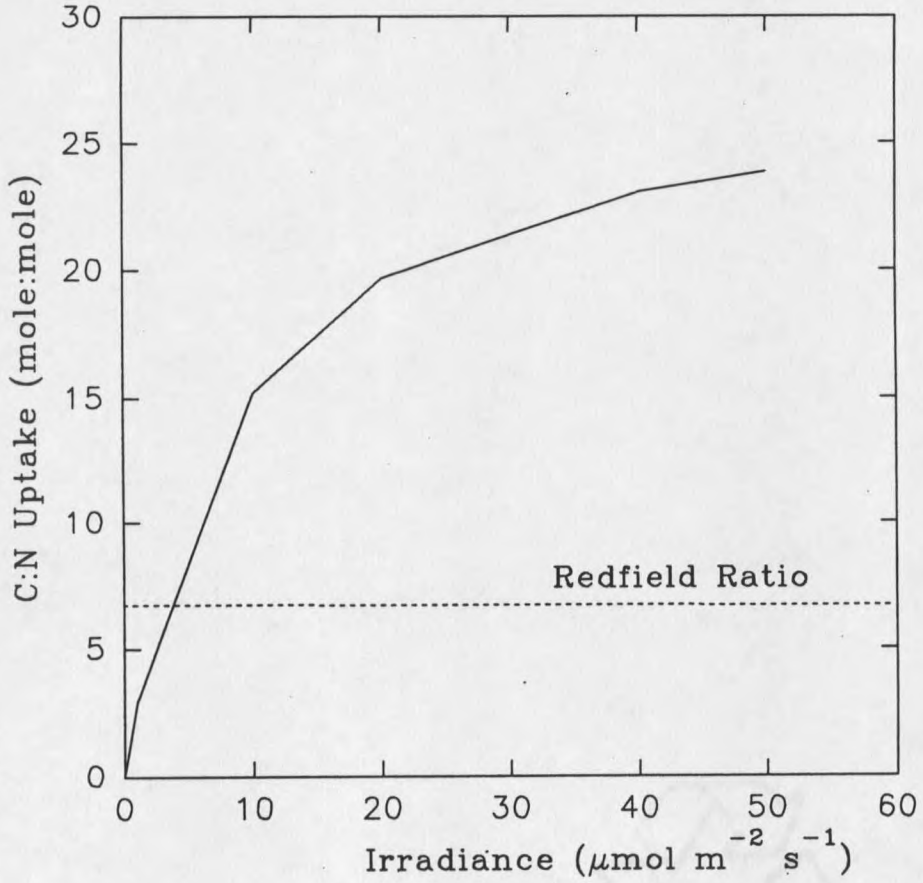

Figure 15. Estimated Carbon : Nitrogen uptake ratios vs irradiance at 5 m east lobe.

significantly less than 6.6. Nitrogen deficiency is likely to occur only when irradiances are above 10 μmole quanta m^{-2} s^{-1} for a majority of the day. In conclusion, mid-summer C:N uptake ratios are likely to be inadequate indicators of phytoplankton nutrient history or physiological state.

CONCLUSION AND AREAS FOR FURTHER RESEARCH

Phytoplankton in Lake Bonney have adapted/acclimated to enhance utilization of irradiance for DIN uptake. This is particularly evident at 5 m in the west lobe where dark uptake of NO_3^- and NO_2^- is significant, I_k of NH_4^+ uptake is much lower than values reported from other systems, and I_t of NH_4^+ uptake is significantly lower than the average <u>in situ</u> daily irradiance during late austral spring and early austral summer.

However, microplankton at 5 m east lobe and 13 m in each lobe are relatively poorly adapted to utilize ambient irradiance for NH_4^+ uptake. The lack of adaptation may be a reflection of the nutritional status of the communities. Relatively high supply rates of NH_4^+ via regeneration and diffusion at 5 m east lobe and 13 m in each lobe may compensate for inefficient utilization of irradiance. The exact mechanism for irradiance control of DIN uptake must be understood for interpretation of NI trends.

GENERAL CONCLUSION

NH_4^+ is the preferred source of DIN for microplankton in Lake Bonney. Regeneration of NH_4^+ is the primary source of DIN in the upper trophogenic zone, and microplankton in this zone have developed several strategies to enhance NH_4^+ uptake. Strategies include high affinity for NH_4^+, "surge" uptake of NH_4^+ pulses, and efficient utilization of irradiance for NH_4^+ uptake.

NO_3^- and NO_2^- are minor sources of DIN in Lake Bonney, but microplankton are still well acclimated/adapted for NO_3^- and NO_2^- uptake. Dark uptake of NO_3^- and NO_2^- was significant in the shallow waters of Lake Bonney, and "surge" uptake of NO_3^- and NO_2^- was also observed.

Perhaps as a result of extreme acclimation/adaptation to <u>in situ</u> supplies of DIN and irradiance, Lake Bonney microbial communities do not appear to be extremely nitrogen deficient. Since several lines of evidence suggest nutrient control of productivity in the upper trophogenic zone, phosphorus should be considered a likely limiting nutrient in this zone.

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