Biological Cycling of Nitrogen in a Rocky Mountain Alpine Lake, with Emphasis on the Physiological and Ecological Effects of Acidification
by ROBERT T ANGELO

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences
Montana State University

Abstract:
This study examined nitrogen cycling interactions occurring among the heterotrophic and autotrophic plankton of a softwater, oligotrophic alpine lake. Its major objectives were (1) to compare the influences of internal (regenerative) and external nitrogen supply processes on water-column primary production, (2) to identify the food web components contributing most to regenerative and assimilative fluxes of nitrogen, and (3) to evaluate the sensitivity of the limnetic nitrogen cycle to lake acidification. Field and laboratory experiments were based on isotopic tracer (15N, 14C, 3H) methodologies, plankton size-fractionation and metabolic inhibitor techniques, and short-term bioassay procedures; supporting data were gathered on lake physicochemical and biological properties. Measured aqueous nutrient concentrations, the results of 14C02-based snowmelt and nutrient enrichment bioassays, and physiological indicators of algal nutrient status collectively demonstrated that phytoplankton nitrogen demand greatly exceeded nitrogen supply. Both NH4^+ and N03^- were quantitatively important forms of assimilatable nitrogen under ambient conditions. Mass balance considerations indicated that within-lake biogeochemical processes constituted a net sink for N03^-, whereas NH4^+ production and consumption rates were approximately in balance on an ecosystem scale. Water-column regenerative and assimilative fluxes of NH4^+ were strongly correlated. Meta- and protozooplankton were the principal sources of regenerated NH4^+; heterotrophic bacterioplankton were net consumers of NH4^+. Experimental reductions in metazooplankton populations markedly enhanced rates of NH4^+ regeneration, apparently by reducing predation pressures on metabolically active nano- and microflagellates. Ammonium regeneration, NH4^+ uptake and bacterial secondary production declined under acidic conditions (pH 5 versus pH 7; HCl), whereas algal N03^- utilization increased and compensated for reductions in NH4^+ uptake. Acidification via HN03 or HN03/H2S04 stimulated increases in total nitrogen uptake and permitted higher rates of photosynthesis than did corresponding additions of HC1 or H2S04. These findings collectively suggested that the efficiency of the NH4^+ regenerative/assimilative cycle constituted a principal determinant of water-column primary production, that higher-level trophic interactions could influence phytoplankton growth through food web. effects on nitrogen cycling, and that lake acidification potentially could disrupt the limnetic nitrogen cycle and increase phytoplankton dependence on allochthonously supplied N03^-.

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by

Robert Thomas Angelo

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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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Date 21 September 1989

This work is gratefully dedicated to my wife, Diane, whose willingness to endure numerous hardships and make many personal sacrifices ultimately enabled me to resume and complete my graduate education, to my children, Andrea and Benjamin, now in the earliest phases of their own academic careers, and to my parents, Samuel and Dorothy Angelo, whose steadfast encouragement and support have figured prominently in all my scholarly endeavors.

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ABSTRACT

This study examined nitrogen cycling interactions occurring among the heterotrophic and autotrophic plankton of a softwater, oligotrophic alpine lake. Its major objectives were (1) to compare the influences of internal (regenerative) and external nitrogen supply processes on water-column primary production, (2) to identify the food web components contributing most to regenerative and assimilative fluxes of nitrogen, and (3) to evaluate the sensitivity of the limnetic nitrogen cycle to lake acidification. Field and laboratory experiments were based on isotopic tracer (^{15}N, ^{14}C, ^{3}H) methodologies, plankton size-fractionation and metabolic inhibitor techniques, and short-term bioassay procedures; supporting data were gathered on lake physicochemical and biological properties. Measured aqueous nutrient concentrations, the results of $^{14}CO_2$-based snowmelt and nutrient enrichment bioassays, and physiological indicators of algal nutrient status collectively demonstrated that phytoplankton nitrogen demand greatly exceeded nitrogen supply. Both NH_4^+ and NO_3^- were quantitatively important forms of assimilatable nitrogen under ambient conditions. Mass balance considerations indicated that within-lake biogeochemical processes constituted a net sink for NO_3^-, whereas NH_4^+ production and consumption rates were approximately in balance on an ecosystem scale. Water-column regenerative and assimilative fluxes of NH_4^+ were strongly correlated. Meta- and protozooplankton were the principal sources of regenerated NH_4^+; heterotrophic bacterioplankton were net consumers of NH_4^+. Experimental reductions in metazooplankton populations markedly enhanced rates of NH_4^+ regeneration, apparently by reducing predation pressures on metabolically active nano- and microflagellates. Ammonium regeneration, NH_4^+ uptake and bacterial secondary production declined under acidic conditions (pH 5 versus pH 7; HCl), whereas algal NO_3^- utilization increased and compensated for reductions in NH_4^+ uptake. Acidification via HNO_3 or HNO_3/H_2SO_4 stimulated increases in total nitrogen uptake and permitted higher rates of photosynthesis than did corresponding additions of HCl or H_2SO_4. These findings collectively suggested that the efficiency of the NH_4^+ regenerative/assimilative cycle constituted a principal determinant of water-column primary production, that higher-level trophic interactions could influence phytoplankton growth through food web effects on nitrogen cycling, and that lake acidification potentially could disrupt the limnetic nitrogen cycle and increase phytoplankton dependence on allochthonously supplied NO_3^-.

CHAPTER 1

INTRODUCTION

During the past quarter century, the impact of human technology and population growth on the functional integrity of freshwater ecosystems has received unprecedented scientific and public attention (*e.g.*, Davis 1964; Thomas 1965; Oden 1968, 1975; NAS 1969; Johnson *et al.* 1970; Bolin 1971; Vallentyne 1972, 1974; Jensen and Snekvik 1972; Grahn *et al.* 1974; Braekke 1976; Hendrey *et al.* 1976; Beamish and van Loon 1977; Alfheim *et al.* 1978; Almer *et al.* 1978; Yan 1979; Fromm 1980; Schindler 1980, 1988a; Vollenweider *et al.* 1980; Yan and Strus 1980; Haines 1981; Harvey *et al.* 1981; Cowling 1982; Lewis 1982; Turk 1983; Dillon *et al.* 1984; Hendrey 1984; Mitchell *et al.* 1985; Sanchez *et al.* 1986; Goldman 1988; Lehman 1988). It is now recognized that mankind's influence on the environmental quality of lakes and streams is manifested over a deceptively wide range of temporal and spatial scales and that few inland waters, however remote, are insulated entirely from the environmental vagaries of industrialization, agricultural expansion, and urbanization. Alpine lakes offer an interesting case in point. Although these lakes collectively rank among the most isolated and pristine of surface water ecosystems, they too are threatened by the growing level of industrial contaminants in atmospheric precipitation, by intensified mining and fossil fuel development activities, and by increasing demophoric demands on freshwater resources (reviewed by Wells 1986; see also Vallentyne

1972; Aamodt 1977; Vollenweider 1979; Dodson 1981; Lewis 1982; Logan *et al.* 1982; Harte *et al.* 1983; Turk and Adams 1983; Eilers *et al.* 1986; Landers *et al.* 1986). Unfortunately, the unfavorable working conditions afforded by the alpine environment have discouraged all but the most simple and descriptive of studies on alpine lake ecology (see Thomasson 1952; Pennak 1955, 1958, 1963, 1968; Rodhe *et al.* 1966; Tilzer 1973; Dodson 1981; Aizako *et al.* 1987; *cf.*, Vincent *et al.* 1984, 1985; Dokulil 1988). Fundamental questions concerning the structure and functioning of these ecosystems remain unanswered: What environmental factors regulate biological productivity and community structure in alpine lakes? Which food web components contribute most to energy flow and nutrient cycling? In what manner do these ecosystems respond to nutrient enrichment, acidification, food web manipulation, and other environmental disturbances? The protection and wise use of alpine aquatic resources ultimately will depend upon our ability to answer such questions.

This study examines nitrogen cycling interactions occurring among the heterotrophic and autotrophic components of an alpine plankton community. It investigates (1) the importance of these interactions to water-column biological production, (2) the roles played by the major planktonic food web constituents in nitrogen cycling, (3) the influences of nutrient enrichment and food web manipulations on nitrogen cycling and algal primary production and (4) the effects of lake acidification on water-column regenerative and assimilative fluxes of inorganic nitrogen.

Historical Overview

Nutrient supply has traditionally been deemed the principal determinant of biological productivity in freshwater ecosystems. Early researchers were well aware of the general stimulatory effects of phosphorus and nitrogen enrichment on algal growth (Rawson 1939: Sawyer 1947; Ohle 1956; Edmondson 1961) and soon developed mathematical models for predicting lake eutrophication responses to nutrient loading (*e.g.*, Vollenweider 1968). Modelling efforts rapidly gained in sophistication and scope of application as researchers began to simulate the effects of multiple abiotic influences on algal growth (*e.g.*, Dillon 1975; Vollenweider and Kerekes 1980) and to capitalize on increasingly comprehensive lake data sets (Vollenweider 1976; Rast and Lee 1978; Schindler 1978a,b; Schindler *et al.* 1978; Oglesby and Schaffner 1978; Canfield and Bachmann 1981). Despite their widespread utilization by researchers and lake managers, however, predictive models based exclusively on nutrient loading and other physicochemical parameters generally accounted for only a moderate fraction of the observed variability in algal biomass and productivity (discussed by Carpenter and Kitchell 1987). By the mid 1970's, researchers began to question whether the physicochemical environment necessarily constituted the most important influence on phytoplankton growth (*e.g.*, Shapiro *et al.* 1975).

Whole-lake fisheries manipulations and plankton size-fractionation studies conducted during the past decade demonstrated that changes in consumer abundance and community structure could have marked effects on algal productivity (Henrikson *et al.* 1980; Shapiro and Wright 1984;

Kitchell and Crowder 1986; Scavia *et al.* 1986a; Carpenter and Kitchell 1988; Elser *et al.* 1988). Carpenter *et al.* (1985) proposed that changes in the abundance of top carnivores were transmitted to virtually all lower trophic components via complex food web interactions or "trophic cascades." They also suggested that physicochemical effects on algal growth, though important, were manifested over different time scales than food web effects. Specifically, nutrient loading and water retention time were credited with setting the long-term potential productivity of a lake, whereas interannual variability around that potential was thought to derive from species interactions and food web effects on nutrient cycling.

The importance ascribed by Carpenter *et al.* (*ibid.*) to nutrient cycling (rather than to grazing activities, *per se*) was based on evidence accumulated over many years from a large number of marine and freshwater studies. Nearly two decades earlier, Dugdale and Goering (1967) had postulated that phytoplankton production in nitrogen-deficient coastal and open oceanic waters was supported primarily by internally recycled NH_4^+ (*i.e.*, NH_4^+ derived from macrozooplankton excretion and microbial ammonification activities) rather than by newly available (*i.e.*, externally derived) forms of assimilatable nitrogen such as N_2 or NO_3^-. The general validity of this scenario in marine systems was substantiated in subsequent investigations (Harrison and Hobbie 1974; McCarthy *et al.* 1975, 1977; Harrison 1978; Caperon *et al.* 1979; Eppley and Peterson 1979; Eppley *et al.* 1979a,b; McCarthy and Goldman 1979; Garside 1981; Glibert 1982; Paasche and Kristiansen 1982; Wheeler *et al.* 1982; Harrison *et al.* 1983, 1987; Goldman 1984a; Koike *et al.* 1986; Kokkinakis and Wheeler

1987; Sahlsten 1987). Furthermore, zooplankton and bacterioplankton remineralization processes were found to provide a quantitatively important source of assimilatable nitrogen and phosphorus in many nutrient-deficient freshwater ecosystems (Hargrave and Geen 1968; Alexander 1970; Barsdate *et al.* 1974; Stanley and Hobbie 1977, 1981; Korstad 1983; Henry 1985; Priscu and Priscu 1987; Priscu *et al.* 1989). It gradually become apparent that primary production, even when limited in the Liebig (1840) sense by the availability of a single nutrient, could be influenced both by allochthonous supply factors and by trophic interactions affecting the cycling of the nutrient among food web components.

Rationale for Present Study

The emphasis placed on aquatic nitrogen cycling in the present study stems from the emerging realization that many high elevation lakes are perennially nitrogen deficient (*e.g.*, Axler *et al.* 1981; Goldman 1981; Vincent *et al.* 1984; Morris and Lewis 1988; Dodds *et al.* 1989). The productivity of such ecosystems presumably is influenced both by the allochthonous supply of assimilatable nitrogen and by the efficiency of the internal nitrogen cycle. Comparisons of allochthonously supported versus internally supported primary production would offer interesting insight into the relative importance of physicochemical and biological controls on alpine lake productivity. Long-term comparisons would reveal the time frames over which the various controls are manifested (*cf.*, Carpenter *et al.* 1985) and disclose any capacity, on the part of the biological community, to intensify nitrogen cycling interactions during

periods of reduced nutrient loading (commensurate with the "boot-strapping" hypothesis of Perry *et al.* 1989).

Organisms responsible for nutrient cycling in alpine lakes have received little scientific attention, and their identification provides an additional impetus for this investigation. The high biological diversity of some alpine lakes (*e.g.*, Wells 1986) suggests that many taxa may participate in the nitrogen cycling process and that patterns of NH_4^+ supply and demand may be relatively complex. The determination of the roles played by major food web components in nutrient cycling would provide an initial (and much sought after) basis for predicting the effects of community compositional changes on resource-limited algal growth (*cf.*, Carpenter and Kitchell 1988).

Physicochemical perturbations resulting in altered rates of NH_4^+ regeneration or inorganic nitrogen uptake theoretically could affect the productivity of nitrogen deficient alpine lakes. Because many high elevation lakes in the western United States and Canada are regarded as extremely sensitive to acidic inputs (Dodson 1981; Logan *et al.* 1982; Gibson *et al.* 1983; Harte *et al.* 1983; Turk and Adams 1983; Galbraith 1984; Mangum 1984; Stuart 1984; Wells 1986), and because some have undergone historical decreases in alkalinity and pH, purportedly owing to acidic precipitation (Lewis 1982), the effects of mineral acid inputs on aquatic nitrogen cycling are clearly of ecological concern. Inputs of NO_3^- (as HNO_3) in acidic precipitation conceivably could ameliorate nutrient constraints on algal growth (see Paerl 1985) or compensate for any adverse effect of lake acidification on nitrogen assimilation (*cf.*, Merezhko *et al.* 1986). However, an increased reliance

by nitrogen-limited phytoplankton on atmospherically derived NO_3^- (or a decreased reliance on regenerated NH_4^+) would tend to reduce internal (*i.e.*, biological) control over phytoplankton growth and could have destabilizing effects on lake productivity (*cf.*, Perry *et al.* 1989). The documentation of such effects would imply that lake metabolic processes are more sensitive to acidification than heretofore acknowledged (see Schindler 1985, 1988a).

Research Objectives

The major objectives of this study were, first, to quantify the influences exerted by internal and external nitrogen supply processes on the biological productivity of a representative alpine lake, second, to identify the food web components contributing most significantly to regenerative and assimilative fluxes of nitrogen within said lake and, third, to evaluate the constancy of the limnetic nitrogen cycle under unusually severe environmental (pH) conditions. Specifically, this study attempted to:

1) Assess the importance of nitrogen as a potentially growth-limiting nutrient within the lake and document any stimulatory (or inhibitory) influences of nitrogen-bearing snowmelt or inflowing stream water on algal photosynthesis.

2) Determine the relative affinities of phytoplankton for regenerated versus nonregenerated nitrogenous nutrients.

3) Quantify *in situ* rates of NH_4^+ regeneration and uptake within the lake water column, documenting the extent to which regenerative processes provided for the nitrogen requirements of phytoplankton.

4) Compare rates of NH_4^+ regeneration and uptake between various size classes of plankton and between eucaryotic and procaryotic organisms present within the water column.

5) Examine the short-term (hour to day) effects of pH reduction on *in situ*, size-fractionated rates of NH_4^+ regeneration and uptake, validating all field findings through carefully controlled and replicated laboratory experiments.

6) Determine the physiological basis for the effects alluded to in (5), above.

7) Determine the influences exerted by different mineral acids and mineral acid combinations on inorganic nitrogen uptake and CO_2 fixation, giving particular attention to any stimulatory effects resulting from HNO_3 enrichment.

8) Ascertain the probable long-term effects of pH reduction on within-lake nitrogen cycling processes.

Organization of Report

The remainder of this report is presented in five chapters. Chapter 2 provides a general description of the major geographical, geological, climatological and biological features of the study area. Chapter 3 describes the experimental methods and routine data collection procedures employed in the study. Chapter 4 provides a general overview of the field and laboratory findings, and Chapter 5 presents a detailed discussion of these findings. Finally, Chapter 6 summarizes the major conclusions stemming from this research.

CHAPTER 2

STUDY AREA

Location and Physical Description

Numerous alpine and subalpine lakes occur within the Beartooth Mountains of southcentral Montana and northwestern Wyoming. Based on recent limnological surveys of this mountain range (Marcuson 1980a-g; Eilers *et al.* 1986; Landers *et al.* 1986), on previous lakes studies conducted in the region (Falter 1966; Wells 1986) and on a field reconnaissance performed by the author in June 1985, Snowbank Lake (latitude 45°2'48" north, longitude 109°29'24" west) appeared to be representative of the range's higher elevation (>3,000 m), lower alkalinity (<200 μeq $CaCO_3$ l^{-1}) water bodies and to provide a logistically feasible field setting for the present study.

Snowbank Lake is one of several alpine lakes in the Hell Roaring Creek watershed of southwestern Carbon County, Montana (Figure 1). The watershed comprises a portion of the Absaroka-Beartooth Wilderness and falls under the administrational jurisdiction of the U.S. Forest Service (Custer National Forest; Red Lodge, Montana, headquarters). Although motorized travel is forbidden within this wilderness region, Snowbank Lake is directly accessible by hiking trail and lies only 5 km from the nearest road (fair-weather jeep trail) and 25 km from the closest highway (Montana/Wyoming Beartooth Highway). The nearest town is Red Lodge,

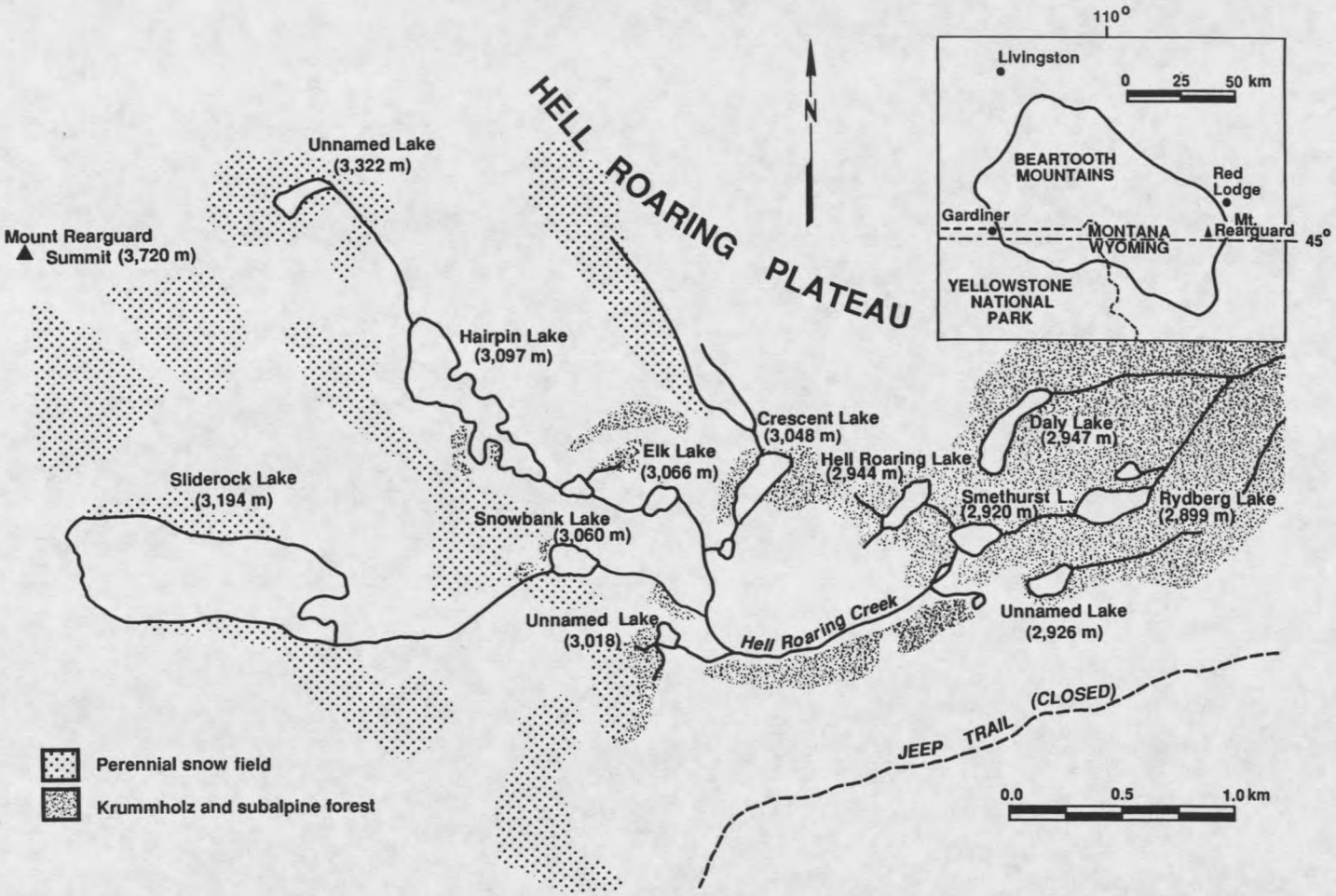

Figure 1. Location and physiographic features of the upper Hell Roaring Creek watershed. Parenthetical values refer to elevation above mean sea level (as determined by Marcuson 1980d).

Montana, located approximately 45 km (by trail and by road) to the northeast.

Snowbank Lake is situated in a glacial valley rock-basin (see Hutchinson 1957) at an elevation of 3,060 m on the southeastern flank of Mount Rearguard (summit elevation 3,720 m). The lake is approximately 3.6 ha in surface area and 11 m deep at its deepest point. Rockslides along the southern shoreline have produced boulder and rubble-dominated substrata to a depth of at least 8 m. Elsewhere, shoreline substrata variably consist of silt, sand, gravel, rubble, boulders and bedrock. Deposits of grey-green copropel have accumulated in the deepest recesses of the lake. A depth contour map is provided in Figure 2; further morphometric details are summarized in Table 1.

Table 1. Snowbank Lake morphometric characteristics.

Surface area	3.6 ha
Maximum length	301 m
Maximum breadth	194 m
Mean breadth	119 m
Shoreline length	889 m
Shoreline development	1.32
Maximum depth	11 m
Mean depth	4.0 m
Mean depth:maximum depth ratio	0.36
Relative depth	5.1%
Volume	1.44×10^5 m^3
Volume development	1.08
Drainage basin area	12.1 ha
Drainage basin area:lake surface area ratio	3.36
Hydrological turnover time (ice-free season)	5-20 days

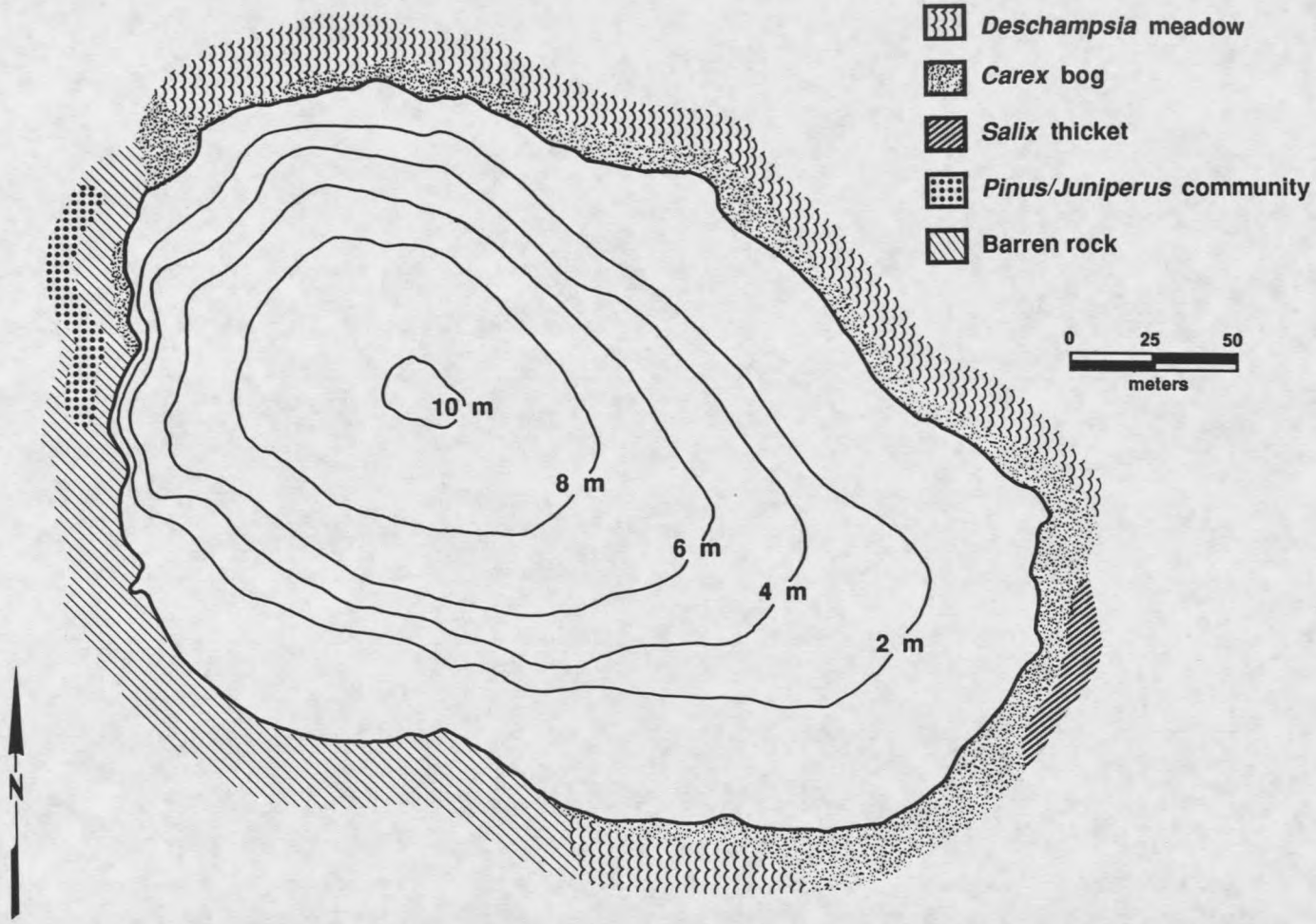

Figure 2. Snowbank Lake bathymetric contours and shoreline vegetation. Contours were determined by triangulation (see Wetzel and Likens 1979) on 25 August 1985; the lake perimeter was determined from aerial photograph (ASCS; 30 July 1971).

Although Snowbank Lake receives runoff from snow fields throughout the ice-free season, a small waterfall on the steep, southwestern bank constitutes a more substantial influent, especially during drier years. The latter influent is derived primarily from subterranean (*i.e.*, boulder field) drainage from Sliderock Lake, a large (32.8 ha), deep (75 m maximum depth) cirque lake located at the head of the watershed (lake elevation 3,194 m). Snowbank Lake drains into a small, unnamed stream which flows east-southeast for 0.6 km to Hell Roaring Creek (Figure 1). The water level of Snowbank Lake is relatively stable, fluctuating less than 10 cm during most open-water seasons.

Geology

Located along the northwestern margin of the Big Horn Basin, the Beartooth Mountains comprise an uplifted mass of northwest-trending, exposed Archean rock, 100 km long by 50 km wide. Relief ranges from approximately 600 m along the southwestern border to over 2,100 m in the northeastern region. The core of these mountains is complexly fractured and consists primarily of granitic gneiss, flanked by metasediments and migmatites with basaltic and metabasaltic intrusions of several ages and felsic porphyry dikes, sheets and stocks of early Laramide age (Eckelmann and Poldervaart 1957; Mueller and Wooden 1982; Mueller *et al.* 1982). Over much of the Beartooth massif, including the Hell Roaring Creek vicinity, glaciation has resulted in a deeply dissected relief and in the formation of numerous lake basins. Poorly consolidated materials have accumulated within many glacial valleys, contributing to rockslides which

commonly extend beneath lake surfaces (*e.g.*, Snowbank and Sliderock lakes).

Soil parent materials in alpine regions of the Beartooth Mountains are thin, heterogeneous and poorly weathered and originate primarily from crystalline rock. In general, soils derived from these materials have low inherent fertility and are excessively drained, coarsely textured, acidic, low in exchangeable base content, and impregnated with much stone, cobble and gravel (*e.g.*, Retzer 1974). On the alpine plateaus, local soil characteristics vary considerably with differences in vegetation, moisture availability, burrowing activity of animals, wind disturbance, insolation, slope, aspect, and cryopedogenic processes. Although profiles with A-B-C horizons occur in some alpine turf soils (see *Watershed Vegetation* section, this chapter), most have only A-C profiles with little vertical differentiation (Johnson and Billings 1962). Permafrost is common throughout alpine regions of the Beartooth Mountains and is closest to the soil surface in damp, peaty locales, such as along the southeastern periphery of Snowbank Lake (see *Watershed Vegetation* section, this chapter).

Climate

In alpine regions of the central Rocky Mountains more than two-thirds of the yearly precipitation may fall as snow, and accumulations from individual winter storms may approach 1 m. Summer and autumn months are relatively dry with most precipitation falling during brief, low intensity showers. Snowfall may occur on any day of the year, and frost

is common on summer nights. Average monthly wind velocities of 13 m s^{-1} are not uncommon; during winter, gusts may exceed 45 m s^{-1} (Retzer 1974).

Weather in the Beartooth Mountains is not monitored routinely above 2,000 m. However, Falter (1966), extrapolating from the data of Larson (1930), the USDA (1941), Baker (1944) and Johnson and Billings (1962), arrived at the following climatological summary for the Beartooth Plateau (elevation 2,911 m): growing season (days above 5.6°C), 42 days; average annual air temperature, -7.8°C; average daily maximum temperature for July, 18.3°C; average daily minimum temperature for January, -21.6°C; total annual precipitation (rainfall equivalent), 69-77 cm; total annual snowfall, 432 cm; average winter wind velocity, 7.5 m s^{-1}; average summer wind velocity, 3.9 m s^{-1}; average annual cloud cover, 55%; and average August cloud cover, 40%. The length of the alpine open-water season is variable among lakes and among years. Although many alpine lakes in the Beartooth Mountains are free of ice from late June to early October, higher elevation (3,300-3,400), sheltered lakes may be open for less than 2 weeks in some years (Marcuson 1978, as cited by Wells 1986).

According to Schaller (1988), yearly snowfall accumulations in the eastern Beartooth Mountains entered a declining trend in 1980 and approached the 30-year low in 1986 and 1987. In the Snowbank Lake vicinity, snow fields were reduced to discontinuous patches during the summers of 1985-87. In each of these years, Snowbank Lake's open-water season extended from the third or fourth week of June to the first or second week of October.

Watershed Vegetation

The Beartooth Mountains comprise an alpine flora of great diversity. Lackschewitz (1984) lists 386 species of alpine vascular plants indigenous to this region, more than reported for any other North American mountain range (Anderson 1984). As discussed by Johnson and Billings (1962), alpine vegetation in the Beartooths is composed of recognizable plant associations which intergrade along environmental (*e.g.*, soil) gradients. Four community types are prominent, including (1) the *Geum* turf, (2) the *Deschampsia* meadow, (3) the *Carex* bog and (4) the *Salix* thicket.

Geum turfs develop on alpine summits, ridges and upper slopes and are dominated by *Geum rossii* (R.Br.) Ser. Various sedges (especially *Carex elynoides* Holm) are locally abundant, as well as *Silene acaulis* L., *Trifolium nanum* Torr., *Artemisia scopulorum* Gray, *Lupinus argenteus* Pursch, *Smelowskia calycina* (Steph.) C.A. Mey, *Polygonum bistortoides* Pursh and *Dryas octopetala* L. *Deschampsia* meadows occur on well drained soils of sheltered uplands, lower mesic slopes, and basins. In these communities, *Deschampsia cespitosa* (L.) Beauv. is the dominant species. Subdominants include *Carex scopulorum* Holm, *P. bistortoides*, *Trifolium parryi* Gray, *Salix arctica* Pall., *Poa alpina* L. and *Potentilla diversifolia* Lehm.

Carex bogs develop on wet alpine soils, such as occur immediately downhill from perennial snow fields. *C. scopulorum* is the dominant member of these communities. Other common species include *Eriophorum callitrix* Cham., *Juncus castaneus* J.E. Smith, *J. biglumis* L., *Polygonum*

viviparum L., *Agrostis humilis* Vasey, *Koenigia islandica* L., *Epilobium alpinum* L., *P. alpina*, *Saxifraga oregana* Howell, *D. cespitosa* and *Ranunculus natana* Meyer. *Salix* thickets occur in alpine valley bottoms, often completely covering such areas with woody growth 25-50 cm in height. *Salix phylicifolia* L. is the most common species, but *S. brachycarpa* Nutt. and *S. glauca* L. occur near the subalpine transition. Certain species, such as *Viola adunca* Sm., are restricted in distribution to willow thickets; in addition, many of the bog species mentioned previously are represented in thicket communities.

A few other forms of vegetation are common in the Beartooth's alpine regions. Lichens, for example, are ubiquitous components of the alpine landscape, growing on rocks, soil and other suitable substrata. Liverworts occur along rivulets draining snow fields. Mosses are represented in each of the major plant communities; however, they are most abundant in *Carex* bogs, where they contribute significantly to the formation of peaty soils. Finally, isolated communities of stunted, wind-flagged trees exist in many sheltered alpine locations. These tree communities may contain one or more of the following species: *Juniperus communis* L., *Abies lasiocarpa* (Hook.) Nutt., *Picea engelmannii* Parry ex. Engelm., *Picea glauca* (Moench) Voss and *Pinus albicaulis* Engelm. (Lackschewitz 1984).

Each of the major community types is represented in the Snowbank Lake drainage basin. A damp area near the southeastern lake margin supports a well developed bog community, willow thickets extend from near the eastern periphery of the lake to a location far downstream, and the ridge to the north of Snowbank Lake supports *Deschampsia* meadow, grading

into *Geum* turf near the ridge top. Although the steep slopes descending to the western and southern shorelines of the lake are comprised primarily of barren rubble, boulders and bedrock, an isolated community of *J. communis* and *P. albicaulis* occurs on a sheltered ledge above the western shore (Figure 2). Vascular hydrophytes are absent from the study area.

General Aquatic Biology

Biological characteristics of alpine lakes in the Beartooth Mountains are known primarily from a few descriptive limnological studies (*e.g.*, Falter 1966; Wells 1986) and fishery surveys (Marcuson 1980a-g). Available data on algal biomass and carbon fixation rates and on limnetic and benthic faunal density suggest that the productivity of the majority of these lakes is very low (conditions ranging from oligo-mesotrophic to ultraoligotrophic). In contrast, biological (especially phytoplankton) diversity is relatively high. Phytoplankton communities tend to be dominated by diatoms during the open-water season; however, desmids, dinoflagellates, cyanophytes and various other algae may at times contribute significantly to community biomass. Zooplankton assemblages generally are dominated by cladocerans and calanoid copepods, and the classic *Daphnia-Diaptomus* association (see Dodds 1917) is common; moreover, most of the alpine lakes appear to contain several species of limnetic rotifers. Benthic macroinvertebrate communities are dominated by chironomid larvae, sphaerid clams, and oligochaete worms. Littoral macrophytes (and organisms dependent upon such vegetation) are exceedingly rare in alpine waters of the Beartooth Mountains.

Although originally barren, many lakes in the Beartooth Mountains now contain fish as a result of private and governmental stocking efforts. Anderson (1984) estimates that 35% of the Beartooth's lakes support fisheries (either periodically restocked or entirely self-sustaining). Of the remaining lakes, 40% are believed to be incapable of supporting viable fish populations, and 25% are maintained intentionally in a natural (barren) state. Fish species present in alpine waters of the Beartooth Mountains include *Salvelinus fontinalis* (Mitchill), *Salmo clarki* Richardson, *S. gairdneri* Richardson, *Thymallus arcticus* (Pallus) and *S. aquabonita* Jordan. The latter two taxa are rare in the Beartooth Mountains, whereas *S. fontinalis*, the most common alpine species, occurs in as many as 13% of the region's lakes (see Anderson 1984).

Other than the data collected over the course of the present study, little information is available on the biological attributes of Snowbank Lake. However, this lake is known to contain a self-sustaining, little exploited, stunted population of *S. fontinalis* (Marcuson 1985), apparently derived from private stocking efforts in the 1930's and supplemented by occasional "swimdowns" from Sliderock Lake (Frazier 1989; see also Figure 1, this report). The lake also contains a conspicuous assemblage of epilithic diatoms, and luxuriant growths of filamentous green algae adhere to the submerged bedrock surface along the steep western shoreline (Figure 2). Further details on the biology of Snowbank Lake are provided in Chapter 4 and in the appendix (tables 28-30).

CHAPTER 3

METHODS

The experiments performed in this study comprised three general categories, including (1) algal limiting-nutrient bioassays, (2) inorganic nitrogen uptake and NH_4^+ isotope dilution experiments, and (3) acidification/physiological response experiments. Limiting-nutrient bioassays evaluated the potential stimulatory effects of nitrogen and phosphorus additions (and precipitation and snowmelt enrichment) on phytoplankton primary production (objective 1; Chapter 1). Nitrogen uptake and isotope dilution experiments focused on the substrate kinetics of NH_4^+ and NO_3^- uptake, on the size-fractionated time-course of NH_4^+ and NO_3^- uptake and NH_4^+ regeneration, on the importance of regenerated NH_4^+ as a potential source of nitrogen for phytoplankton growth and metabolism, and on the relative importance of procaryotic versus eucaryotic plankton as agents of NH_4^+ regeneration (objectives 2-4). Finally, the acidification/response experiments examined the effects of pH reduction on the size-fractionated uptake and regeneration of NH_4^+, on the time course of NH_4^+ and NO_3^- uptake and NH_4^+ regeneration, on inorganic carbon fixation, chlorophyll *a* production and photosynthate partitioning by phytoplankton, on bacterioplankton secondary production, and on other parameters of physiological and ecological interest (objectives 5-8).

This chapter describes the methods and materials used in these experiments and in the routine collection of lake physicochemical and

biological data. All field and laboratory experiments and supporting limnological measurements discussed in this chapter were conducted during the 1985-87 open-water seasons.

Sampling Procedures and Routine Measurements

Sampling Procedures

Water was sampled at selected depths in the lake's central basin with the aid of an inflatable raft and a 2-liter PVC Van Dorn sampler. Samples for measurement of pH, alkalinity and specific conductance were collected from a depth of 2 m. Additional samples were collected at 2-m depth intervals and from inlet and outlet streams and later analyzed for NH_4^+, NO_3^-, soluble reactive phosphorus (SRP), particulate carbon and nitrogen and chlorophyll *a* (discussed below). Linear polyethylene (LPE) containers were used for the collection, transport and storage of all water chemistry samples. These containers were cleaned prior to each field trip by rinsing twice with 80°C tap water, twice with 0.1 N HCl and 6 times with freshly deionized water; in addition, all containers were rinsed with lake water immediately before sample collection (*cf.*, Fitzwater *et al.* 1982).

Depth-integrated zooplankton samples were obtained by vertical tows with a conical net (63-μm mesh), equipped with a solid cod end to minimize abrasive damage of specimens. Additional zooplankton samples and all phytoplankton samples were collected from a depth of 2 m using a 2-liter Van Dorn sampler (1985-87) or a hose sampler designed for this study (1987 only). The hose sampler consisted of a 2.5-m length of flexible polyethylene tubing, fitted on one end with a glass funnel and

attached by the opposite end to the stoppered opening of a 20-liter LPE carboy. A second, shorter length of tubing extended from the carboy to the air intake of a heavy-duty foot pump. The funnel end of the longer tube was lowered over the side of the raft to a depth of 2 m; manual operation of the pump forced water upward and into the carboy at 2-4 liters min^{-1}. The diameter of the tubing (0.5-cm I.D.) and the funnel stem (0.4-cm I.D.) accommodated even the largest zooplankton in Snowbank Lake (see *Plankton Biomass and Community Composition*, Chapter 4; *cf.*, Boltovskoy *et al.* 1985).

Routine Measurements

Water-Column Transparency. A standard 20-cm black and white Secchi disc was used to measure the lake's transparency. Secchi measurements were conducted between 1000 and 1400 hours MDT, unless severe weather conditions dictated otherwise. All measurements were made on the shaded side of the raft, and the mean of the disappearance and reappearance depths was recorded.

Temperature and Dissolved Oxygen. Temperature and dissolved O_2 were measured at 1-m depth intervals with a YSI model 57 portable meter, coupled to a YSI combination thermistor/O_2 membrane probe. Thermistor readings periodically were checked against those of an NBS-traceable reference thermometer. The O_2 probe and meter were air calibrated in the field immediately before use in accordance with manufacturer's instructions.

Alkalinity and pH. Measurements of pH were performed in the field on freshly collected, duplicate samples using a Corning model 103 portable meter and an Orion model 91-05 combination glass/calomel reference electrode, following standardization with NBS-traceable commercial buffers (pH 7.0 and pH 4.0). Probe response time and slope were monitored throughout the study: probes failing to provide 98.0-100.0% of the theoretical Nernstian slope or a stable response (<0.01 pH unit drift min^{-1}) within 5 min were replaced. Methods used for pH measurement and electrode maintenance conformed to those of Pickering (1980). Alkalinity titrations were conducted potentiometrically (Corning meter and Orion probe) on 100-ml samples using a 0.020 N HCl titrant dispensed by microliter burette. Total-inflection-point alkalinity was calculated via standard graphical procedures (Stumm and Morgan 1981).

Specific Conductance. Measurements of specific conductance were performed at the Montana State University (MSU) limnological laboratory within one day of sample collection, using a YSI model 32 meter and YSI model 3403 conductivity cell. Conductivity meter/cell performance was evaluated by measuring the resistance of a KCl solution standard, and cell constant was redetermined periodically (APHA 1975).

Dissolved Inorganic Nutrients. Dissolved nutrient analyses were performed on samples filtered under low vacuum (approximately 10 cm Hg) through precombusted (475°C; 24 hr) Whatman GF/C filters. Filtrate was stored on ice in darkness during transport to the MSU laboratory, then frozen (-20°C) pending colorimetric analysis. SRP was analyzed via the blue sol-forming reduction of phosphomolybdate complexes by ascorbic acid

(Murphy and Riley 1962; Stainton *et al.* 1977). Ammonium concentrations were determined by the blue indophenol reaction between NH_4^+, hypochlorite and alkaline phenol (Solórzano 1969, as modified by Priscu 1982). Concentrations of NO_3^- + NO_2^- were measured via the red diazotization reaction between NO_2^-, sulfanilamide and N-1-napthylethylenediamine dihydrochloride, following hydrazine reduction of NO_3^- (Kamphake *et al.* 1967, as modified by Priscu 1982). At intervals during the study, tests were conducted which excluded the hydrazine reduction step to provide a measure of NO_2^- concentration; levels of NO_2^- were consistently below the minimum limit of detection (0.03 μM).

All dissolved nutrient samples were analyzed in duplicate. Standards comprised 25% of all nutrient samples, and spikes comprised an additional 5%. Recovery efficiencies for SRP and NH_4^+ averaged approximately 98% during the course of the study; NO_3^- recoveries averaged only 80%, requiring that apparent concentrations be multiplied by a factor of 1.25 (Table 2). Reagent grade chemicals and ASTM Type I quality (18 megohm) water were used in the preparation of all stock solutions and standards.

Table 2. Accuracy and precision of analytical methods used for determination of dissolved inorganic nutrient concentrations in Snowbank Lake.

Parameter	Spike recovery (mean % ± SD)	Replicability (mean CV ± SD)	Number of replicate groups
NH_4^+	97.8 ± 3.5	5.4 ± 1.7	5
NO_3^-	80.0 ± 4.3	6.9 ± 1.2	5
SRP	97.6 ± 5.7	2.8 ± 0.7	5

Particulate Carbon and Nitrogen. Particulate carbon (PC) and particulate nitrogen (PN) samples were obtained by filtering lake water (250 ml sample^{-1}) through precombusted GF/C filters under low vacuum (10 cm Hg). Filters were folded in half to prevent the loss of concentrated seston, wrapped in aluminum foil, stored on ice during transfer to the laboratory, and frozen (-20°C) pending determination of PC and PN content. Analyses were conducted via flash-combustion gas chromatography (Carlo Erba model 1106 elemental analyzer) using procedures similar to those of Hilton *et al.* (1986). However, in an attempt to increase the service life of the quartz combustion columns and to prevent their accidental clogging, a 7-mm diameter subsample was excised from each filter (using an acid-cleaned cork borer) and analyzed. The PC and PN loadings on filter discs were representative of loadings on entire filters (Table 3). All PC and PN analyses were standardized relative to known masses of sulfanilamide (1985 only) or acetanilide (1986-87), measured to ± 1 μg with a Cahn model 29 electrobalance; moreover, all PC and PN data were corrected for background carbon and nitrogen contamination of filters and encapsulating tin foil (combustion catalyst).

Chlorophyll *a*. Chlorophyll *a* samples were obtained by filtering lake water through GF/C filters under low vacuum (10 cm Hg). Filters were folded, wrapped in aluminum foil, stored on ice during transit to the laboratory, and frozen (-20°C) pending chlorophyll *a* determination. Analyses were conducted fluorometrically (Turner model 111 fluorometer) on samples homogenized in a glass/teflon tissue homogenizer and extracted

Table 3. Precision of filter-subsample method for determination of particulate nitrogen and particulate carbon concentrations. Four 7-mm diameter discs were moved from each of 3 filters (precombusted Whatman GF/C; 25-mm dia.) and analyzed via flash-combustion gas chromatography. Seston contained on each filter was derived from the same lake water sample. The far right column provides a measure of variability between sample aliquots. Note influence of sample volume on precision of method.

Parameter	Filter 1	Filter 2	Filter 3	All filters
Particulate Nitrogen				
Mean concentration (μM)	5.7	4.6	4.5	4.9
Standard deviation (μM)	0.8	0.3	0.2	0.7
Coefficient of variation (%)	14.0	6.5	4.4	14.3
Particulate Carbon				
Mean concentration (μM)	45.2	39.4	51.4	45.3
Standard deviation (μM)	7.3	3.6	3.8	6.0
Coefficient of variation (%)	16.2	9.1	7.4	13.2
Sample volume filtered (ml)	196	250	250	--

(4°C; darkness) overnight in 90% acetone (Lorenzen 1967). Concentrations were corrected for phaeopigment interference by measuring fluorescence both before and after the acidification of acetone extracts with 1 N HCl (1% v:v). Standard solutions were prepared by diluting pure *Anacystis nidulans* chlorophyll *a* (Sigma) in 90% acetone. Stock concentrations were confirmed spectrophotometrically using the following monochromatic equation (*cf.*, Lorenzen 1967):

$$C = (E_a - E_b) \frac{\tau}{\tau - 1} \frac{1{,}000}{SAC} \frac{v}{V\,l} \qquad (1)$$

where C = concentration of chlorophyll *a* ($\mu g\ l^{-1}$)

E_a = pre-acidification extract absorbance at 665 nm minus absorbance at 750 nm

E_b = post-acidification extract absorbance at 665 nm minus absorbance at 750 nm

r = 1.7 = maximum acid ratio ($E_a:E_b$) for phaeopigment-free chlorophyll *a* standard in 90% acetone

SAC = 89 $l \cdot g\ cm^{-1}$ = specific absorption coefficient at 665 nm for chlorophyll *a* in 90% acetone

v = volume of extract (ml)

V = volume of lake water filtered (l)

l = path-length of cuvette (cm).

Plankton Identification and Enumeration. Immediately after collection, phytoplankton samples were preserved with Lugol's solution (1% v:v), and zooplankton samples were preserved with Kahle's solution (0.4% v:v; 1985 only) or with buffered formalin (4% v:v; 1986-87), as recommended by Steedman (1976) and Wetzel and Likens (1979). Samples were stored in darkness at 1-4°C pending identification and enumeration of specimens. Phytoplankton samples (100 ml each) were concentrated by settling in 20-cm sedimentation chambers for 5-7 days, then counted with the aid of a Zeiss inverted microscope (Lund *et al.* 1958). Net-concentrated samples were examined qualitatively for crustacean zooplankton using a dissecting microscope; for more difficult identifications, a compound microscope was used following glycerin clearing of specimen soft body tissues (Brooks 1957).

Limiting-Nutrient Bioassays

Nutrient Enrichment Experiments

These laboratory experiments examined the effects of nutrient additions on $^{14}CO_2$ uptake by Snowbank Lake phytoplankton. They were commenced upon return from the field using lake water collected 6 hr earlier from a depth of 2 m (Van Dorn sampler, 1986; hose sampler, 1987) and transported (in darkness, at prevailing lake water temperature ± 2°C) in a 20-liter LPE carboy. Treatments (in triplicate; 250-ml samples in LPE bottles) included unamended lake water controls and samples augmented with PO_4^{3-} (0.3 μM as KH_2PO_4), NH_4^+ (5.0 μM as NH_4Cl) or, on one occasion (7 September 1987), both nutrients simultaneously. Parallel treatments were performed on lake water screened through 80-μM mesh Nitex netting to remove the large colonial alga, *Volvox tertius* Meyer (see *Plankton Biomass and Community Composition*, Chapter 4). All samples were preincubated at 300 $\mu E\ m^{-2}\ s^{-1}$ (GE soft-white) and the prevailing lake temperature for approximately 24 hr, then inoculated with 2 μCi of ^{14}C-$NaHCO_3$ solution and incubated for an additional 6-18 hr. Incubations were terminated by filtration (GF/C). Filters containing ^{14}C-labeled seston were transferred to glass scintillation vials, to which 10 ml of Scinterverse E scintillation cocktail (Fisher) were added. The ^{14}C activity of the concentrated seston was determined via standard liquid scintillation spectrometry (Steeman Nielsen 1951, 1952; Wetzel and Likens 1979); activity measurements were converted from counts min^{-1} (cpm) to disintegrations min^{-1} (dpm) through the sample channels ratio method,

using acetone as the quenching agent and ^{14}C-toluene as the radioactivity standard (*cf.*, Schindler and Holmgren 1971).

In a related experiment, the stimulatory effects of major nitrogenous nutrients on ^{14}C uptake were compared. Treatments (triplicate 100-ml samples in 250-ml LPE containers) included unamended lake water controls and lake water samples augmented with 3.6 μM nitrogen, in the form of NH_4^+, NO_3^- or urea. Following preincubation (300 $\mu E\ m^{-2}\ s^{-1}$, prevailing lake temperature, 24 hr), each sample was inoculated with 1 μCi of ^{14}C-$NaHCO_3$ solution, incubated (preincubation conditions) for 11 hr, and filtered (GF/C). The ^{14}C activity of seston retained on filters was assayed by liquid scintillation spectrometry.

Snowmelt Enrichment Experiments

These laboratory experiments examined the stimulatory effect of snow field runoff and snowfall on $^{14}CO_2$ uptake by phytoplankton. Meltwater was collected in LPE containers from small streams flowing through snow caves (June 1987) or draining the surfaces of snow fields (September 1987). On one occasion (September 1987) freshly fallen snow was collected in clean LPE containers (see *Sampling Procedures*, this chapter) and permitted to melt (0-2°C) during transit to the laboratory. All meltwater and snow collections were made within 50 m of the lake shoreline. Treatments for the June experiment included unamended lake water controls and a lake water:meltwater dilution series of 7:1, 3:1, 1:1, 1:3, and 1:7. Snowmelt was not filtered in this experiment, and it was believed that the presence of particulate matter, including snow algae (*Chlamydomonas* spp.; see Smith 1950) might have affected the

bioassay results. Hence, the September experiment included unamended lake water controls and a lake water:filtered (muffled GF/C) meltwater series (3:1, 1:1, 1:3), in addition to a lake water:nonfiltered meltwater treatment (3:1) for comparison. Also, a treatment comprised of lake water and nonfiltered water from freshly melted snow (3:1 ratio) was included in the September experiment. Samples (3 per treatment, 200 ml each, in clear plastic flasks) were inoculated with 2 μCi of ^{14}C-$NaHCO_3$ and incubated 25 hr (June) or 14 hr (September) at 300 $\mu E\ m^{-2}\ s^{-1}$ and the prevailing lake temperature. Incubations were terminated by filtration, and the ^{14}C activity of the concentrated seston was determined by standard scintillation spectrometry. Seston ^{14}C activities, expressed as percent control activity, were corrected for differences in pH (*i.e.*, $^{14}CO_2$ specific activity) and phytoplankton density between dilution treatments (see Table 4 and *Comparative Effects of Mineral Acids on Inorganic Carbon Uptake*, this chapter).

Table 4. Chemical comparison of lake water, snow field runoff, snow, and ^{14}C-$NaHCO_3$ inoculant used in 2 September 1987 meltwater enrichment experiment.

Chemical parameter	Medium			
	Lake water	Runoff	Snow	Inoculant
pH (at 25°C)	7.0	6.8	6.7 (unstable)	9.6
Alkalinity (meq l^{-1}, $CaCO_3$)	124	22	18	1,430
Dissolved inorganic C (μM)	158	33	25	1,292
NH_4^+ (μM)	0.08	6.31	6.76	presumed nil
NO_3^- (μM)	0.78	8.01	4.53	presumed nil
SRP (μM)	0.12	0.54	0.14	presumed nil

Nitrogen Uptake and Isotope Dilution Experiments

Substrate Kinetics of NH_4^+ and NO_3^- Uptake

The substrate kinetics of NH_4^+ and NO_3^- uptake by natural phytoplankton assemblages were examined on 8 occasions during the study. On each occasion, a 20-liter lake water sample was collected from a depth of 2 m by repeated use of a 2-liter Van Dorn sampler (1985-86) or by use of the hose sampler (1987). The carboy containing the sample was transferred immediately to a tent on shore. Aliquots were removed for later determination of NH_4^+, NO_3^-, SRP, chlorophyll *a*, PC and PN concentrations and for later identification and enumeration of plankton. Six 250-ml subsamples were inoculated with progressively larger volumes of 99 atom-% $^{15}NH_4^+$ stock solution (Bio-Rad ^{15}N-$(NH_4)_2SO_4$, 1985-86; MSD ^{15}N-NH_4Cl, 1987) to produce aqueous enrichments of 0.1, 0.4, 0.7, 2.0, 3.3 and 6.7 μM; similarly, 6 subsamples were inoculated with 99 atom-% $^{15}NO_3^-$ stock solution (Stohler ^{15}N-$NaNO_3$) to produce an identical series of concentrations. The inoculated subsamples were incubated at the depth of collection (3 August 1986 only) or at the water surface under neutral density screening (40% surface irradiance). Incubations lasted approximately 5 hr and were terminated by low-vacuum filtration (10 cm Hg; precombusted GF/C). Filters were stored on ice in darkness during transfer to the laboratory, then frozen (-20°C) pending analysis for seston ^{15}N enrichment. The ^{15}N analyses involved conversion of all particulate nitrogen to N_2 via Dumas combustion (Priscu 1984), followed by ^{15}N:^{15}N + ^{14}N ratio (or ^{15}N atom-%) determination by optical emission spectrometry (Timperly and Priscu 1986). All ^{15}N atom-% measurements were

made relative to $^{15}N/^{14}N$ serine standards of known isotopic composition (Figure 3).

For each NH_4^+ and NO_3^- experimental concentration (spike plus ambient concentration), uptake rates were normalized to PN concentration using the following model (*cf.*, Dugdale and Wilkerson 1986; see also *Limitations of Nutrient Uptake Models*, Chapter 5, this report):

$$P = \frac{^{15}N_{xs}\ PN}{(^{15}N_{enr} - {}^{15}N_s)\ t} \qquad (2)$$

where P = uptake rate ($\mu M\ hr^{-1}$)

PN = particulate nitrogen concentration (μM)

$^{15}N_{enr}$ = $^{15}N:^{15}N + ^{14}N$ ratio of NH_4^+ or NO_3^- fraction at start of incubation

$^{15}N_s$ = $^{15}N:^{15}N + ^{14}N$ ratio of particulate nitrogen and end of incubation

$^{15}N_{xs}$ = $^{15}N_s$ minus the naturally occurring $^{15}N:^{15}N + ^{14}N$ ratio (0.00365)

t = duration of incubation (hr).

Ammonium uptake rates calculated via equation 2 required correction for changes in $^{15}NH_4^+$ specific activity (*i.e.*, isotope dilution) resulting from $^{14}NH_4^+$ regeneration within the experimental vessel (see Garside 1984). The following equation from Kanda *et al.* (1987) was employed:

$$\frac{P_c}{P} = \frac{-1 + (1 - \frac{P\ t}{S})^{1-\frac{r}{P_c}}}{\frac{r\ P\ t}{P_c\ S} - \frac{P\ t}{S}} \qquad (3)$$

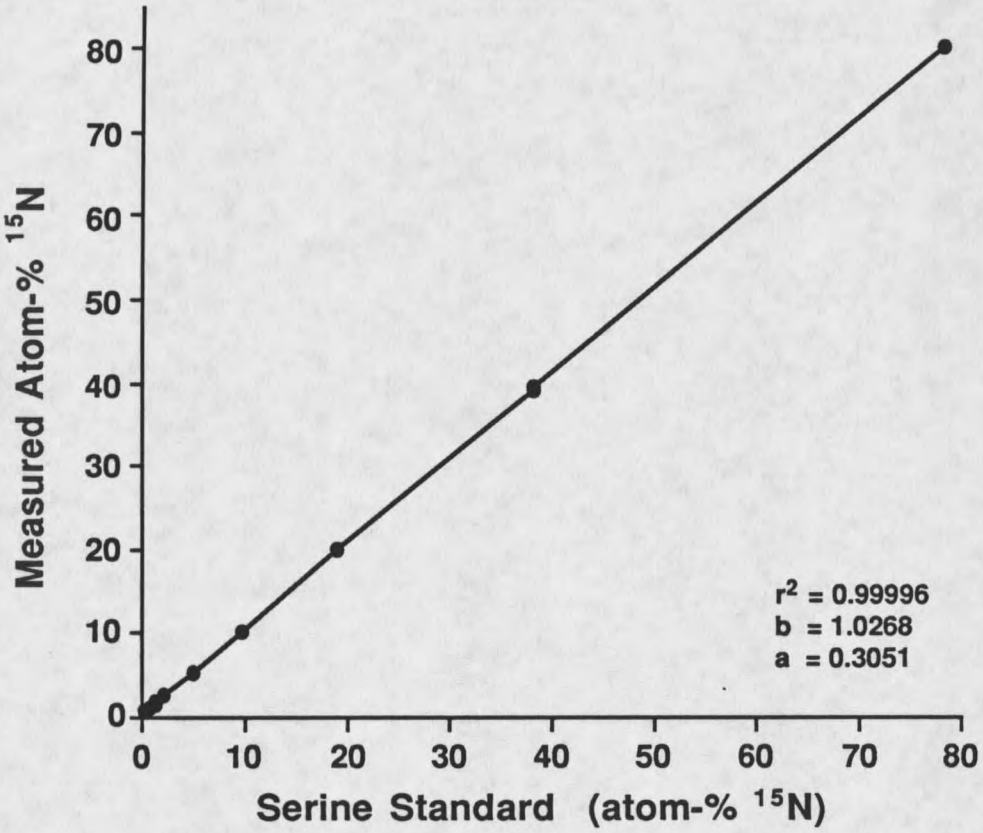

Figure 3. Typical standard curve for ^{15}N atom-% measurements performed by optical emission spectrometry.

where P_c = NH_4^+ uptake rate (μM hr^{-1}) corrected for isotope dilution

r = NH_4^+ regeneration rate (μM hr^{-1}; discussed below)

S = NH_4^+ concentration (μM) at start of incubation.

Rather than assign an arbitrary value to the ratio $r:P_c$ to facilitate the analytical solution of equation 3 (*cf.*, Kanda *et al.* 1987), r was determined empirically through concurrent regeneration time-course experiments or acidification-response experiments (*i.e.*, control treatments; see below). Hence, the equation could be rewritten in the form:

$$0 = \frac{-1 + (1 - k_1)^{1-\frac{k_2}{P_c}}}{\frac{k_1 k_2}{P_c} - k_1} - \frac{P_c}{k_3} \qquad (4)$$

where $k_1 = P\ S^{-1}\ t$, $k_2 = r$, and $k_3 = P$. The root of this function was solved numerically using a bisection-based technique, similar to that described by Press *et al.* (1986).

Substrate kinetic constants (K_t and P_{max}) for volumetric NO_3^- uptake and for volumetric regeneration-corrected NH_4^+ uptake were calculated numerically by applying Marquardt's algorithm to the following equation (see Michaelis and Menten 1913; *cf.*, Li 1983):

$$P_c = P_{max} \frac{S}{K_t + S} \qquad (5)$$

where P_c = uptake rate (μM hr^{-1}) corrected for isotope dilution

P_{max} = maximum dilution-corrected uptake rate (μM)

S = NH_4^+ concentration (μM)

K_t = NH_4^+ concentration at which $P_c = P_{max} \div 2$.

Substrate Competition Experiments: NH_4^+ versus NO_3^-

Effects of NH_4^+ Concentration on NO_3^- Uptake. This laboratory experiment examined the influence of NH_4^+ concentration on phytoplankton uptake of NO_3^-. It was commenced upon return from the field, using lake water collected 6 hr earlier from a depth of 2 m (hose sampler) and transported (in darkness, at prevailing lake water temperature ± 2°C) in a 20-liter LPE carboy. Treatments (in triplicate; 250-ml samples in LPE bottles) included unamended lake water controls, formalin (4% v:v) kills, and samples enriched with 0.7, 1.4, 3.6 or 7.1 μM NH_4^+. Following a 6 hr preincubation at 300 $\mu E\ m^{-2}\ s^{-1}$ and the prevailing lake water temperature (at the time and depth of collection), all treatments were inoculated with 99 atom-% $^{15}NO_3^-$ (0.3 μM added concentration), then incubated for 24 hr under preincubation conditions. Incubations were terminated by filtration (muffled GF/C), and the ^{15}N enrichment of the seston was determined via emission spectrometry.

Effects of NO_3^- Concentration on NH_4^+ Uptake. This experiment was performed concurrently with the experiment described in the preceding paragraph. Experimental conditions were identical, except that the roles of NO_3^- and NH_4^+ were reversed; *i.e.*, treatments included formalin kills and 0, 0.7, 1.4, 3.6 and 7.1 μM NO_3^- enrichments, each inoculated with 99 atom-% $^{15}NH_4^+$ (0.3 μM added concentration).

Time Course of NH_4^+ Uptake and Regeneration

Ammonium uptake and regeneration time-course experiments were performed on 4 occasions in 1987. In each experiment, a 20-liter lake

water sample was collected by hose sampler from a depth of 2 m and transferred immediately to a tent on shore. Aliquots were removed for later analysis of various chemical and biological properties (see *Kinetics of NH_4^+ and NO_3^- Uptake*, this chapter). Three 2-liter subsamples were transferred to 4-liter polyethylene bottles and inoculated with 20 atom-% $^{15}NH_4^+$ for an added concentration of 3.6 μM NH_4^+. Immediately after inoculation, a 250-ml aliquot was removed from each subsample and filtered through a precombusted GF/C filter; both filter and filtrate were retained for later analytical determinations (discussed below). The remaining volume was incubated at the water surface under neutral density screening at 40% surface irradiance. (Incubation periods varied among experiments but in all cases were between 5 and 22 hr.) At intervals during the incubation, 250-ml aliquots were removed and filtered (GF/C). As before, filters and filtrate were retained for chemical analysis. All filters and filtrate samples accumulated during the time-course experiment were stored on ice in darkness pending transfer to the MSU limnological laboratory.

Laboratory determinations of seston PC and PN content were accomplished by subjecting a 7-mm subsample from each filter to flash-combustion gas chromatographic analysis (see *Routine Measurements*, this chapter). Based on this analysis, a section of the filter corresponding to 0.7 μmol of seston nitrogen was subjected to Dumas combustion, then analyzed for ^{15}N enrichment by emission spectrometry (note: combustion tube loadings of 0.7 μmol PN, or 0.35 μmol N_2 following Dumas combustion, were found to provide the strongest emission signal and the highest signal-to-noise ratio; *cf.*, Timperley and Priscu 1986; see also *Kinetics*

of NH_4^+ and NO_3^- Uptake, this chapter). From each filtrate sample, two 10-ml aliquots were removed and analyzed for NH_4^+ content. The NH_4^+ fraction in the remaining volume of filtrate was isolated for isotopic analysis using a molecular sieve-based extraction procedure adapted from Lipschultz (1984). This method entailed the addition of 0.1 mg activated (200°C; 4 hr) zeolite powder (Ionsiv W-85; Union Carbide) per ml of filtrate. The zeolite suspension was mixed thoroughly, and 30 min was allowed for zeolite "entrapment" of NH_4^+. At the end of this period, the suspension was again mixed, and the zeolite-NH_4^+ complex was filtered onto a muffled GF/C filter and dried overnight at 30°C. The efficiency of zeolite as an NH_4^+ scavenging agent was calculated via the following equation:

$$\% \text{ efficiency} = 100\ [1 - ([NH_4^+]_{post} \div [NH_4^+]_{pre})] \qquad (6)$$

where $[NH_4^+]_{pre}$ refers to the aqueous NH_4^+ concentration before zeolite extraction and $[NH_4^+]_{post}$ refers to the aqueous NH_4^+ concentration immediately following extraction. Based on an estimated extraction efficiency of 93% (Table 5), a portion of the NH_4^+-enriched zeolite (corresponding to 0.7 μmol seston nitrogen) was subjected to Dumas combustion and analyzed for ^{15}N enrichment via emission spectrometry. A consistent discrepancy between the expected and observed ^{15}N atom-% enrichment of zeolite was noted following the extraction of $^{15}NH_4^+$-augmented lake water samples of known $^{15}NH_4^+/^{14}NH_4^+$ composition; *i.e.*, the extraction procedure appeared to discriminate between ^{15}N and ^{14}N isotopes. The extent of this discrimination was estimated from the following relation:

$$\% \text{ discrimination} = 100\ [(^{15}N_{exp} - {}^{15}N_{obs}) \div {}^{15}N_{exp}] \quad (7)$$

where $^{15}N_{exp}$ refers to the expected ^{15}N atom-% enrichment of the zeolite, and $^{15}N_{obs}$ refers to the observed ^{15}N atom-% enrichment. A correction coefficient of 1.19 was applied to all zeolite ^{15}N atom-% data, based on an estimated isotopic discrimination factor of 16.14% (Table 5). Further tests revealed that both extraction efficiency and isotopic discrimination were influenced strongly by the electrolytic composition of the water sample (implying that correction coefficients used in conjunction with the zeolite extraction procedure were specific to Snowbank Lake, *i.e.*, that such coefficients must be determined, and applied, on a site-specific, study-by-study basis; see Table 5 and Figure 4).

Table 5. Ammonium extraction efficiency and ^{15}N isotope discrimination by zeolite in filtered (GF/C) surface water samples of widely contrasting ionic content. Surface water samples containing known ambient concentrations of $^{14}NH_4^+$ and augmented with 7.1 μM $^{15}NH_4^+$ (9.61 atom-%) were subjected (in triplicate) to zeolite extraction. Extraction efficiency and isotopic discrimination were calculated (as percentages) via equations 6 and 7, respectively.

Water body sampled	Specific conductance (μmhos cm^{-1})	Extraction efficiency ($\bar{\%} \pm$ SE)	Isotopic discrimination ($\bar{\%} \pm$ SE)
Snowbank Lake	16	93.16 ± 0.89	16.14 ± 0.01
MSU campus pond	558	49.13 ± 2.27	53.67 ± 0.03
Ross Sea, Antarctica	49,416	5.50 ± 0.00	83.07 ± 0.01

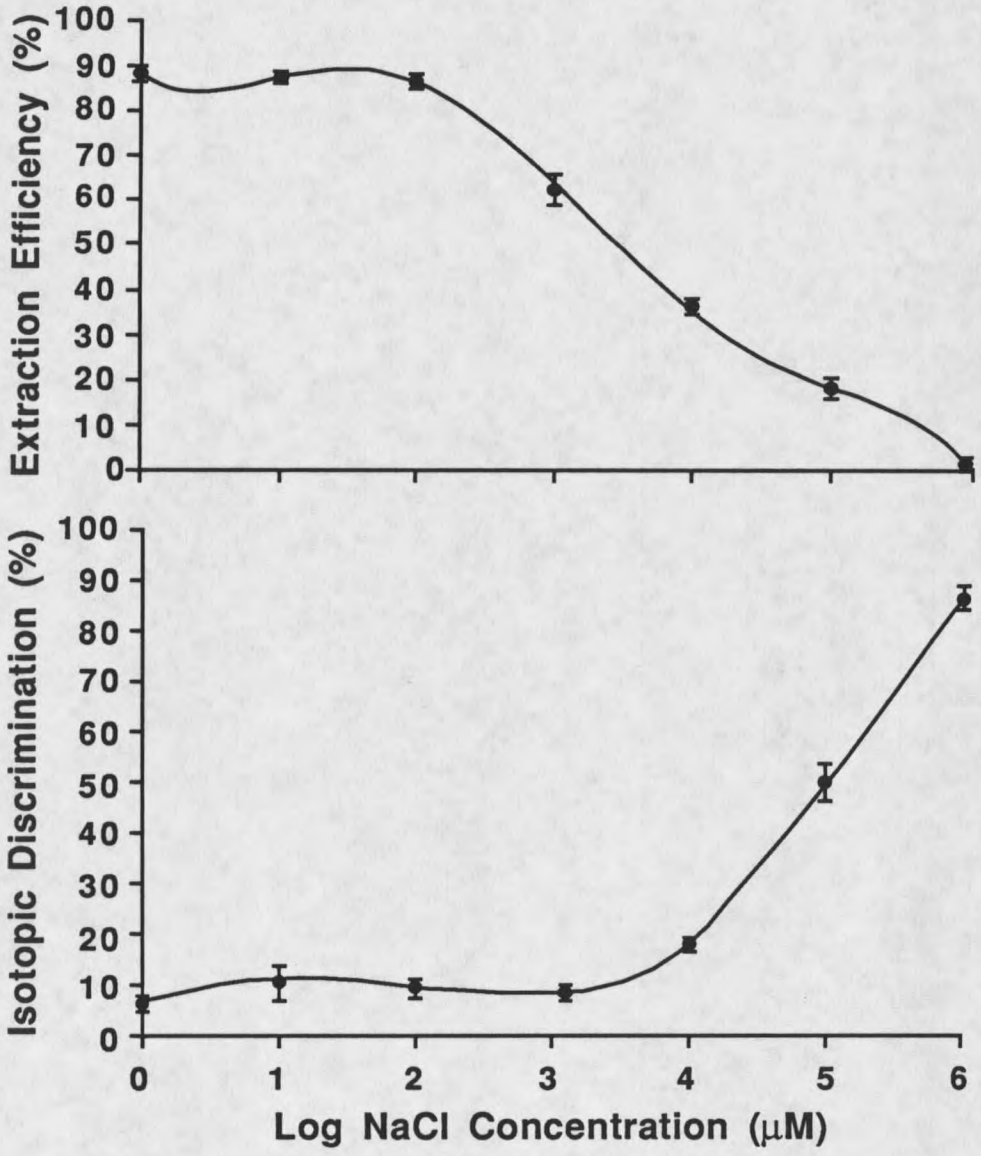

Figure 4. Ammonium extraction efficiency and isotopic discrimination by zeolite as functions of NaCl concentration. Values shown represent treatment means ± standard error and are corrected for $^{14}NH_4^+$ contamination of added NaCl. Curves are based on fifth-order polynomial fitting of experimental data.

Ammonium regeneration rates were calculated using the following equations (Blackburn 1979; Caperon *et al.* 1979; Laws 1984):

$$r = \frac{ln(R_t/R_o)\ (S_o-S_t)}{ln(S_t/S_o)\ t} \quad ; \quad S_t \neq S_o \tag{8}$$

$$r = \frac{ln(R_o/R_t)\ S_o}{t} \quad ; \quad S_t = S_o \tag{9}$$

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

R_o = ratio of ^{15}N to total nitrogen in the NH_4^+ phase at start of time step, minus naturally occurring ratio of 0.00365

R_t = ratio of ^{15}N to total nitrogen in the NH_4^+ phase at end of time step, minus naturally occurring ratio of 0.00365

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

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

t = duration of time step.

Ammonium uptake rates were corrected for isotope dilution using the following equations (Laws 1984; see also Blackburn 1979; Caperon *et al.* 1979; Glibert *et al.* 1982):

$$P_c = \frac{\rho\ \bar{PN}}{\bar{R}\ t} \tag{10}$$

$$\bar{R} = \frac{R_oS_o - R_tS_t}{u\ t} \tag{11}$$

$$u = r - [(S_t-S_o)/t] \tag{12}$$

where P_c = dilution-corrected NH_4^+ uptake rate ($\mu M\ hr^{-1}$), based on incorporation of ^{15}N into particulate matter

$\overline{PN}$ = average of particulate nitrogen concentrations at start and end of time step (see *Limitations of Nutrient Uptake Models*, Chapter 5)

ρ = ratio of ^{15}N to total nitrogen in particulate matter at end of time step (less 0.00365) minus ratio at start of time step (less 0.00365)

$\bar{R}$ = "average" value of R during time step (discussed below)

u = apparent NH_4^+ uptake rate ($\mu M\ hr^{-1}$), based on change in NH_4^+ concentration and $^{15}NH_4^+$ specific activity.

The variable $\bar{R}$ also was calculated as an arithmetic average (Dugdale and Wilkerson 1986) and compared with values calculated via equation 11; differences in the computed $\bar{R}$ values were statistically insignificant ($p > 0.05$, t-test). Estimates of uptake rates at ambient substrate levels were calculated via equation 5 (*cf.*, MacIsaac and Dugdale 1972; Williams 1973; Hoppe 1978; Axler *et al.* 1982). Values for P_{max} and K_t were derived from the uptake kinetic experiments considered previously. The kinetic experiments and time course experiments were performed concurrently using subsamples removed from the same 20-liter lake water sample.

Metabolic Inhibitor Experiments

These laboratory experiments were designed to compare NH_4^+ regeneration rates between procaryotic and eucaryotic components of the lake plankton community. The antibiotics chloramphenicol and cyclohexamide (inhibitors of protein synthesis in procaryotes and eucaryotes,

respectively) were used to terminate or radically impede NH_4^+ metabolism among affected groups of organisms (*cf.*, Wheeler and Kirchman 1986). The first of two such experiments (26 June 1987) was geared toward microbial components less than approximately 1 μm in size and involved the filtration of all experimental water through precombusted GF/C filters. Treatments (in triplicate; 500-ml samples in LPE bottles) included filtered controls, formalin (4% v:v) kills, samples amended with 50 mg cyclohexamide liter^{-1}, and samples amended with 50 mg chloramphenicol liter^{-1}. After a 2-hr preincubation at 185 μE m^{-2} s^{-1} and the prevailing lake water temperature, samples were inoculated with 20 atom-% $^{15}NH_4^+$ for an added concentration of 3.6 μM NH_4^+. Two 10-ml aliquots were removed from each sample for determination of NH_4^+ concentration, and a 230-ml aliquot was subjected immediately to zeolite extraction. The remaining volume was incubated (preincubation conditions) for 6 hr, then tested for NH_4^+ concentration and subjected to zeolite extraction. Regeneration rates were calculated on the basis of measured changes in NH_4^+ concentration and ^{15}N enrichment (equations 8 and 9).

The second inhibitor experiment (2 September 1987) involved a somewhat different protocol. Modifications included (1) the inclusion of both nonfiltered and filtered (GF/C) lake water samples in parallel treatments, (2) the doubling of antibiotic concentrations, (3) an increase in preincubation period to 30 hr and (4) the termination of incubations via filtration (GF/C; nonfiltered treatments only) or via zeolite extraction (filtered treatments). The increases in antibiotic concentration and in preincubation period were intended to ensure the metabolic inhibition of the large eucaryotic zooplankton (*e.g.*, *Daphnia*)

present in the nonfiltered lake water treatments, as well as the inhibition of any carapace or gastrointestinal microbial communities associated with these zooplankton.

Acidification-Response Experiments

Effects of pH Reduction on Size-Fractionated Uptake and Regeneration of NH_4^+

These experiments were conducted on 5 occasions during the study. On each occasion, a 20-liter lake water sample was collected from a depth of 2 m, either through repeated use of a 2-liter capacity Van Dorn sampler (1986) or via the hose sampler (1987). The carboy containing the sample was transferred immediately to a tent on shore. Two 250-ml subsamples were filtered through precombusted GF/C filters; the filters and filtrate were retained for later laboratory determination of NH_4^+, NO_3^-, SRP, chlorophyll *a*, PC and PN concentrations. A 1-liter subsample provided zooplankton and phytoplankton specimens for later identification and enumeration (see *Plankton Identification and Enumeration*, this chapter). Six 500-ml subsamples, including 3 which had been gravity filtered through 63-μm mesh Nitex netting, were acidified to 100 ± 2 times the ambient H^+ activity using predetermined volumes of 0.02 N HCl (see *Alkalinity and pH*, this chapter). These 6 subsamples comprised the experimental group, consisting of triplicate screened and triplicate non-screened subsamples. A control group was prepared in the same manner, except that pH was unaltered. Following a 10-min equilibration period, each of the 12 subsamples was inoculated with 20 atom-% $^{15}NH_4^+$ for an added concentration of 3.6 μM NH_4^+. Immediately after isotope addition, a

250-ml aliquot was filtered (precombusted GF/C), and the filter and filtrate were retained for later laboratory analysis. The remaining volume was incubated in an LPE bottle at the depth of collection (3 August 1986 only) or at the water surface under neutral density screening (40% surface irradiance). After 3-10 hr, incubations were terminated by filtration (muffled GF/C); filters and filtrate were retained, as before.

All filters and filtrate were stored on ice in darkness during transfer to the laboratory, then frozen (-20°C) pending analysis for PC and PN content, seston ^{15}N enrichment, NH_4^+ concentration and $^{15}NH_4^+$ atom-% enrichment. Ammonium regeneration rates were calculated via equations 8 and 9; NH_4^+ uptake rates were corrected for isotope dilution and substrate enrichment using equations 5 and 10-12.

Effects of pH Reduction on Time Course of Nitrogen Uptake and Regeneration

These laboratory experiments examined the influence of acidification on the time course of NH_4^+ regeneration and uptake and on the time course of NO_3^- uptake. Four 4-liter LPE containers were filled with 3.8 liters of lake water, and the pH of two of these samples was decreased 2.0 ± 0.1 pH units by the addition of 0.02 N HCl (required volume predetermined by electrometric titration). After a 15-min equilibration period, one unamended sample and one acidified sample were inoculated with 99 atom-% $^{15}NH_4^+$ (3.3 μM added concentration); the other 2 samples were inoculated with 99 atom-% $^{15}NO_3^-$ (3.3 μM added concentration). The samples were swirled gently for approximately 30 s to ensure uniform mixing. A 200-ml aliquot was removed from each sample and filtered (muffled GF/C), and

filter and filtrate were frozen (-20°C) pending chemical analysis. Samples were incubated at 300 $\mu E\ m^{-2}\ s^{-1}$ and the prevailing lake temperature (at the depth and time of collection). Aliquots were removed and filtered at intervals of 1, 3, 7, 15 and 23 hr; filters and filtrate were frozen pending chemical analysis. All filters were analyzed for PC and PN content and seston ^{15}N enrichment; filtrate from $^{15}NH_4^+$-enriched samples was analyzed for NH_4^+ concentration and ^{15}N atom-% enrichment. Nitrate uptake rates were calculated according to equation 2. Ammonium regeneration rates and uptake rates (corrected for isotope dilution) were calculated via equations 8-12.

Threshold of pH-Related Effects on Nitrogen Uptake

This laboratory experiment examined the influence of aqueous $[H^+]$ on NH_4^+ and NO_3^- uptake over a comparatively broad pH range; specifically, it attempted to identify a threshold pH value above which impacts on nitrogen uptake did not occur, at least in the time frame of the experiment. The pH of 36 200-ml lake water samples was adjusted (0.02 N HCl) in groups of 6 to provide the following series of pH values: pH 7.4 (control), pH 7.0, pH 6.0, pH 5.0, pH 4.0 and pH 3.0. After a 5 min equilibration period, 3 samples in each pH category were inoculated with $^{15}NH_4^+$ (99 atom-%; 6.7 μM added concentration); the remaining samples were inoculated with $^{15}NO_3^-$ (99 atom-%; 6.7 μM added concentration). Following incubation at 185 $\mu E\ m^{-2}\ s^{-1}$ and the prevailing lake temperature for 28 hr, the samples were filtered (muffled GF/C) and the filters frozen pending analysis of seston ^{15}N enrichment by emission spectrometry. Enrichment of

the seston was expressed as a percentage of the control enrichment and compared among treatments.

Comparative Effects of Mineral Acids on Algal Nitrogen Uptake and Chlorophyll *a* Concentration

These laboratory experiments examined the effects of various mineral acids and mineral acid combinations on algal NH_4^+ and NO_3^- uptake and on chlorophyll *a* concentration (an indicator of algal biomass). Twenty-four 500-ml lake water samples were acidified (2.0 ± 0.1 pH units) in groups of 6 using 0.02 N HCl (reagent grade), HNO_3 (Ultrex grade, J.T. Baker), H_2SO_4 (Ultrex grade), or HNO_3 plus H_2SO_4 (1:1 N, Ultrex grade). Six additional unacidified samples served as controls. After a 5 min equilibration period, 3 samples in each acidification and control category were inoculated with $^{15}NH_4^+$ (99 atom-%; 6.7 μM added concentration), and the remaining 3 samples in each category were inoculated with $^{15}NO_3^-$ (99 atom-%; 6.7 μM added concentration). Incubations lasted 14 hr (8 July 1987) or approximately 30 hr (6 and 27 September 1987) and were terminated by filtration (muffled GF/C). Seston retained on filters was analyzed for chlorophyll *a* content and for ^{15}N enrichment using methods described previously. In treatments involving additions of both $^{15}NO_3^-$ and HNO_3, uptake competition between $^{15}NO_3^-$ and $^{14}NO_3$ required that corrections be made for reduced aqueous $^{15}NO_3$ specific activity. Given the likely saturation of NO_3^- uptake mechanisms at $^{15}NO_3^- + {}^{14}NO_3$ concentrations exceeding 6.7 μM (see *Kinetics of NH_4^+ and NO_3^- Uptake*, Chapter 4), reductions in measured rates of specific uptake (*i.e.*, V, where V = P ÷ PN; see equation 2) resulting from the dissociation of HNO_3 were deemed

proportional to the decrease in $^{15}NO_3^-$:$^{15}NO_3^-$ + $^{14}NO_3^-$ ratio and were corrected for using the following equation:

$$\text{corrected uptake} = \frac{\text{apparent uptake}}{a \div b} \quad (13)$$

where a = $^{15}NO_3^-$:total NO_3^- ratio, given complete dissociation of added HNO_3

b = $^{15}NO_3^-$:total NO_3^- ratio, assuming (hypothetically) no dissociation of added HNO_3.

Comparative Effects of Mineral Acids on Algal Photosynthesis

These laboratory experiments evaluated the relative influences of various mineral acids on inorganic carbon uptake by phytoplankton. Treatments (6 replicates each; 60-ml lake water samples in 70-ml glass serum vials) included unamended controls and experimental treatments amended with HCl, HNO_3, H_2SO_4 or HNO_3 plus H_2SO_4 (1:1 N). Following the addition of the mineral acids to experimental samples (2.0 ± 0.1 pH unit reduction; 30 min equilibration period), all vials were sealed with gas-tight rubber septa and injected (via tuberculin syringe) with 0.1 ml of 10 μCi ml^{-1} ^{14}C-$NaHCO_3$ solution. Incubations were conducted at the prevailing lake temperature and 185 $\mu E\ m^{-2}\ s^{-1}$ for 26 hr (6-7 September 1987) or 29 hr (28-29 September 1987) and terminated by filtration (GF/C). The ^{14}C activity of the seston retained on filters was determined by liquid scintillation spectrometry, using methods described previously in this chapter (see *Limiting-Nutrient Bioassays*).

Comparisons of inorganic carbon uptake between lake water controls (*ca.* pH 7) and experimental treatments (*ca.* pH 5) required that corrections be made for $[H^+]$-related effects on inorganic carbon concentration and, following the sealing of the sample containers and inoculation, $^{14}CO_2$ specific activity. Accordingly, the pH and alkalinity of the ^{14}C-$NaHCO_3$ inoculant were determined potentiometrically, permitting total inorganic carbon concentration (C_T) to be estimated via the following relation (Pagenkopf 1978):

$$C_T = \frac{\text{alkalinity} - [OH^-]}{\alpha_1 + 2\alpha_2} \tag{14}$$

where

$$\alpha_1 = \frac{K_1 K_{a1} [H^+]}{K_1 K_{a1} K_{a2} + K_1 K_{a1} [H^+] + K_1 [H^+]^2 + K_{a1} [H^+]^2} \tag{15}$$

$$\alpha_2 = \frac{K_1 K_{a1} K_{a2}}{K_1 K_{a1} K_{a2} + K_1 K_{a1} [H^+] + K_1 [H^+]^2 + K_{a1} [H^+]^2} \tag{16}$$

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} = \frac{K_{a1}}{K} \tag{17}$$

$$K = \frac{[H_2CO_3]}{[CO_{2,aq}]} \simeq 10^{-2.80} \tag{18}$$

$$K_{a1} = \frac{[H^+][HCO_3^-]}{[CO_{2,aq}]} \simeq 10^{-6.35} \tag{19}$$

$$K_{a2} = \frac{[H^+][CO_3^-]}{[HCO_3^-]} \simeq 10^{-10.25} \tag{20}$$

Similarly, a knowledge of the pH and alkalinity of the experimental and control treatments allowed C_T to be estimated for all samples using

equations 14-20. The measured radioactivity of ^{14}C-labeled cells from the experimental treatments was relativized to that from the circumneutral controls using the following equation:

$$\text{Corrected DPM} = \text{DPM}\ \frac{(C_{T,i}\ v_i/(C_{T,pH7}\ v_{pH7} + C_{T,i}\ v_i))}{(C_{T,i}\ v_i/(C_{T,pH5}\ v_{pH5} + C_{T,i}\ v_i))} \qquad (21)$$

where DPM = measured activity (disintegrations per min) of ^{14}C-labeled seston

$C_{T,i}$ = inorganic carbon concentration (μM) of ^{14}C-$NaHCO_3$ inoculant

$C_{T,pH7}$ = inorganic carbon concentration (μM) of circumneutral controls

$C_{T,pH5}$ = inorganic carbon concentration (μM) of acidified treatments

v_i = added inoculant volume (ml)

v_{pH7} = control sample volume (ml)

v_{pH5} = experimental sample volume (ml).

Effects of pH Reduction on End Products of Photosynthesis

This experiment examined phytoplankton incorporation of $^{14}CO_2$ into major metabolic end products of photosynthesis (EPPS), including protein, polysaccharide, lipid, and low molecular weight metabolites (LMWM). Treatments (6 replications each; 60-ml samples in 75-ml serum bottles) included unamended controls and samples acidified 2.0 ± 0.1 pH units with 0.02 H HCl. Using a tuberculin syringe, 0.1 ml of ^{14}C-$NaHCO_3$ solution (approximately 1 μCi) was injected into each sample through an air-tight rubber septum. The samples were mixed by repeated inversion, then

incubated at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 10°C (the prevailing lake temperature) for 29 hr. Incubations were terminated by filtration (GF/C), and filters containing the radioactive seston were transferred to glass scintillation vials containing 1.2 ml deionized water, 1.5 ml chloroform and 3.0 ml methanol. The contents of each vial were vortexed 60 s, incubated at 4°C for 10 min, and filtered through another GF/C filter; the latter filter was rinsed under vacuum with 1.5 ml chloroform. All filtrate was collected in a glass centrifuge tube, in preparation for lipid and LMWM fractionation. Particulate matter retained on the filter contained protein and polysaccharide fractions. Procedures used for further fractionation of metabolites are described below.

Protein. Filters containing protein and polysaccharide fractions were placed in glass scintillation vials, resuspended in 4 ml ice-cold 5% trichloroacetic acid (TCA) and heated at 95°C for 30 min. The contents of each vial were filtered through a GF/C filter, which was rinsed under vacuum with 10 ml TCA solution and transferred to another scintillation vial. Tissue solubilizer (9 parts 0.5 M Protosol, New England Nuclear:1 part deionized H_2O; 0.5 ml total) was added to the vial to enhance suspension and dissolution of carbonaceous materials. The contents of each vial were incubated for 2 hr at 20°C, dried overnight at 50°C, resuspended in 0.5 ml deionized water, and augmented with 0.15 ml glacial acetic acid (to reduce pH and chemiluminescence) and 10 ml Scinterverse E scintillation cocktail. The activity of the protein isolate was determined by liquid scintillation spectrometry; measurements were converted from cpm to dpm via the sample channels ratio method,

using acetone as the quenching agent and ^{14}C-toluene as the radioactivity standard (*cf.*, Priscu and Priscu 1984b).

Polysaccharide. Filtrate derived from the protein isolation procedure was collected in a glass centrifuge tube and vortexed 60 s. Two ml were transferred to a glass scintillation vial, evaporated at 50°C, resuspended in 0.5 ml deionized water, and augmented with 10 ml Scinterverse E scintillation cocktail. The activity of this polysaccharide isolate was determined by liquid scintillation spectrometry (see *Protein*, above).

Lipid. The filtrate derived from the initial chloroform- and methanol-based fractionation procedure was collected in a glass centrifuge tube, augmented with 1.5 ml deionized water, vortexed 60 s, and centrifuged at 800-1,000 g for 10 min, resulting in a biphasic solution containing an upper methanol-soluble fraction and a lower chloroform-soluble fraction. One ml was removed from the chloroform phase, transferred to a glass scintillation vial and evaporated to dryness at 50°C. Ten ml of Scinterverse E scintillation cocktail were added to the vial prior to determination of ^{14}C activity (see *Protein*, above).

Low Molecular Weight Metabolites. A 3-ml aliquot was removed from the methanol phase, transferred to a glass scintillation vial, augmented with 0.2 ml of 3 N HCl (to drive off residual ^{14}C-labeled dissolved inorganic carbon), dried overnight at 50°C, and resuspended in 0.5 ml deionized water. Ten ml of Scinterverse E scintillation cocktail were

added to the vial, and the ^{14}C activity of the LMWM isolate was determined via scintillation spectrometry (see *Protein*, above).

Effects of pH Reduction on Nitrogen Incorporation into Protein

These laboratory experiments examined the incorporation of NH_4^+-N and NO_3^--N into phytoplankton protein and were conducted concurrently with the EPPS experiment. Treatments (4 replications each; 200-ml samples in 250-ml LPE bottles) included lake water controls and samples acidified 2.0 ± 0.1 pH units with 0.02 N HCl. After a 5 min equilibration period, samples were inoculated with $^{15}NH_4^+$ or $^{15}NO_3^-$ (99 atom-%) for a final concentration of 6.7 μM added NH_4^+ or NO_3^-. Incubations were conducted at 300 $\mu E\ m^{-2}\ s^{-1}$ and 10°C (the prevailing lake temperature) and terminated after 29 hr by filtration (muffled GF/C). Each filter was transferred to a glass scintillation vial containing 10 ml ice-cold 5% TCA to precipitate protein. The contents of the vial were vortexed (60 s) and filtered (muffled GF/C). The filters were rinsed under vacuum (approximately 10 cm Hg) with two 10-ml aliquots of ice-cold 5% TCA (to rinse away non-proteinaceous compounds) and dried overnight at 50°C. Following Dumas combustion, the ^{15}N enrichment of the protein isolate was determined by optical emission spectrometry (see *Nitrogen Uptake and Isotope Dilution Experiments*, this chapter).

Effects of pH Reduction on Bacterial Secondary Production

The influence of $[H^+]$ on bacterioplankton secondary production was examined using the ^3H-methyl thymidine incorporation technique of Fuhrman and Azam (1982), as modified by Murray *et al.* (1986). Treatments

(7 replicates each; 10 ml samples in glass scintillation vials) included lake water controls, formalin (5% v:v) kills, and samples acidified 2.0 ± 0.1 pH units with 0.02 N HCl. Each sample was inoculated with 14.6 μCi of tritiated thymidine (20 nM final concentration) and incubated in darkness at 10°C (the prevailing lake temperature) for 1.0 hr (7 September) or 0.7 hr (28 September). Incubation was terminated by the addition of 10 ml ice-cold 10% TCA. The contents of each vial were filtered (0.2 μm Nucleopore) and the filter rinsed under vacuum (10 cm Hg) with two 15-ml aliquots of ice-cold 5% TCA. The filters were transferred to a glass scintillation vial to which 10 ml Scinterverse E scintillation cocktail was added. The ^{3}H activity of the protein/nucleotide isolate was assayed by liquid scintillation spectrometry. Measurements were corrected for quench via the sample channels ratio method, using acetone as the quenching agent (*cf.*, Murray *et al.* 1986).

CHAPTER 4

RESULTS

Water-Column Physicochemical Properties

Snowbank Lake physicochemical data from 1985-87 are summarized in Table 6. Variations observed during this period in Secchi depth and in water-column profiles for temperature, dissolved O_2, dissolved inorganic nutrients, PC, PN and chlorophyll *a* are depicted in figures 5-7.

Secchi depth ranged from 4.5 to 7.0 m during the study (Figure 5). No significant temporal correlation was observed between Secchi depth and whole-lake (*i.e.*, depth-integrated, volume-weighted) mean concentrations of chlorophyll *a*, PC or PN, suggesting that nonbiogenic factors (*e.g.*, inorganic suspensoids) played a predominant role in light attenuation. Secchi depth gradually increased during the 1987 ice-free season but exhibited no distinct trend in 1986.

Water temperature in Snowbank Lake typically decreased with depth in a gradual manner. However, the water column approached an isothermal condition in late summer 1986, following a period of weak thermal stratification (Figure 5). Whole-lake mean temperatures ranged from 5.0°C on 20 September 1986 to 8.9°C on 1 September 1987. Water temperature at the experimental depth (2 m) was consistently within 0.5°C of the whole-lake mean temperature.

Table 6. Summary of physicochemical properties of Snowbank Lake water column, 1985-87. The first value of each datum pair pertains to the experimental depth; the second value refers to the water-column depth-integrated, volume-weighted mean. Single values pertain to the experimental depth, unless stated otherwise.

	Sampling date							
Parameter	8/24/85	7/26/86	8/24/86	9/20/86	6/26/87	7/18/87	9/1/87	9/25/87
Secchi depth (m)	4.5	6.5	5.3	6.0	4.5	5.9	6.2	7.0
Temperature (°C)	9.0/8.8	8.0/7.5	8.5/8.5	5.0/5.0	8.2/7.8	6.4/6.3	9.2/8.9	7.9/7.5
Specific conductance (μmhos cm^{-1})	10.1	9.6	10.2	12.2	9.4	9.8	12.7	16.8
Dissolved O_2 (μM)	250/253	256/260	381/384	263/263	306/307	--	263/263	312/305
Whole-lake O_2 saturation (%)	101	91	153	97	109	--	112	120
pH	7.00	6.94	7.21	7.12	6.79	--	7.38	7.38
Total alkalinity (μeq l^{-1} as $CaCO_3$)	106	91	110	110	94	--	124	142
NH_4^+ (μM)	0.42/0.42	0.07/0.07	0.09/0.12	0.09/0.12	0.16/0.09	0.16/0.19	0.08/0.14	0.11/0.15
NO_3^- (μM)	--	0.27/0.27	0.21/0.29	0.31/0.28	0.16/0.17	0.36/0.37	0.78/0.61	0.34/0.28
SRP (μM)	--	0.11/0.12	0.14/0.14	0.08/0.08	0.03/0.04	0.12/0.13	0.12/0.13	0.10/0.09
$NH_4^+ + NO_3^-$:SRP ratio	--	3.09/2.83	2.14/2.93	5.00/5.00	10.67/6.50	4.33/4.31	7.17/5.77	4.50/4.78
Particulate nitrogen (μM)	--	2.91/3.01	3.62/3.38	3.77/5.41	1.63/2.50	1.06/1.42	12.37/6.49	2.94/1.75
Particulate carbon (μM)	--	29.3/33.7	36.5/35.8	35.7/32.5	17.4/23.8	15.3/21.0	116.1/66.2	46.2/36.6
Particulate carbon: nitrogen ratio	--	10.1/11.2	10.1/10.6	9.5/6.0	10.7/9.5	14.4/14.8	9.4/10.2	15.7/20.9
Chlorophyll *a* (μg l^{-1})	2.3/3.8	1.0/1.7	2.3/2.7	2.0/2.0	1.6/2.5	0.9/1.9	7.4/5.5	5.9/4.5

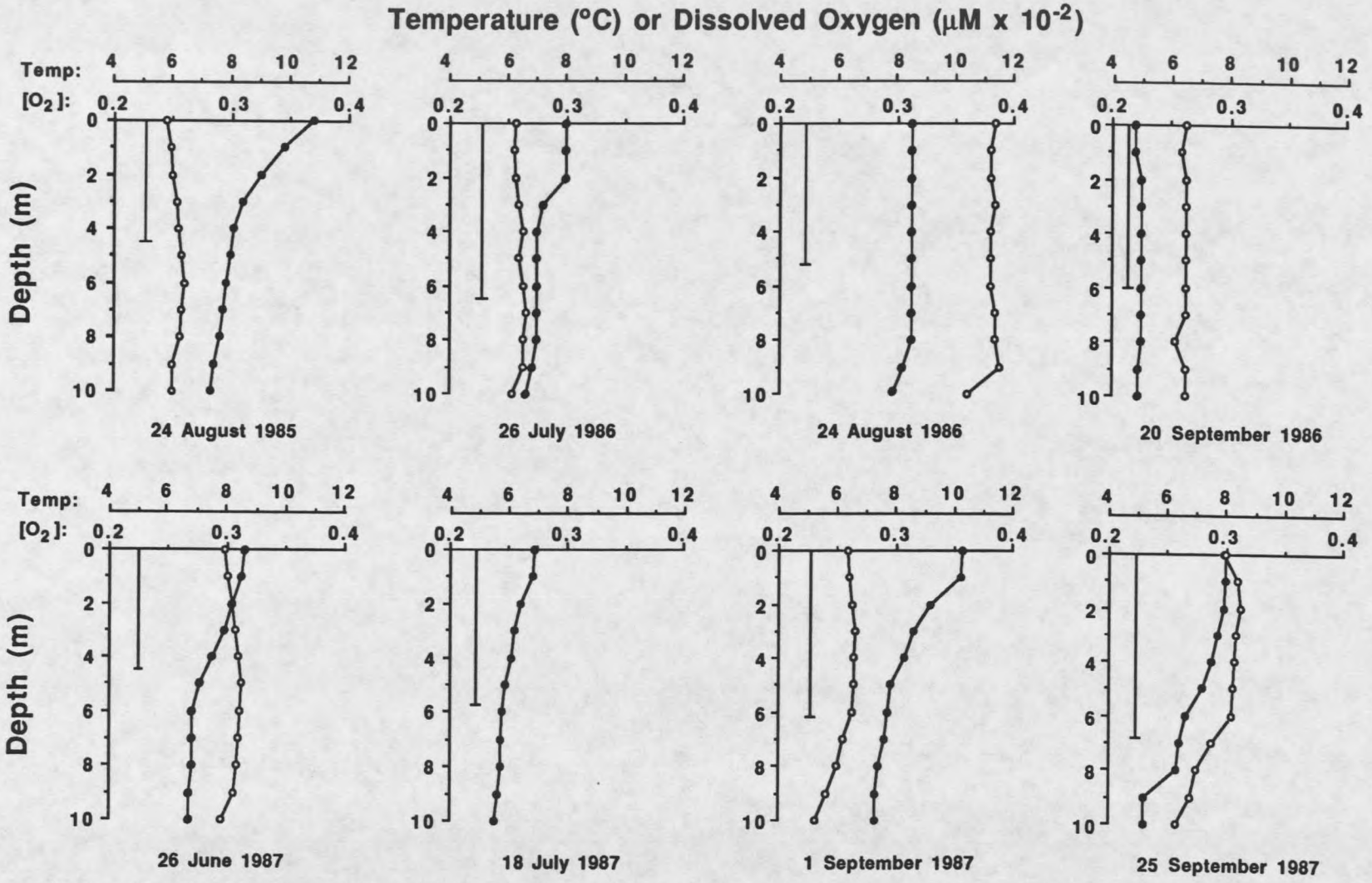

Figure 5. Vertical profiles of temperature (•) and dissolved O_2 (○), and Secchi depth (⊥), 1985-87 ice-free seasons. An equipment malfunction precluded the collection of O_2 data on 18 July 1987.

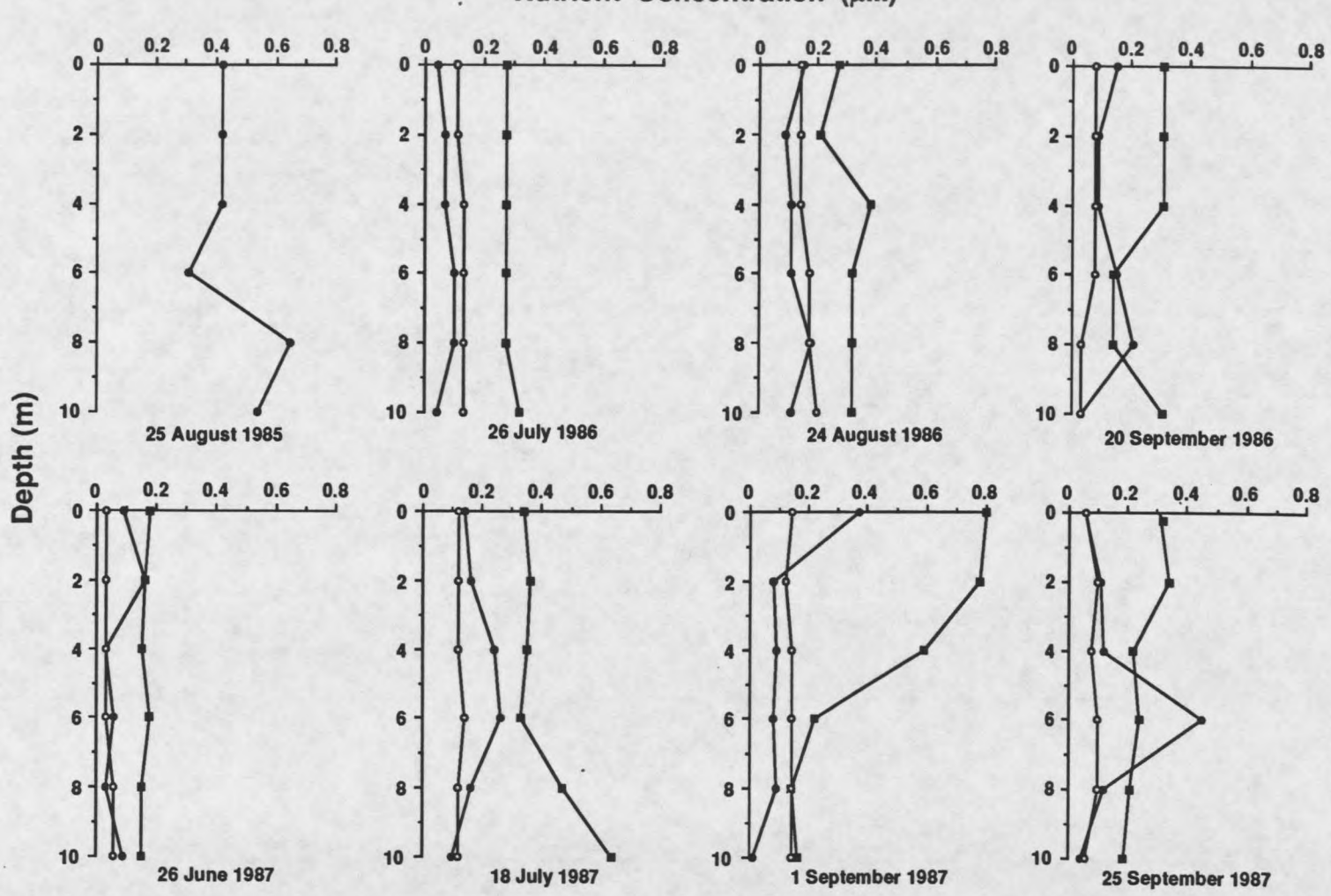

Figure 6. Depth profiles of NH_4^+ (•), NO_3^- (▪) and SRP (o) concentrations, 1985-87 ice-free seasons. Nitrate and SRP samples from 25 August 1985 were inadvertently destroyed during processing.

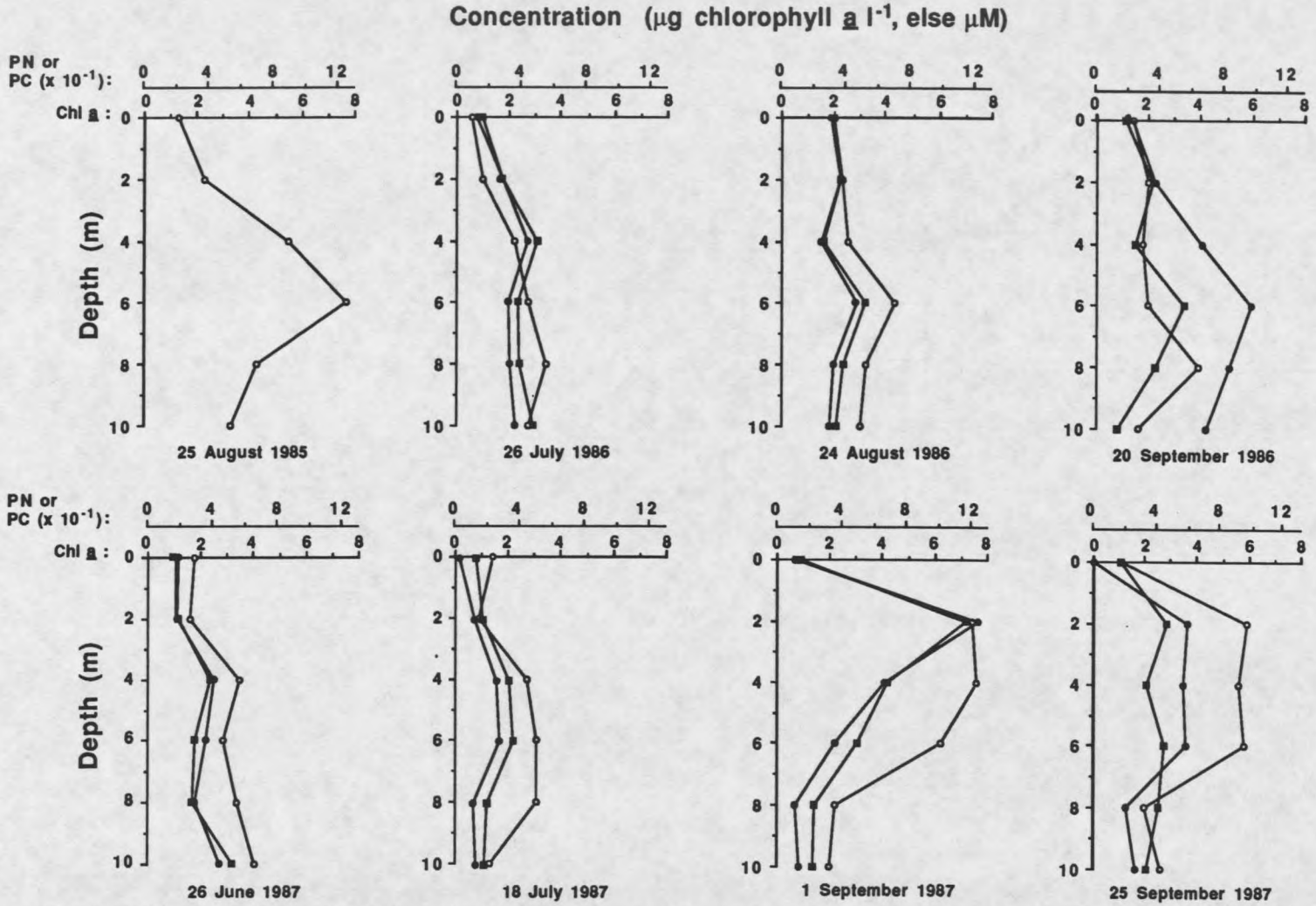

Figure 7. Depth profiles of chlorophyll *a* (o), particulate nitrogen (•) and particulate carbon (▪) concentrations, 1985-87 ice-free seasons. Note differences in scale between parameters. Samples for particulate carbon and nitrogen were not collected on 25 August 1985.

Concentrations of dissolved O_2 generally exhibited little change with depth. However, slight decreases (31-47 μM) in O_2 concentration below 6 m were documented on 1 and 25 September 1987 (Figure 5). These dates coincided with those of maximum observed chlorophyll *a* concentration and phytoplankton biomass in overlying (0-6 m) waters (discussed below), suggesting that atypically high rates of photosynthesis in shallower waters (and, perhaps, correspondingly high rates of decomposition in deeper waters) were responsible for the observed clinograde profiles. During the 3 years of study, measured whole-lake concentrations of dissolved O_2 ranged from 91 to 153% of saturation (mean = 112%; values relativized to whole-lake mean temperature and lake elevation; see Mortimer 1981).

Extremes in lake water pH recorded during the study were pH 6.79 on 28 June 1987 and pH 7.38 on both 1 and 25 September 1987. Despite the circumneutral nature of the lake, total alkalinity was low, ranging from 91 to 142 μeq liter^{-1} as $CaCO_3$. In 1987, pH and alkalinity gradually increased during the open-water season. Specific conductance also increased during this period, indicative of a net gain in the electrolytic content of the water column. The low conductance and alkalinity values recorded for Snowbank Lake confirmed that it was a dilute system of low chemical buffering capacity and, therefore, potentially sensitive to acidic inputs (Table 7).

Whole-lake concentrations of NH_4^+, NO_3^- and SRP averaged approximately 0.1, 0.3 and 0.1 μM, respectively, during the 1986-87 ice-free seasons. Concentrations of NH_4^+ typically decreased below a depth of 8 m; conversely, NO_3^- concentrations tended to increase below this depth,

Table 7. Categories of lake sensitivity to acidic inputs (adapted from Harvey *et al.* 1981).

Lake category	Lake water property	
	Total alkalinity (μeq l^{-1} as $CaCO_3$)	Conductance (μmhos cm^{-1}, 25°C)
Highly sensitive	0-200	0-35
Moderately sensitive	200-400	22-78
Least sensitive	>400	>60
Snowbank Lake (range):	91-142	9.4-16.8

suggestive of appreciable rates of nitrification in sediments and overlying waters. Vertical changes in SRP concentration generally were less pronounced than those noted for NH_4^+ or NO_3^- concentration (Figure 6). Nutrient levels in inflowing stream water were substantially less than those in snow field runoff (Figure 8). Nutrient levels in outflowing stream waters were nearly identical to those within the lake water column (2-m depth; see Table 6).

Depth profiles for PN, PC and chlorophyll *a* paralleled one another on each sampling date (Figure 7). On a temporal (sampling date-to-sampling date), whole-lake basis, however, correlation between PN and chlorophyll *a* was poor relative to that between PC and PN or between PC and chlorophyll *a* (Table 8). During the 1986-87 open-water seasons, whole-lake PC:PN atomic ratios ranged from 6.0 to 20.9 (mean ± SE = 11.9 ± 1.8), compared to a ratio of approximately 6.6 normally associated with balanced algal growth (Redfield 1934, 1958; Redfield *et al.* 1963; see

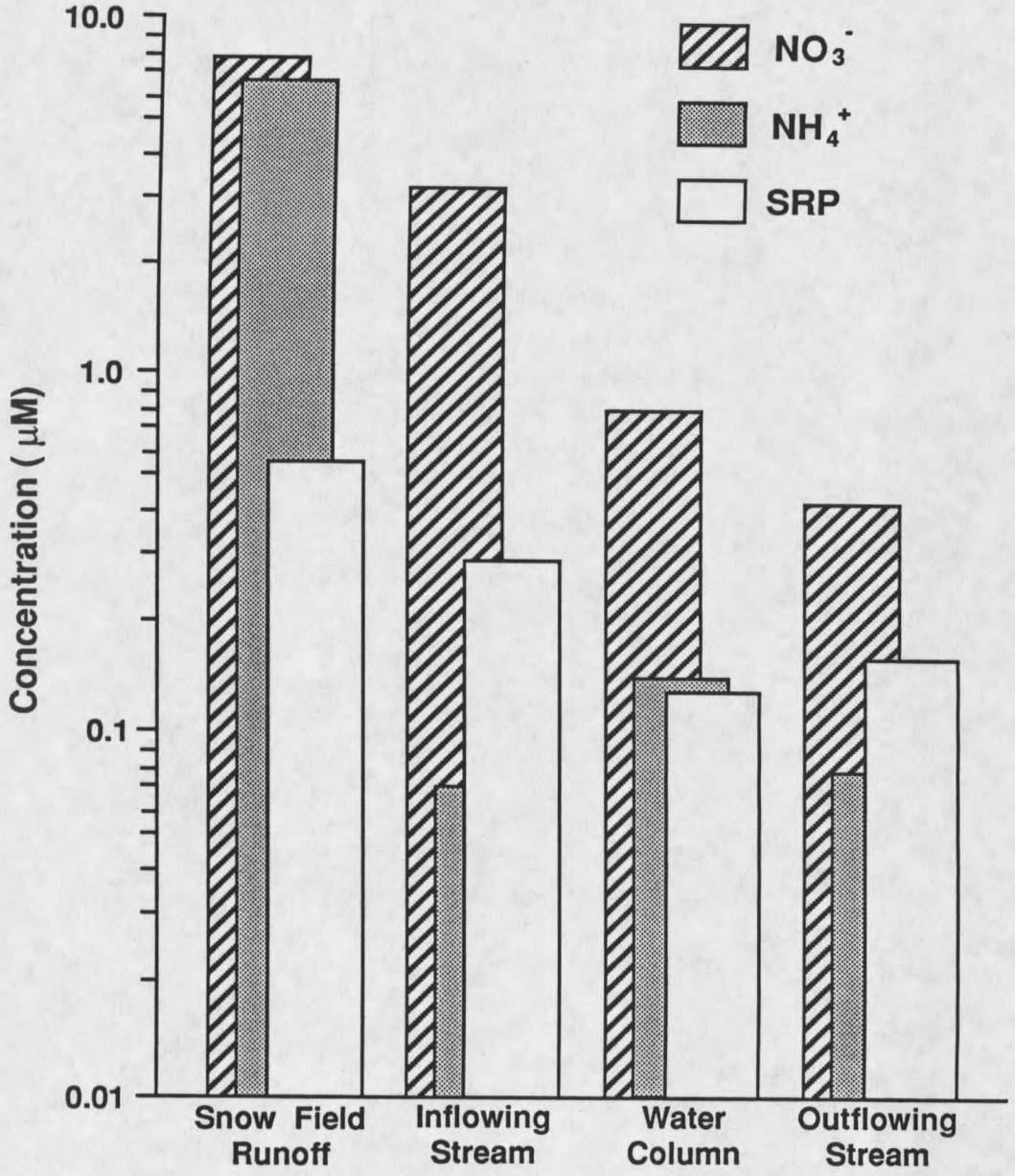

Figure 8. Comparison of dissolved inorganic nutrient concentrations in snow field runoff, lake water column, and inflowing and outflowing streams, 1 September 1987. Water-column values represent depth-integrated, volume-weighted mean concentrations. Note logarithmic scale of vertical axis.

Table 8. Temporal, regression-based comparisons of particulate nitrogen, particulate carbon and chlorophyll *a* concentrations within the Snowbank Lake water column, 1986-87.

	x variable (as $\mu g\ l^{-1}$)	y variable (as $\mu g\ l^{-1}$)	r	r^2	Slope	Intercept (as $\mu g\ l^{-1}/\mu M$)
Experimental depth:	Particulate N	Particulate C	0.98	0.96	7.60	78.07/6.51
	Chlorophyll *a*	Particulate N	0.78	0.61	16.22	7.70/0.55
	Chlorophyll *a*	Particulate C	0.88	0.77	140.58	84.54/7.05
Whole-lake average:	Particulate N	Particulate C	0.76	0.58	5.13	181.85/15.15
	Chlorophyll *a*	Particulate N	0.39	0.15	6.78	27.06/1.93
	Chlorophyll *a*	Particulate C	0.82	0.66	99.40	132.53/11.04

also *Evidence for Nitrogen Limitation of Water-Column Primary Production*, Chapter 5). Chlorophyll *a* concentration appeared to peak in late August or early September during the years studied; greatest concentrations were documented in September 1987, owing largely to the development of a dense population of the dinoflagellate, *Peridinium cinctum* (Muell.) Ehrenberg (see *Plankton Biomass and Community Composition*, this chapter). Data on chlorophyll *a*, dissolved inorganic nitrogen, and total alkalinity have been reexpressed in Table 9 to provide a comparative assessment of Snowbank Lake's trophic status.

Plankton Biomass and Community Composition

A complete listing of plankton taxa collected from Snowbank Lake is provided in the appendix (tables 29 and 30). In all, 104 species of phytoplankton were identified by light microscopy. Metazoan zooplankton included 6 species of rotifers and 4 species of arthropods; occasionally, free-living nematodes were collected from the lake's open waters. Ciliated protozoans were noted only rarely in lake water samples; unfortunately, preservation techniques used in the study were not amenable to ciliate identification.

As shown in Figure 9, chlorophycean and bacillariophycean algae were represented by the greatest numbers of phytoplankton species. Desmids were especially diverse; for example, 7 species of *Staurastrum* were collected in 1987 alone (appendix, Table 30). From the standpoint of algal biomass (as approximated by biovolume), chlorophycean and dinophycean algae were clearly most important. A distinct seasonality was noted in the biomass of both classes: chlorophyceans tended to comprise a large

Table 9. Trophic status of Snowbank Lake as indicated by chlorophyll *a* and dissolved inorganic nitrogen concentrations and by seasonal change in total alkalinity (based on Vollenweider 1968; 1979).

	Productivity index			
Lake productivity designation	Change in alkalinity in epilimnion over summer (meq l^{-1})	Inorganic N ($\mu g\ l^{-1}$)	Mean chlorophyll ($\mu g\ l^{-1}$)	Peak chlorophyll ($\mu g\ l^{-1}$)
Ultra-oligotrophic	<0.2	<200	--	--
Oligotrophic	--	--	0.3-4.5	1.3-10.6
Oligo-mesotrophic	0.6	200-400	--	--
Mesotrophic	--	--	3-11	4.9-49.5
Mesoeutrophic	0.6-1.0	300-650	--	--
Eutrophic	--	500-1,500	3-78	9.5-275
Hypereutrophic	>1.0	>1,500	100-150	--
Snowbank Lake:	0.019 (2 m; 1986) 0.048 (2 m; 1987)	6.3 (whole-lake mean; 1986-87)	3.1 (whole lake mean; 1985-87)	5.5 (whole-lake mean; 9/1/87)

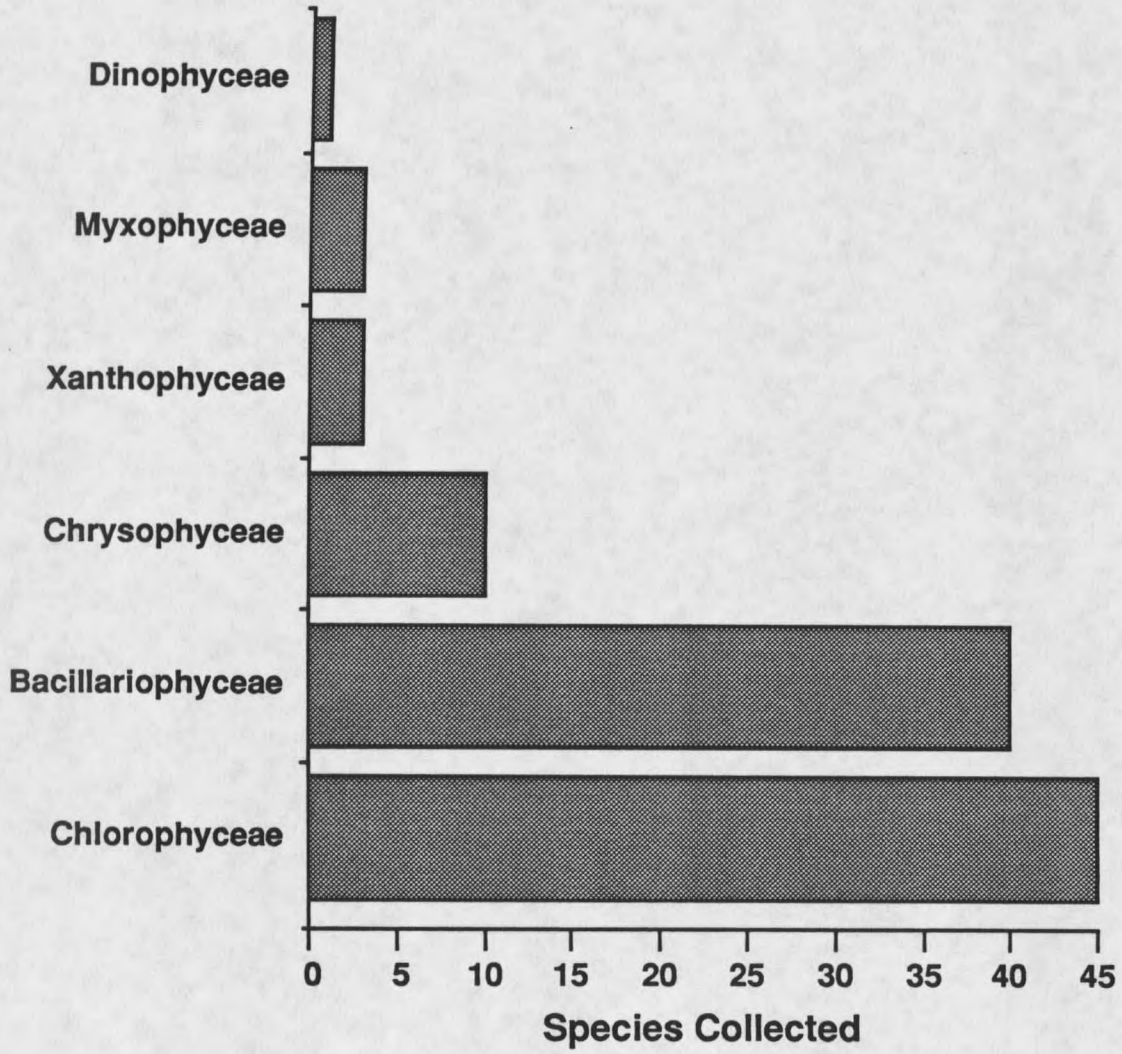

Figure 9. Phytoplankton community composition relativized to numbers of species collected during the period 1985-87. A complete listing of phytoplankton taxa collected from Snowbank Lake is provided in the appendix (Table 30).

fraction of community biovolume early in the open-water season, gradually diminishing in importance as the dinoflagellate, *P. cinctum*, assumed prominence (Figure 10). By mid to late September in both 1986 and 1987, *P. cinctum* comprised more than 80% of the total algal biovolume. The following additional species at times made important contributions to algal community biomass (*i.e.*, present at biovolumes $\geq 10^4$ μm^3 ml^{-1}): the chlorophyceans *Volvox tertius* Meyer, *Schroederia setigera* Lemmermann, *Sphaerocystis schroeteri* Chodat, *Botryococcus* sp., *Cosmarium tinctum* var. *subretusum* Messik and *Staurastrum paradoxum* var. *cingulum* West *et* West; the chrysophyceans *Mallomonas* spp., *Synura uvella* Ehrenberg, *Dinobryon sertularia* Erhenberg and *Chromulina pascheri* Hofeneeder; the diatoms *Tabellaria fenestrata* (Lyngb.) Kuetzing, *Asterionella formosa* Hassall, *Fragilaria construens* (Ehr.) Grunow, *F.* sp., *Navicula* spp., *Epithemia* sp. and *Hantzschia virgata* (Roper) Grunow; the xanthophyceans *Botrydiopsis arhiza* Borzi and *Tribonema bombycinum* (Ag.) Derbes *et* Solier; and the blue-green algae *Chroococcus dispersus* (Keissl.) Lemmermann and *Rivularia* sp.

Crustacean zooplankton were represented by the calanoid copepod, *Diaptomus (Hesperodiaptomus) shoshone* Forbes, and by the cladocerans, *Daphnia pulex* Leydig, *D. rosea* Sars and *D. schodleri* Sars. *D. shoshone* was the most abundant macrozooplankter; densities (including nauplii, copepodids and adults) ranged from 10 to 25 individuals $liter^{-1}$ at the experimental depth during daylight hours. Daphnid densities were variable but never exceeded 16 individuals $liter^{-1}$ (2-m depth; daylight). *D. schodleri* was the most abundant daphnid, followed by *D. rosea* and *D. pulex*. Planktonic rotifers were represented by the genera *Filinia*,

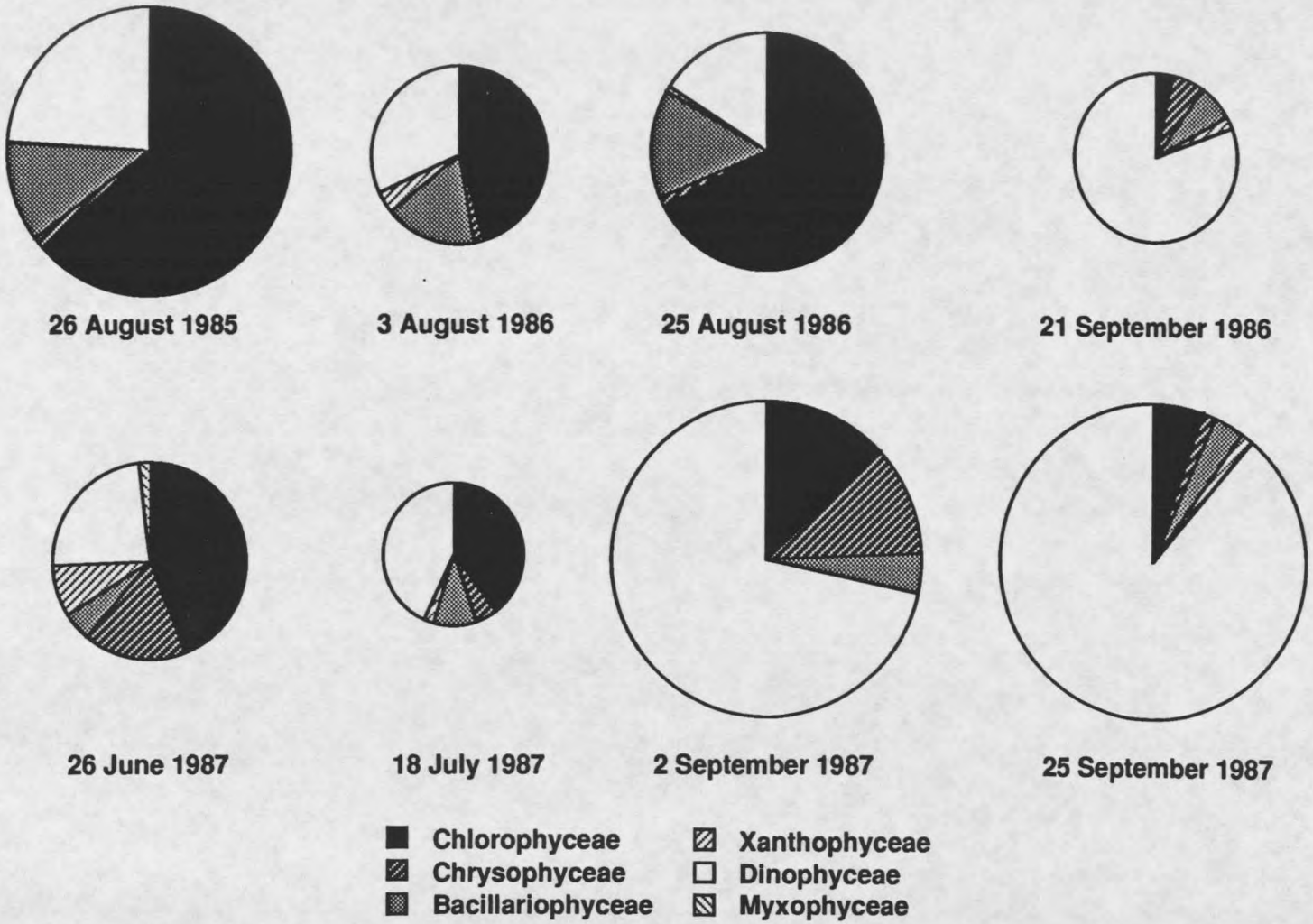

Figure 10. Phytoplankton community composition and total biomass relativized to biovolume, 1985-87. Total (relative) biovolume is indicated by diameter of subfigures.

Polyarthra, *Brachionus*, *Kellicottia*, *Keratella* and *Notholca*. Rotifer densities (2-m depth; daylight) were typical of clear, mountain lakes, ranging from 5 to 22 individuals liter^{-1} (*cf.*, Pennak 1978).

Influence of Nutrient and Meltwater Additions on Algal Photosynthesis

Influence of Nitrogen and Phosphorus Additions

Phytoplankton uptake of $^{14}CO_2$ was increased significantly ($p < 0.05$, ANOVA/LSD) by additions of NH_4^+ or $NH_4^+ + PO_4^{3-}$ but not by the addition of PO_4^{3-} alone (figures 11 and 12). The exclusion of *Volvox tertius* (which represented >97% of the algal biomass removed via size fractionation) from NH_4^+- and $NH_4^+ + PO_4^{3-}$-enriched treatments dampened this stimulatory effect. On 26 June 1987, uptake of $^{14}CO_2$ by *V. tertius* appeared disproportionately large in relation to the population's biomass (Table 10). This disproportionality was even greater in the 2 September 1987 $NH_4^+ + PO_4^{3-}$ treatment (see Figure 12), when *V. tertius* accounted for 55% of the increase in $^{14}CO_2$ uptake while comprising only 7% of the phytoplankton biovolume. In samples containing *V. tertius*, simultaneous additions of NH_4^+ and PO_4^{3-} stimulated greater increases in $^{14}CO_2$ uptake than did additions of NH_4^+ alone ($p < 0.05$; see Figure 12).

No enhancement of $^{14}CO_2$ uptake was observed over a 24-hr period in samples augmented with forms of nitrogen other than NH_4^+ (Figure 13). Additions of NH_4^+ increased $^{14}CO_2$ uptake by approximately 20% ($p < 0.05$), whereas uptake was reduced 14% in urea treatments ($p < 0.05$) and was statistically unchanged in NO_3^- treatments ($p > 0.05$).

Table 10. Enhancement of algal $^{14}CO_2$ uptake via NH_4^+ addition in samples with and without *Volvox tertius*.

Sampling date	Increase in $^{14}CO_2$ uptake (%)		Percent community uptake due to *V. tertius* (100[a-b]/a)	*V. tertius* as percent community biomass
	(a) all plankton	(b) *V. tertius* excluded		
24 August 1986	65	20	69	62
26 June 1987	193	143	26	11
2 September 1987	56	53	5	7

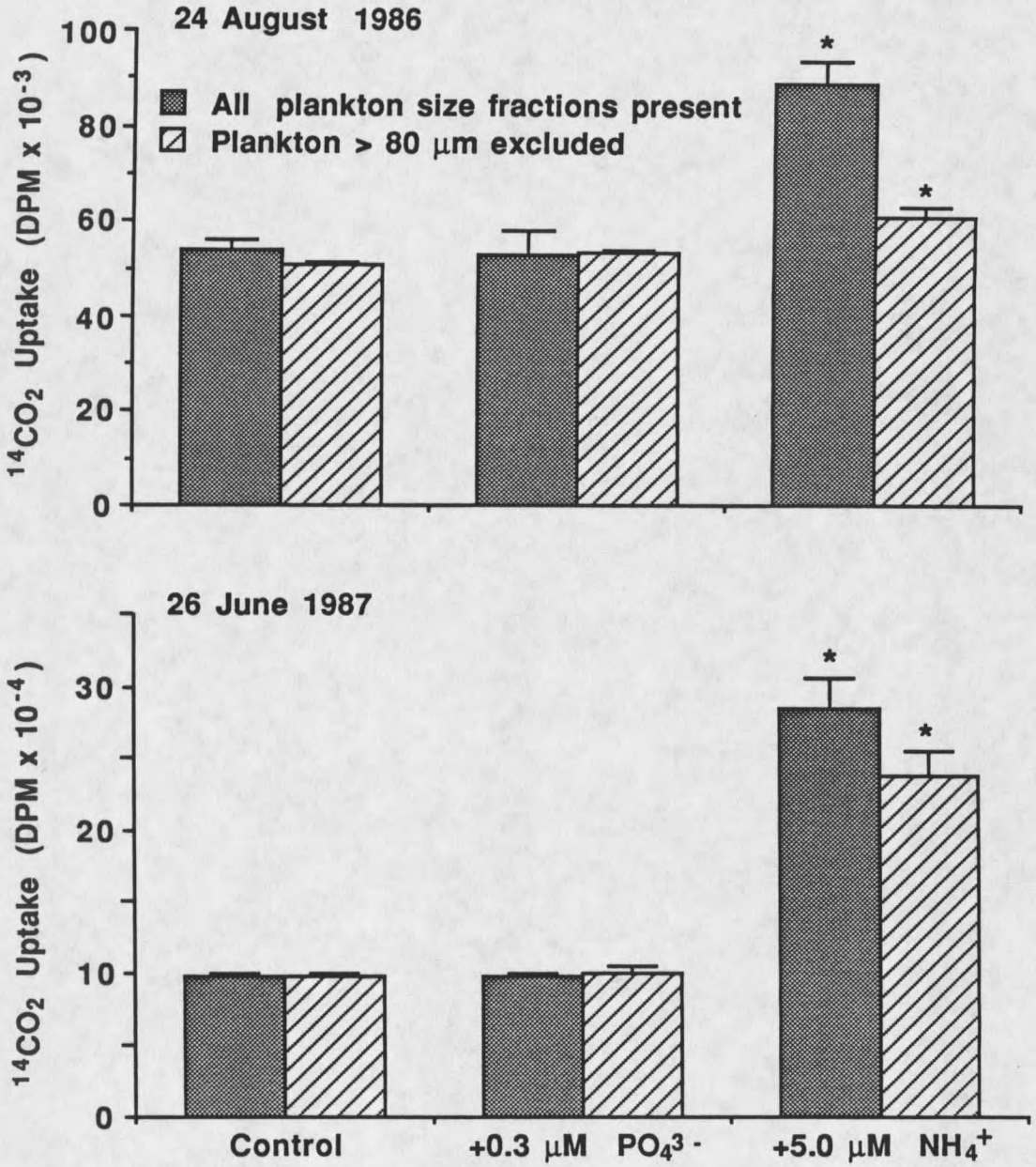

Figure 11. Influence of NH_4^+ or PO_4^{3-} enrichment on phytoplankton uptake of $^{14}CO_2$, 24 August 1986 and 26 June 1987. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

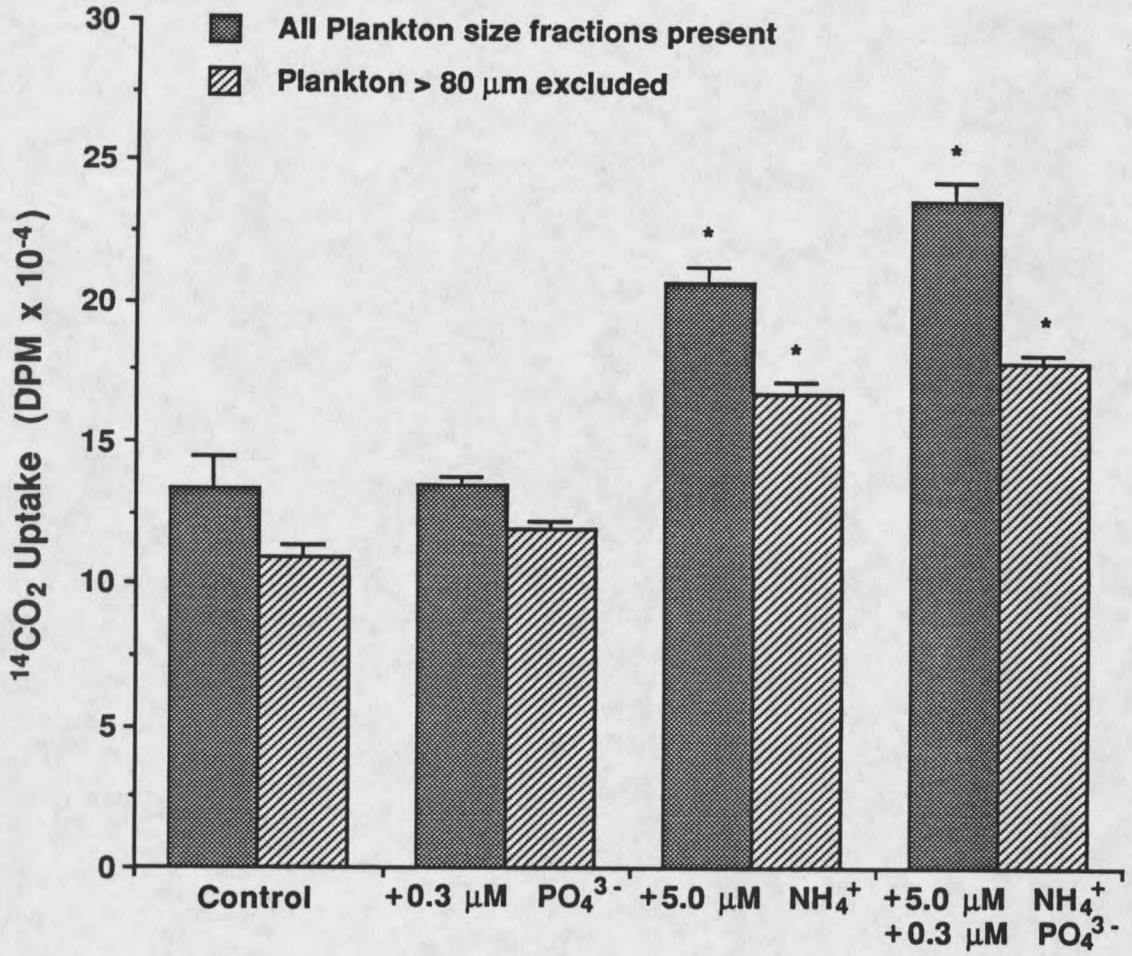

Figure 12. Influence of NH_4^+ and/or PO_4^{3-} enrichment on phytoplankton uptake of $^{14}CO_2$, 2 September 1987. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

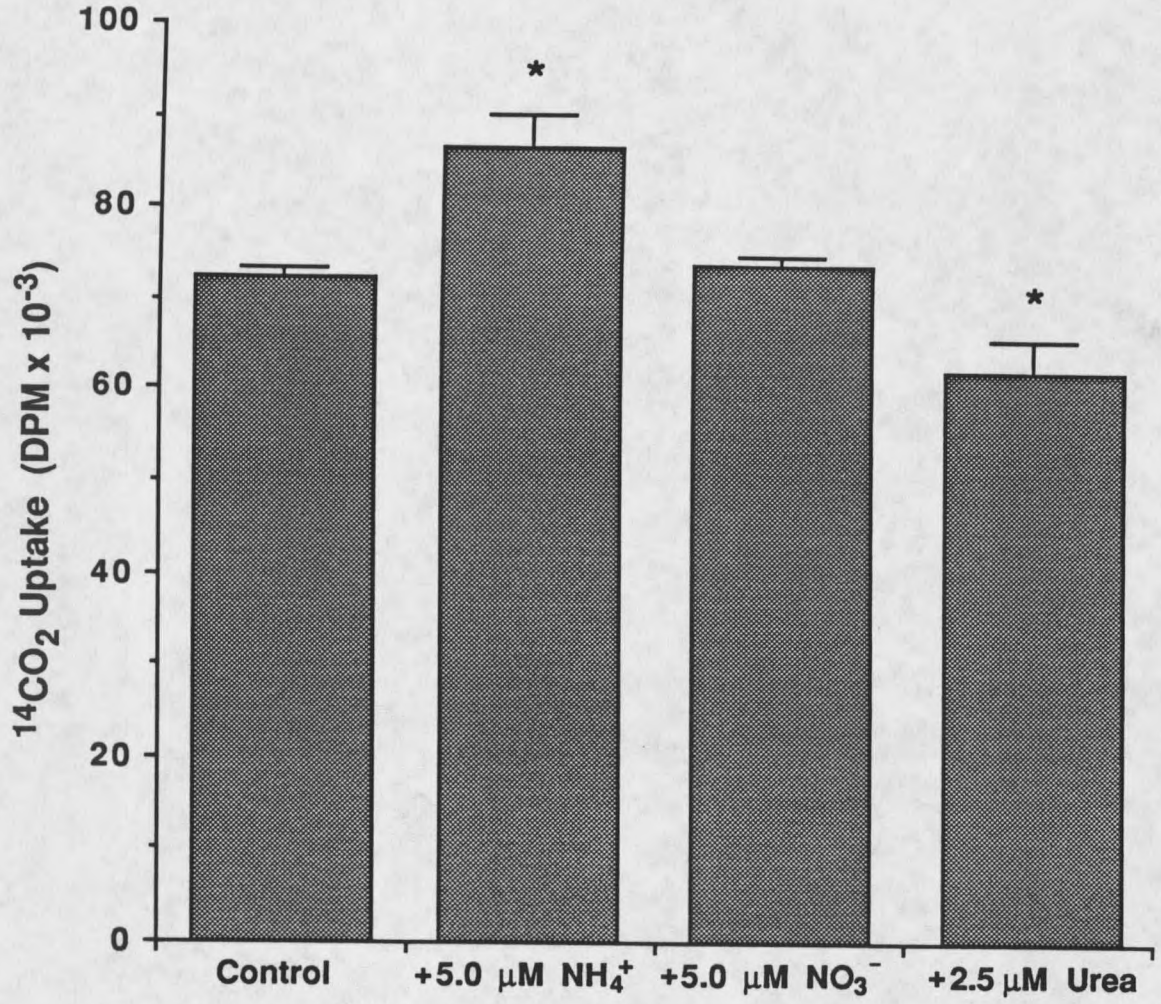

Figure 13. Comparison of effects of NH_4^+, NO_3^- and urea enrichments on phytoplankton uptake of $^{14}CO_2$, 2 September 1987. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

Influence of Meltwater Additions

Dilution of lake water with meltwater from snow fields or from freshly fallen snow significantly increased algal cellular incorporation of $^{14}CO_2$ ($p < 0.05$, ANOVA/LSD; see Figure 14). However, this effect was dampened as the dilution water:lake water ratio increased from 1:3 to 3:1, suggestive of osmoregulatory constraints on phytoplankton metabolic activity. Concentrations of NH_4^+, NO_3^- and SRP were much greater in dilution waters than in lake water (Table 4; Figure 8), presumably accounting for the stimulatory effect of meltwater on algal photosynthesis (see *Evidence for Nitrogen Limitation of Water-Column Primary Production*; Chapter 5).

Planktonic Uptake and Regeneration of Nitrogen

Substrate Kinetics of NH_4^+ and NO_3^- Uptake

Serial additions of NH_4^+ to lake water samples stimulated an asymptotic increase in NH_4^+ uptake on 7 of 8 sampling occasions (Figure 15). Additions of NO_3^- generally resulted in minimal increases in NO_3^- uptake, indicating that ambient concentrations of this substrate were near saturation relative to the uptake capabilities of the phytoplankton community. Because even the lowest NO_3^- enrichments in the kinetics experiments appeared to exceed K_t, estimates of this parameter were of questionable accuracy (see Table 11). Conventionally interpreted, however, the estimates suggested that algal affinity for NO_3^- sometimes matched or even exceeded that for NH_4^+, a possibility which will receive critical evaluation in Chapter 5. Based on assumed Michaelis-Menten uptake kinetics (Chapter 3) and on calculated values of K_t and P_{max}

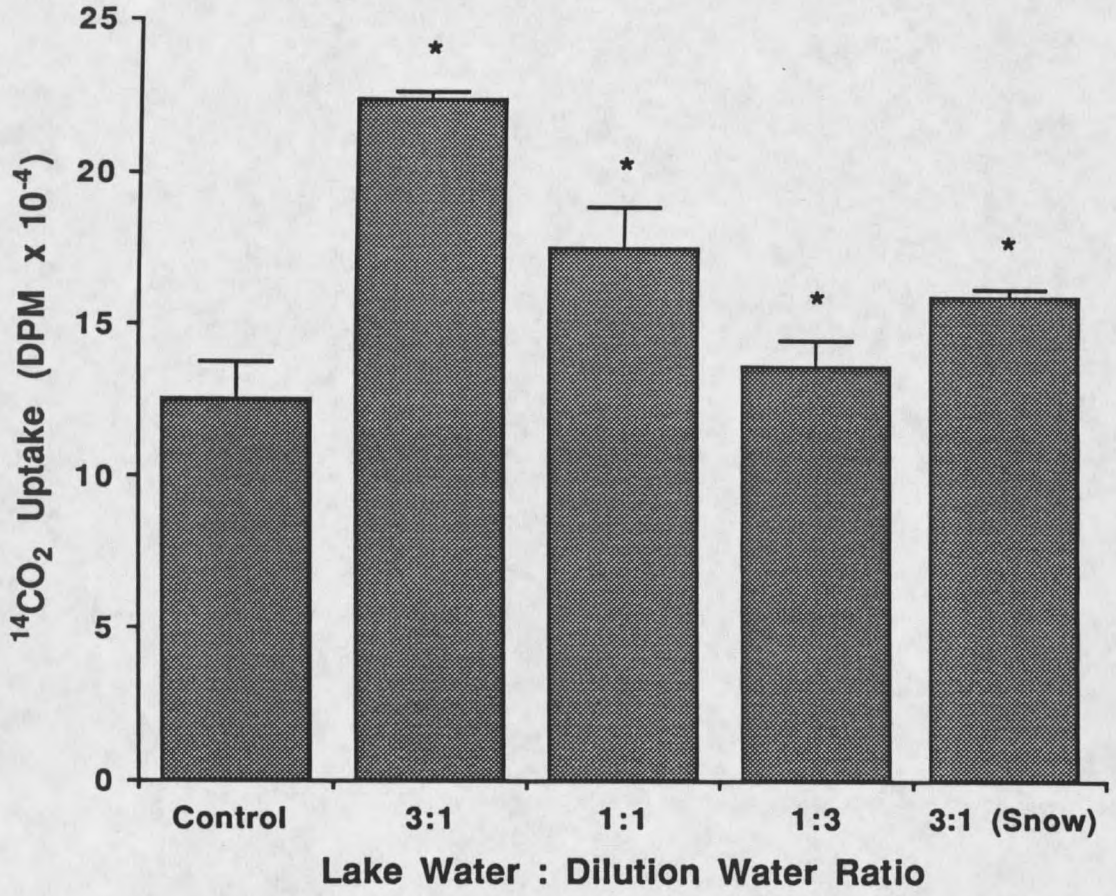

Figure 14. Influence of snowmelt and precipitation additions on phytoplankton uptake of $^{14}CO_2$, 2 September 1987. This experiment included lake water controls, a lake water:snow field runoff dilution series, and a lake water:snowmelt treatment (far right column). DPM values (treatment means and associated standard errors) have been corrected for differences in phytoplankton density and $^{14}CO_2$ specific activity between treatments. A comparison of the chemical characteristics of lake water, runoff water and snow used in this experiment is provided in Table 4.

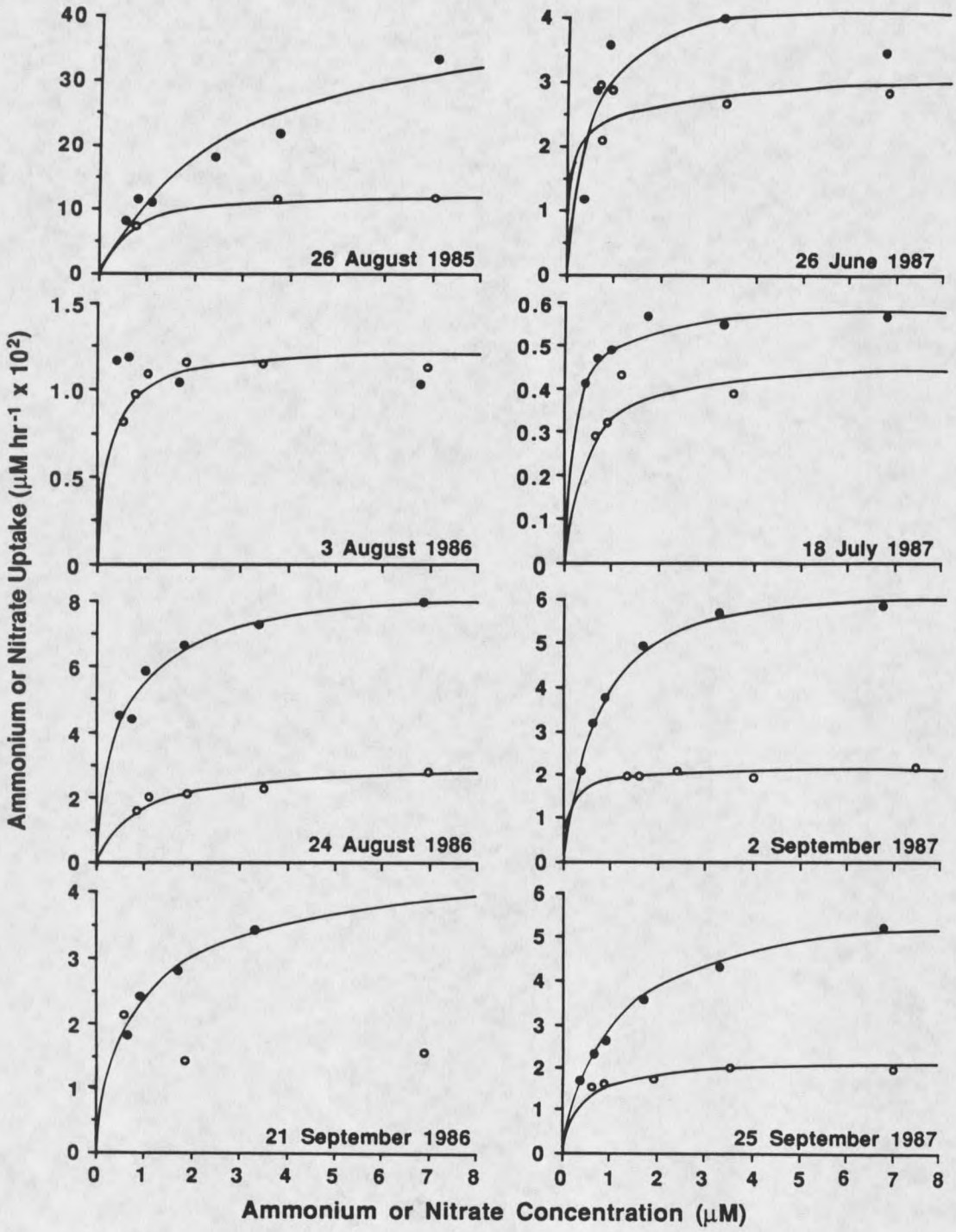

Figure 15. Phytoplankton uptake of NH_4^+ (•) and NO_3^- (∘) versus substrate concentration. All NH_4^+ uptake data are corrected for isotope dilution. Regression lines are based on equation 5 (Chapter 3), fitted with Marquart's algorithm. Regression lines are not depicted for 3 August 1986 NH_4^+ data or for 21 September 1986 NO_3^- data.

Table 11. Substrate kinetic constants calculated for phytoplankton uptake of NH_4^+ and NO_3^-, 1985-87. Values (± standard deviations) for maximum uptake rate (P_{max}), maximum specific uptake rate (V_{max}) and the half-saturation constant (K_t) were calculated via Marquardt's algorithm. All NH_4^+ uptake data were corrected for isotope dilution before calculation of P_{max}, V_{max} and K_t.

Date Parameter	P_{max} ($\mu M\ hr^{-1}$)	V_{max} (hr^{-1})	K_t (μM)
8/26/85			
NH_4^+	0.4665 ± 0.0787	0.0359 ± 0.0061	3.3880 ± 1.1832
NO_3^-	0.1300 ± 0.0056	0.0100 ± 0.0004	0.5945 ± 0.1265
8/03/86			
NH_4^+	0.0103 ± 0.0004	0.0092 ± 0.0004	0.0544 ± 0.0005
NO_3^-	0.0123 ± 0.0005	0.0110 ± 0.0004	0.2171 ± 0.0632
8/24/86			
NH_4^+	0.0846 ± 0.0041	0.0448 ± 0.0022	0.5037 ± 0.0943
NO_3^-	0.0288 ± 0.0020	0.0152 ± 0.0011	0.6357 ± 0.1789
9/21/86			
NH_4^+	0.0426 ± 0.0030	0.0087 ± 0.0006	0.7756 ± 0.1612
NO_3^-	0.0138 ± 0.0012	0.0028 ± 0.0002	0.1880 ± 0.0469
6/26/87			
NH_4^+	0.0440 ± 0.0036	0.0308 ± 0.0025	0.3605 ± 0.1225
NO_3^-	0.0293 ± 0.0025	0.0205 ± 0.0017	0.0906 ± 0.0831
7/18/87			
NH_4^+	0.0059 ± 0.0002	0.0086 ± 0.0003	0.1889 ± 0.0316
NO_3^-	0.0044 ± 0.0008	0.0064 ± 0.0012	0.2736 ± 0.2530
9/02/87			
NH_4^+	0.0666 ± 0.0021	0.0151 ± 0.0005	0.6714 ± 0.0700
NO_3^-	0.0211 ± 0.0009	0.0048 ± 0.0002	0.0988 ± 0.0922
9/25/87			
NH_4^+	0.0587 ± 0.0018	0.0200 ± 0.0006	1.0560 ± 0.0917
NO_3^-	0.0208 ± 1.14E8	0.0071 ± 3.88E7	0.2521 ± 6.08E9

(Table 11), rates of NH_4^+ uptake at ambient NH_4^+ concentrations appeared to range from 9.5% to 56.3% of P_{max} (mean = 23.6% during course of study), whereas rates of NO_3^- uptake ranged from 25.3% to 88.6% of P_{max} (mean = 58.6%). Maximum uptake rates for NH_4^+ exceeded those for NO_3^- by 2.4 times, on average (the ratio $P_{max,NH_4^+}:P_{max,NO_3^+}$ ranged from 0.84 on 3 August 1986 to 3.59 on 26 August 1985; see Table 11). At ambient nutrient levels, estimated ratios of NO_3^- uptake:NO_3^- + NH_4^+ uptake (*i.e.*, f-ratios; see Eppley 1981) averaged 0.6 or, alternatively, 0.7, depending on the method used in their calculation (Table 12); the ratio $P_{NH_4^+}/(P_{NH_4^+} + P_{NO_3^+}):[NH_4^+]/([NH_4^+] + [NO_3^-])$ (the Relative Preference Index; see McCarthy *et al.* 1977) generally was near unity (mean ± standard error = 0.98 ± 0.11, excluding an unusually high value of 2.78 on 2 September 1987), indicating that NH_4^+ (NO_3^-) utilization in Snowbank Lake was roughly proportional to NH_4^+ (NO_3^-) availability.

Table 12. Temporal comparison of f-ratios calculated by two alternative methods, 1986-87. Method 1 uses equation 5 (Chapter 3) and numerically generated values for K_t and P_{max} (Table 11) to correct for the effect of substrate enrichment on algal NO_3^- uptake. Method 2 assumes P_{NO_3} equals P_{max} on each sampling occasion (Figure 11) and provides an upper estimate of the f-ratio.

Basis of NO_3^- uptake calculation	Sampling date							1986-87 mean (± SE)
	8/03	8/24	9/21	6/26	7/18	9/02	9/25	
Method 1:	0.54	0.37	0.65	0.59	0.48	0.73	0.69	0.58 ± 0.08
Method 2:	0.68	0.70	0.75	0.69	0.62	0.75	0.79	0.71 ± 0.03

Substrate Competition: NH_4^+ versus NO_3^-

Results from the substrate competition experiments indicated that NO_3^- uptake was inhibited in a progressive manner by serial increases in NH_4^+ concentration (Figure 16). Overall, a 56% reduction in NO_3^- uptake was documented as NH_4^+ concentration increased from 0.1 to 7.3 μM ($p < 0.05$; t-test). Significant reductions in NH_4^+ uptake in response to increasing NO_3^- concentration were also documented but were less pronounced (24% maximum decrease; $p < 0.05$). Nitrate concentrations above approximately 0.9 μM had little additional effect on NH_4^+ uptake ($p > 0.05$; see Figure 17).

Eucaryotic versus Procaryotic Regeneration and Uptake of NH_4^+

The results of the metabolic inhibitor experiments conducted on 2 September 1987 are depicted in Figure 18. Ammonium uptake was reduced 45.7% in chloramphenicol treatments and 84.1% in cyclohexamide treatments relative to controls ($p < 0.0001$, ANOVA/LSD), implying that both procaryotic and eucaryotic plankton contributed substantially to measured rates of NH_4^+ uptake in Snowbank Lake. Rates of NH_4^+ regeneration in cyclohexamide treatments were only 0.2% of control rates ($p = 0.0001$, ANOVA/LSD), suggesting that eucaryotic plankton were almost exclusively responsible for NH_4^+ regeneration. Chloramphenicol treatments experienced only an 11.2% reduction in regeneration activity ($p > 0.05$, ANOVA/LSD), again implicating eucaryotes as the principal planktonic agents of NH_4^+ regeneration. In the 26 June 1987 experiment, isotope dilution was not detected in filtered (GF/C) controls or in filtered antibiotic treatments, implying that NH_4^+ regeneration by planktonic organisms

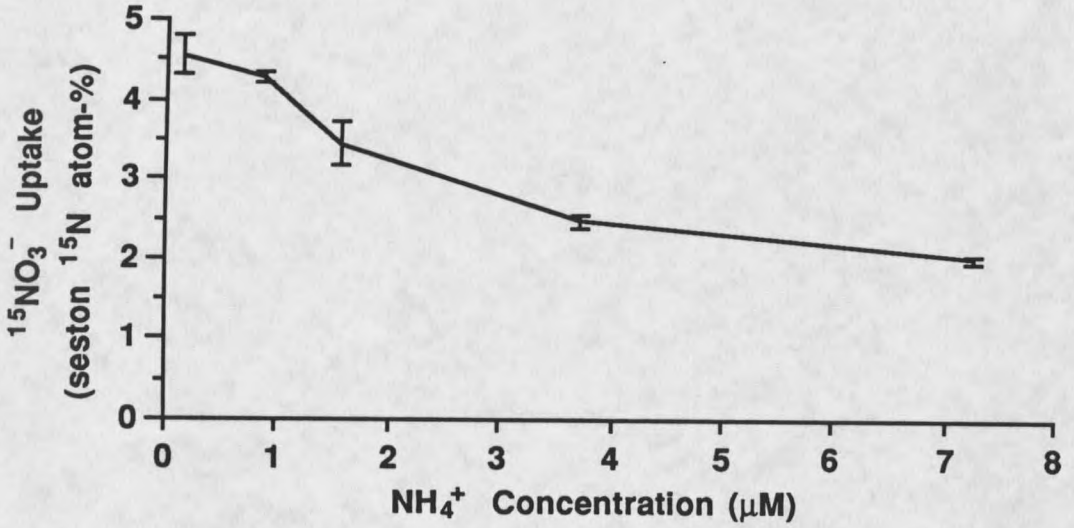

Figure 16. Influence of NH_4^+ concentration on phytoplankton uptake of $^{15}NO_3^-$. Data represent ^{15}N enrichments (mean ± 1 standard error) of the seston 30 hr after the addition of $^{15}NO_3^-$ inoculant.

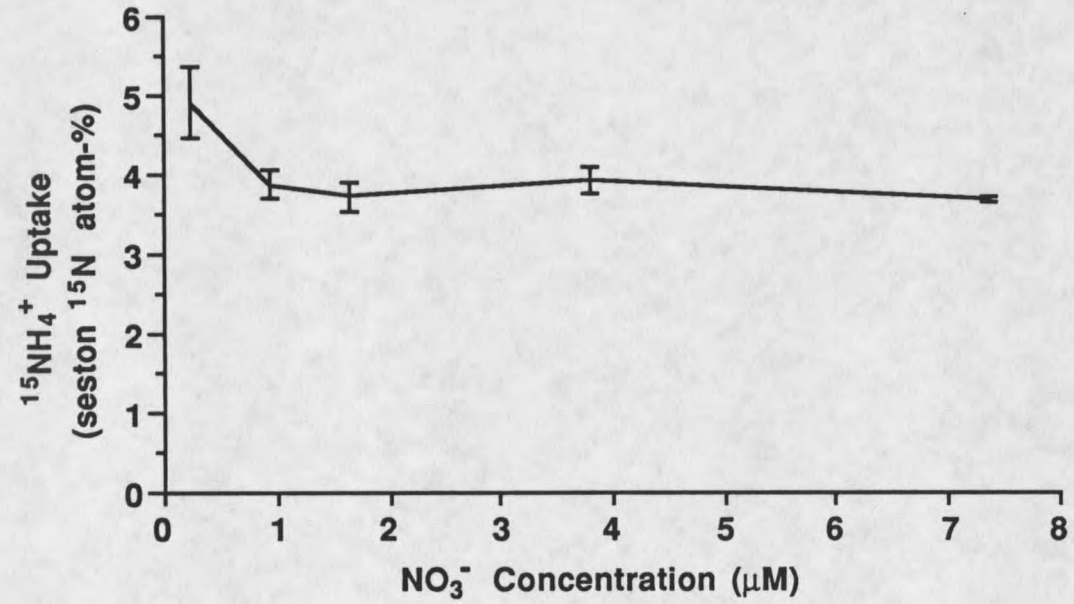

Figure 17. Influence of NO_3^- concentration on phytoplankton uptake of $^{15}NH_4^+$. Data represent ^{15}N enrichments (mean ± 1 standard error) of the seston 30 hr after the addition of $^{15}NH_4^+$ inoculant.

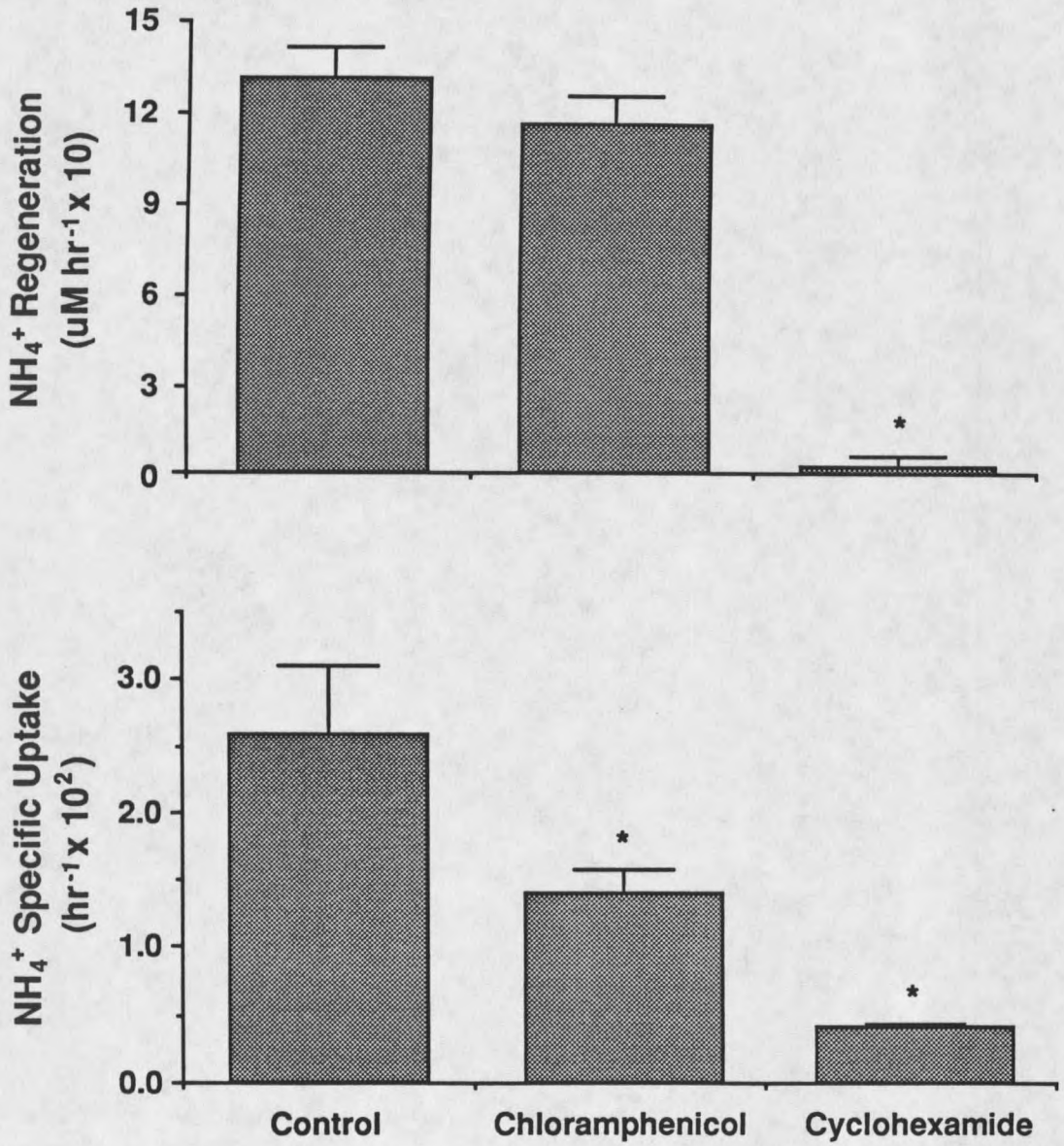

Figure 18. Effects on chloramphenicol and cyclohexamide additions on the regeneration and specific uptake of NH_4^+ by Snowbank Lake plankton. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

smaller than approximately 1 μm was quantitatively unimportant in Snowbank Lake.

Time Course of NH_4^+ Uptake and Regeneration

Figure 19 depicts changes in size-fractionated NH_4^+ regeneration and uptake, NH_4^+ concentration and $^{15}NH_4^+$ specific activity, and seston ^{15}N atom-% excess occurring over a 6.2-hr *in situ* incubation (26 August 1985). Ammonium depletion, $^{15}NH_4^+$ dilution, and algal ^{15}N accumulation proceeded more slowly in screened treatments than in nonscreened treatments; NH_4^+ uptake rates exhibited no clear trends over time, regardless of size class (*i.e.*, slope of regression lines not significantly different from zero at $\alpha = 0.05$). On average, uptake rates in screened treatments were only 21% of those in nonscreened treatments. This reduction was disproportionate to the algal biomass removed via screening (approximately 58%), suggesting that biomass-specific NH_4^+ uptake was greater for *V. tertius* than for smaller (<63 μm) phytoplankton. Regeneration rates in nonscreened samples remained nearly constant for the first 5 hr, then declined by roughly 90% between 5.0 and 6.2 hr. Regeneration rates in screened samples generally increased during the incubation and surpassed rates in nonscreened samples after approximately 4.7 hr. Calculated over the entire 6.2-hr incubation (*i.e.*, based on initial and final values of S, R, ρ and PN; see equations 8-12, Chapter 3), volumetric regeneration rate:uptake rate ratios were 0.25 for the "intact" plankton assemblage and 1.46 for the isolated (<63 μm) size fraction.

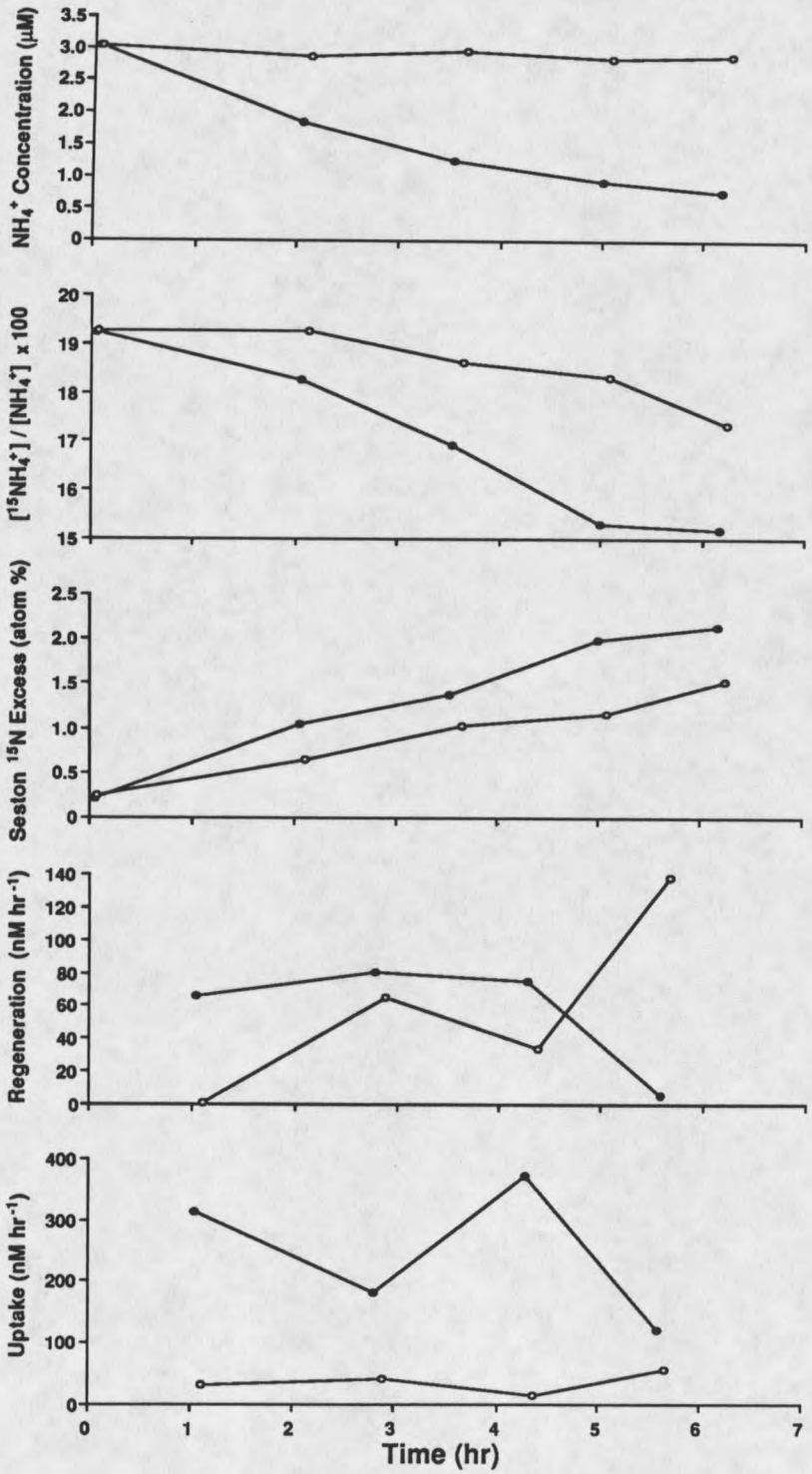

Figure 19. Size-fractionated time course of NH_4^+ regeneration, uptake, aqueous concentration and ^{15}N specific activity and particulate ^{15}N atom-% excess, 26 August 1985 field experiment. Fractions include all plankton (•) and plankton smaller than 63 μm (○).

The results of the field time-course experiments conducted in 1987 are presented in Figure 20 and in tables 13-16. Particulate nitrogen concentration and seston ^{15}N atom-% excess increased during incubations, whereas NH_4^+ concentration, ^{15}N atom-% enrichment of NH_4^+, and volumetric NH_4^+ uptake gradually decreased. Time course patterns of NH_4^+ regeneration were variable among experiments, exhibiting rapid, nonlinear decreases on 26 June and 2 September and a gradual increase on 25 September. On 18 July, NH_4^+ regeneration was not detected over the course of a 5.2-hr incubation; moreover, NH_4^+ uptake rates were the lowest recorded during the study, averaging only 0.0066 μM hr^{-1}. (These low rates presumably reflected unusually low levels of plankton metabolic activity and biomass, as they coincided with the second coldest water temperature, the lowest algal biovolume, and the lowest concentrations of PN, PC and chlorophyll *a* recorded during the study; see Table 6 and figures 5, 7 and 10). Correction for isotope dilution increased measured NH_4^+ uptake rates by as much as 18% in these experiments (tables 13-16). Loss of $^{15}NH_4^+$ from the aqueous fraction was 2.9-4.5 times greater than accounted for by ^{15}N uptake into the particulate fraction, a discrepancy similar in magnitude to those reported in previous ^{15}N-based studies of algal nitrogen uptake (*e.g.*, Glibert *et al.* 1982; see also *Limitations of Nutrient Uptake Models*, Chapter 5, this report). When calculated over entire incubation periods (*i.e.*, when based only on initial and final values of S, R, ρ and PN), regenerative and assimilative fluxes of NH_4^+ closely paralleled each other during the 1987 ice-free season (Pearson $r = 0.97$, $p = 0.03$; see Figure 21).

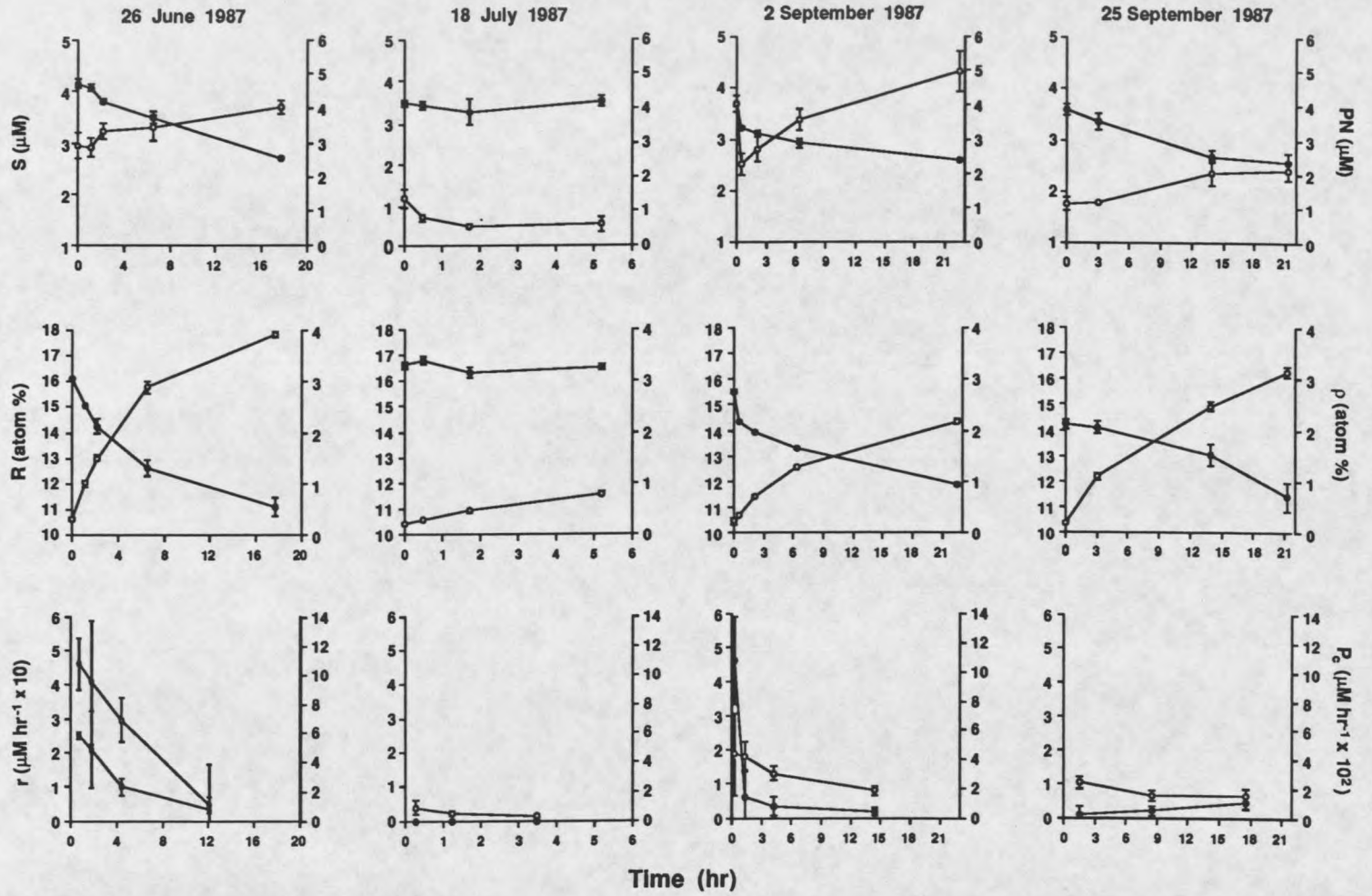

Figure 20. Time course of NH_4^+ regeneration (r), uptake (P_c), aqueous concentration (S) and ^{15}N specific activity (R), and particulate nitrogen concentration (PN) and ^{15}N atom-% excess (ρ), 1987 field experiments. Data (treatment means ± 1 standard error) denoted by darkened circles (•) pertain to left axes; data denoted by open circles (o) pertain to right axes.

Table 13. Time course of NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}NH_4^+$ specific activity (R), particulate nitrogen concentration (PN), particulate ^{15}N atom-% excess (ρ), and the ratios $r:P_c$, $P_c:P$ (where P is NH_4^+ uptake uncorrected for isotope dilution) and $u:P_c$ (where u is NH_4^+ uptake based only on changes in S and R), 26 June 1987. Ammonium fluxes are in units of $\mu M\ hr^{-1}$, concentrations in μM, ^{15}N enrichments in atom-%, time (t) in hr, and error terms in ± standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	4.15±0.08	2.91±0.38	16.12±0.00	0.32±0.03					
0.57					0.2500±0.0115	0.1123±0.0153	2.23	1.04	2.85
1.14	4.07±0.06	2.86±0.28	15.04±0.00	1.01±0.06					
1.65					0.2086±0.1113	0.1013±0.0365	2.06	1.03	4.58
2.16	3.81±0.03	3.32±0.27	14.25±0.29	1.50±0.07					
4.37					0.0996±0.0274	0.0791±0.0130	1.26	1.06	2.17
6.58	3.49±0.12	3.44±0.37	12.63±0.32	2.89±0.11					
12.16					0.0359±0.0133	0.0292±0.0048	1.23	1.07	3.62
17.74	2.71±0.02	4.05±0.19	11.09±0.35	3.92±0.06					
Overall (t_0 - $t_{17.74}$):					0.0712±0.0079	0.0519±0.0037	1.37	1.18	2.94

Table 14. Time course of NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}NH_4^+$ specific activity (R), particulate nitrogen concentration (PN), particulate ^{15}N atom-% excess (ρ), and the ratios $r:P_c$, $P_c:P$ (where P is NH_4^+ uptake uncorrected for isotope dilution) and $u:P_c$ (where u is NH_4^+ uptake based only on changes in S and R), 18 July 1987. Ammonium fluxes are in units of $\mu M\ hr^{-1}$, concentrations in μM, ^{15}N enrichments in atom-%, time (t) in hr, and error terms in ± standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	3.45±0.08	1.37±0.26	16.62±0.13	0.22±0.01					
0.27					not detected	0.0092±0.0051	--	1.00	--
0.48	3.40±0.10	0.81±0.11	16.78±0.14	0.28±0.03					
1.25					not detected	0.0058±0.0019	--	1.00	--
1.74	3.24±0.31	0.54±0.08	16.33±0.23	0.46±0.03					
3.47					not detected	0.0034±0.0014	--	1.00	--
5.20	3.49±0.14	0.62±0.21	16.52±0.10	0.79±0.04					
Overall (t_0-$t_{5.20}$):					not detected	0.0066±0.0012	--	1.00	--

Table 15. Time course of NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}NH_4^+$ specific activity (R), particulate nitrogen concentration (PN), particulate ^{15}N atom-% excess (ρ), and the ratios $r:P_c$, $P_c:P$ (where P is NH_4^+ uptake uncorrected for isotope dilution) and $u:P_c$ (where u is NH_4^+ uptake based only on changes in S and R), 2 September 1987. Ammonium fluxes are in units of $\mu M\ hr^{-1}$, concentrations in μM, ^{15}N enrichments in atom-%, time (t) in hr, and error terms in ± standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	3.68±0.03	4.04±0.24	15.51±0.20	0.23±0.07					
0.29					0.4617±0.1295	0.0437±0.0279	10.57	1.04	28.71
0.58	3.22±0.03	2.26±0.31	14.35±0.25	0.35±0.03					
1.33					0.0618±0.0742	0.0423±0.0098	1.46	1.01	2.89
2.07	3.13±0.03	2.71±0.35	13.94±0.42	0.71±0.03					
4.24					0.0339±0.0275	0.0304±0.0052	1.12	1.02	2.63
6.40	2.93±0.09	3.56±0.31	13.28±0.33	1.28±0.02					
14.41					0.0193±0.0113	0.0189±0.0033	1.02	1.06	2.14
22.42	2.59±0.03	4.99±0.58	11.87±0.52	2.17±0.06					
Overall (t_0-$t_{22.42}$):					0.0370±0.0067	0.0286±0.0025	1.29	1.13	2.99

Table 16. Time course of NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}NH_4^+$ specific activity (R), particulate nitrogen concentration (PN), particulate ^{15}N atom-% excess (ρ), and the ratios $r:P_c$, $P_c:P$ (where P is NH_4^+ uptake uncorrected for isotope dilution) and $u:P_c$ (where u is NH_4^+ uptake based only on changes in S and R), 25 September 1987. Ammonium fluxes are in units of $\mu M\ hr^{-1}$, concentrations in μM, ^{15}N enrichments in atom-%, time (t) in hr, and error terms in ± standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	3.59±0.10	1.14±0.19	14.21±0.20	0.19±0.02					
1.52					0.0097±0.0252	0.0245±0.0043	0.40	1.00	3.49
3.04	3.36±0.16	1.19±0.02	14.09±0.24	1.09±0.06					
8.47					0.0221±0.0135	0.0152±0.0036	1.45	1.04	5.58
13.89	2.68±0.12	2.05±0.36	13.01±0.42	2.47±0.09					
17.57					0.0474±0.0214	0.0155±0.0047	3.06	1.06	4.11
21.24	2.56±0.18	2.11±0.28	11.39±0.57	3.14±0.10					
Overall (t_0-$t_{21.24}$):					0.0317±0.0123	0.0177±0.0020	1.79	1.11	4.53