



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
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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 , ^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 ^{14}C -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 assimilable 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 $\text{HNO}_3/\text{H}_2\text{SO}_4$ 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^{-} .

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by

Robert Thomas Angelo

A thesis submitted in partial fulfillment
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in

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16 October 1989
Date

John C. Prue
Chairperson, Graduate Committee

Approved for the Major Department

30 November 1989
Date

Robert S. Moore
Head, Major Department

Approved for the College of Graduate Studies

December 7, 1989
Date

Benny L. Parsons
Graduate Dean

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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 , ^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 $^{14}\text{CO}_2$ -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 $\text{HNO}_3/\text{H}_2\text{SO}_4$ 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 demographic 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 became 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 "bootstrapping" 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.*, Merezko *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 $\mu\text{eq CaCO}_3 \text{ l}^{-1}$) 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 administrative 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,

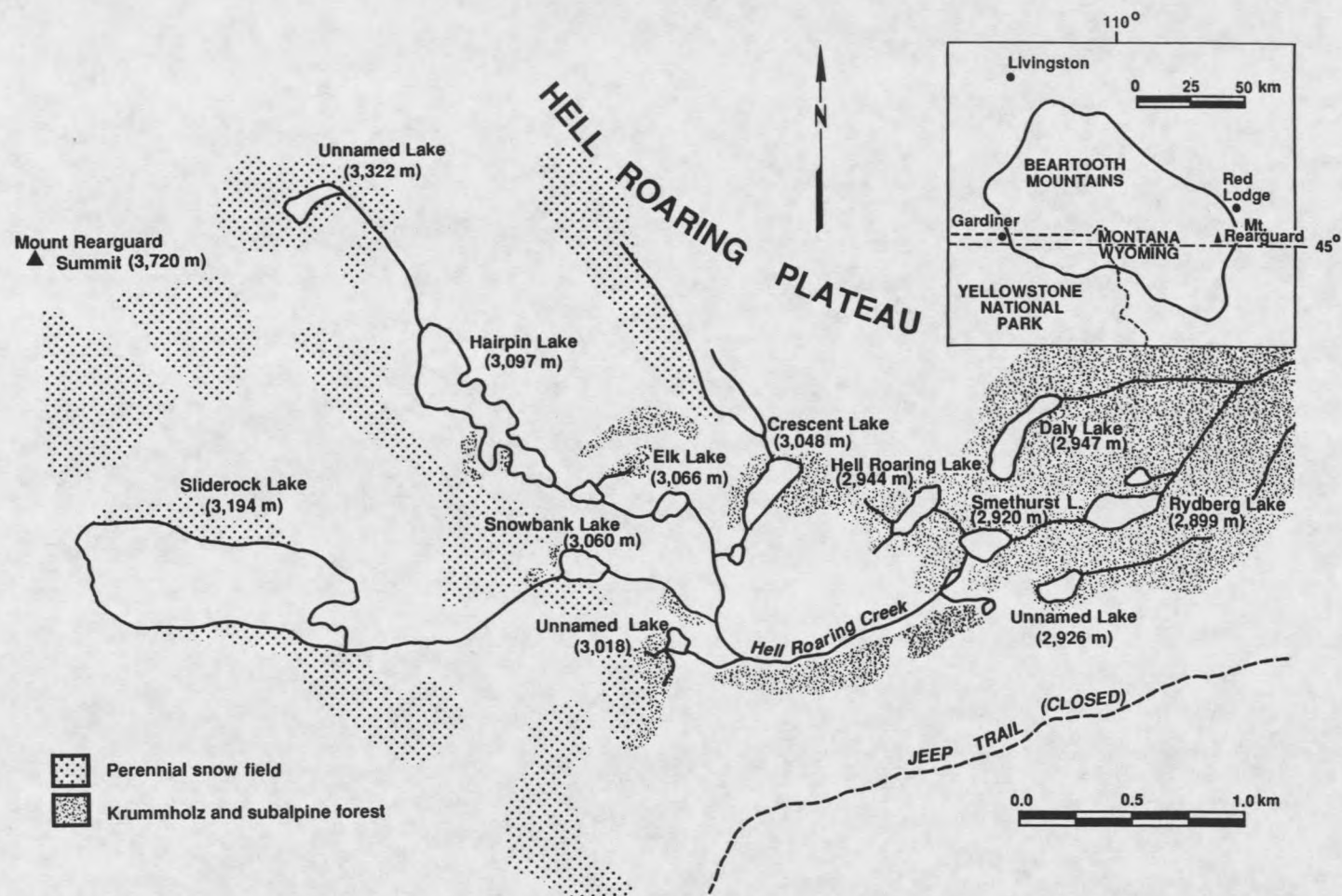


Figure 1. Location and physiographic features of the upper Hell Roaring Creek watershed. Parenthetical values refer to elevation above mean sea level (as determined by Marcuson 1980d).

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	$1.44 \times 10^5 \text{ m}^3$
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

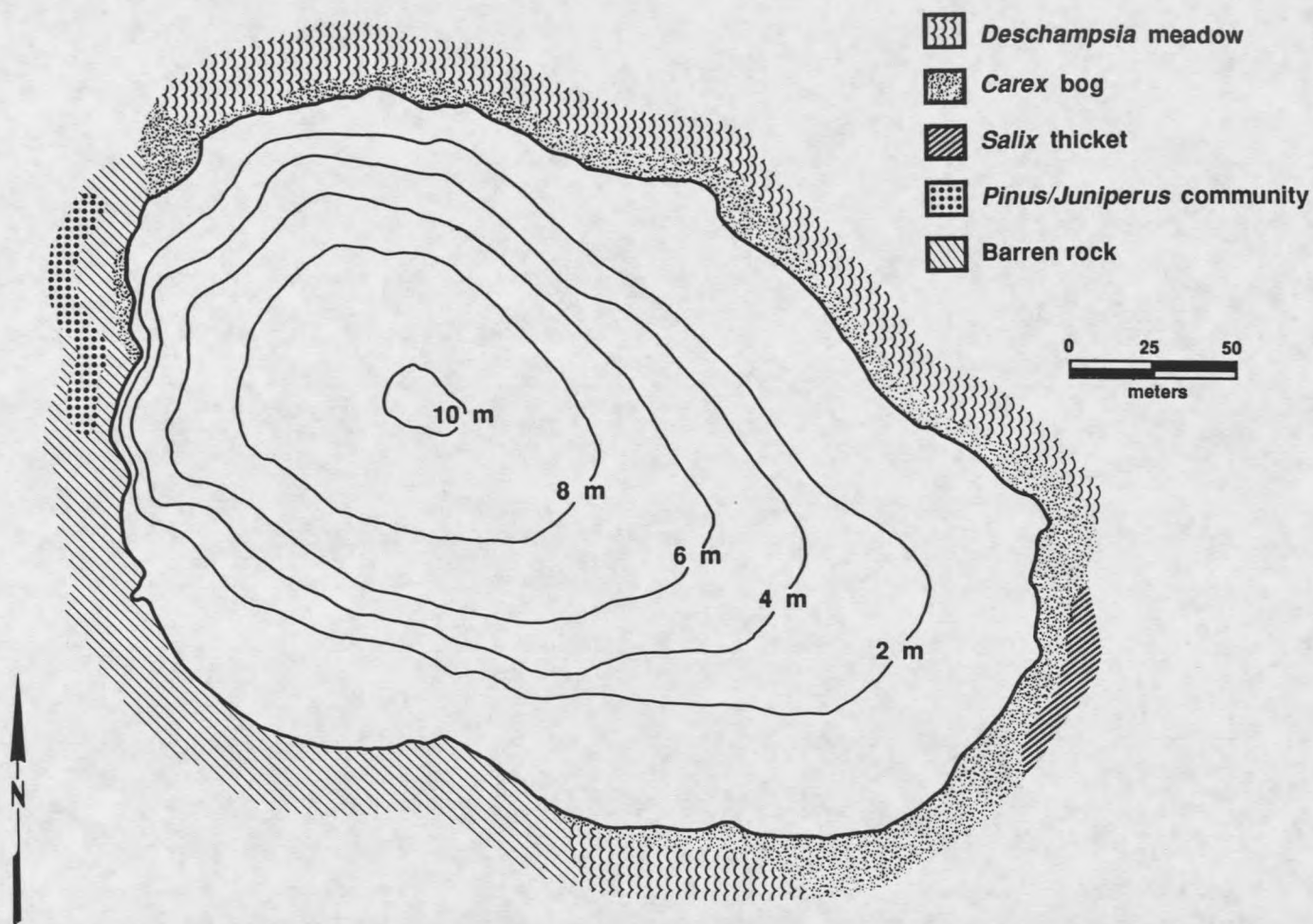


Figure 2. Snowbank Lake bathymetric contours and shoreline vegetation. Contours were determined by triangulation (see Wetzel and Likens 1979) on 25 August 1985; the lake perimeter was determined from aerial photograph (ASCS; 30 July 1971).

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* Pursh, *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 $\text{NO}_3^- + \text{NO}_2^-$ were measured via the red diazotization reaction between NO_2^- , sulfanilamide and N-1-naphthylethylenediamine 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 ($0.03 \mu\text{M}$).

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 % $\pm$ SD)	Replicability (mean CV $\pm$ SD)	Number of replicate groups
NH_4^+	97.8 $\pm$ 3.5	5.4 $\pm$ 1.7	5
NO_3^-	80.0 $\pm$ 4.3	6.9 $\pm$ 1.2	5
SRP	97.6 $\pm$ 5.7	2.8 $\pm$ 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 $\pm 1 \mu\text{g}$ 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):

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

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

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

$SAC = 89 \text{ l} \cdot \text{g cm}^{-1}$ = 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 $^{14}\text{CO}_2$ 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 $\pm 2^\circ\text{C}$) 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-} ($0.3 \mu\text{M}$ as KH_2PO_4), NH_4^+ ($5.0 \mu\text{M}$ as NH_4Cl) or, on one occasion (7 September 1987), both nutrients simultaneously. Parallel treatments were performed on lake water screened through $80\text{-}\mu\text{M}$ 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 $300 \mu\text{E m}^{-2} \text{s}^{-1}$ (GE soft-white) and the prevailing lake temperature for approximately 24 hr, then inoculated with $2 \mu\text{Ci}$ 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 Scintiverse 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 $3.6\ \mu\text{M}$ nitrogen, in the form of NH_4^+ , NO_3^- or urea. Following preincubation ($300\ \mu\text{E m}^{-2}\ \text{s}^{-1}$, prevailing lake temperature, 24 hr), each sample was inoculated with $1\ \mu\text{Ci}$ 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 $^{14}\text{CO}_2$ 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 ($0\text{--}2^\circ\text{C}$) 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 $\mu\text{E m}^{-2} \text{ s}^{-1}$ 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., $^{14}\text{CO}_2$ 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-% $^{15}\text{NH}_4^+$ stock solution (Bio-Rad $^{15}\text{N}-(\text{NH}_4)_2\text{SO}_4$, 1985-86; MSD $^{15}\text{N}-\text{NH}_4\text{Cl}$, 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-% $^{15}\text{NO}_3^-$ stock solution (Stohler $^{15}\text{N}-\text{NaNO}_3$) 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 $^{15}\text{N}:^{15}\text{N} + ^{14}\text{N}$ ratio (or ^{15}N atom-%) determination by optical emission spectrometry (Timperly and Priscu 1986). All ^{15}N atom-% measurements were

made relative to $^{15}\text{N}/^{14}\text{N}$ 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):

$$P = \frac{{}^{15}\text{N}_{\text{xs}} \text{ PN}}{({}^{15}\text{N}_{\text{enr}} - {}^{15}\text{N}_e) t} \quad (2)$$

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

PN = particulate nitrogen concentration (μM)

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

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

${}^{15}\text{N}_{\text{xs}}$ = ${}^{15}\text{N}_e$ minus the naturally occurring $^{15}\text{N}:^{15}\text{N} + {}^{14}\text{N}$ ratio (0.00365)

t = duration of incubation (hr).

Ammonium uptake rates calculated via equation 2 required correction for changes in $^{15}\text{NH}_4^+$ specific activity (i.e., isotope dilution) resulting from $^{14}\text{NH}_4^+$ regeneration within the experimental vessel (see Garside 1984). The following equation from Kanda et al. (1987) was employed:

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

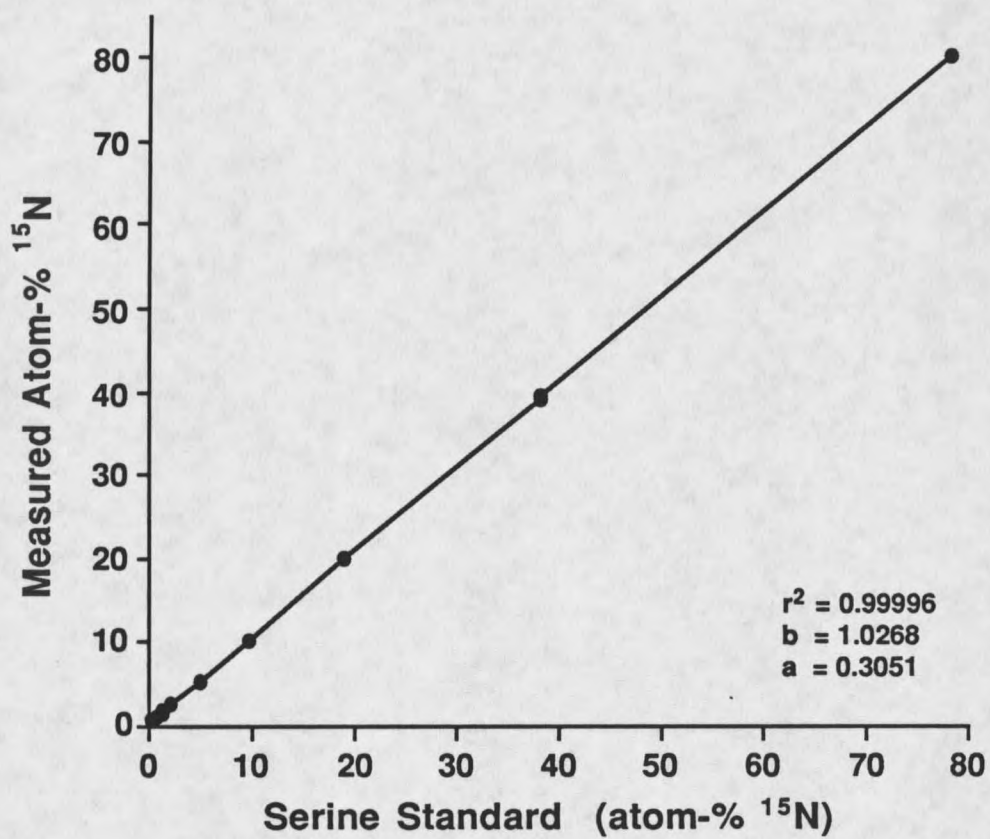


Figure 3. Typical standard curve for ^{15}N atom-% measurements performed by optical emission spectrometry.

where P_c = NH_4^+ uptake rate ($\mu\text{M hr}^{-1}$) corrected for isotope dilution
 r = NH_4^+ regeneration rate ($\mu\text{M hr}^{-1}$; 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:

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

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):

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

where P_c = uptake rate ($\mu\text{M hr}^{-1}$) 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:NH₄⁺ versus NO₃⁻

Effects of NH₄⁺ Concentration on NO₃⁻ Uptake. This laboratory experiment examined the influence of NH₄⁺ concentration on phytoplankton uptake of NO₃⁻. 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 $\pm 2^{\circ}\text{C}$) 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 NH₄⁺. Following a 6 hr preincubation at 300 $\mu\text{E m}^{-2} \text{s}^{-1}$ and the prevailing lake water temperature (at the time and depth of collection), all treatments were inoculated with 99 atom-% ¹⁵NO₃⁻ (0.3 μM added concentration), then incubated for 24 hr under preincubation conditions. Incubations were terminated by filtration (muffled GF/C), and the ¹⁵N enrichment of the seston was determined via emission spectrometry.

Effects of NO₃⁻ Concentration on NH₄⁺ Uptake. This experiment was performed concurrently with the experiment described in the preceding paragraph. Experimental conditions were identical, except that the roles of NO₃⁻ and NH₄⁺ were reversed; i.e., treatments included formalin kills and 0, 0.7, 1.4, 3.6 and 7.1 μM NO₃⁻ enrichments, each inoculated with 99 atom-% ¹⁵NH₄⁺ (0.3 μM added concentration).

Time Course of NH₄⁺ 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-% $^{15}\text{NH}_4^+$ for an added concentration of $3.6 \mu\text{M NH}_4^+$. 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 $0.7 \mu\text{mol}$ of seston nitrogen was subjected to Dumas combustion, then analyzed for ^{15}N enrichment by emission spectrometry (note: combustion tube loadings of $0.7 \mu\text{mol PN}$, or $0.35 \mu\text{mol N}_2$ 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:

$$\% \text{ efficiency} = 100 [1 - ([\text{NH}_4^+]_{\text{post}} \div [\text{NH}_4^+]_{\text{pre}})] \quad (6)$$

where $[\text{NH}_4^+]_{\text{pre}}$ refers to the aqueous NH_4^+ concentration before zeolite extraction and $[\text{NH}_4^+]_{\text{post}}$ 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 $0.7 \mu\text{mol}$ 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 $^{15}\text{NH}_4^+$ -augmented lake water samples of known $^{15}\text{NH}_4^+ / ^{14}\text{NH}_4^+$ 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:

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

where ${}^{15}\text{N}_{\text{exp}}$ refers to the expected ${}^{15}\text{N}$ atom-% enrichment of the zeolite, and ${}^{15}\text{N}_{\text{obs}}$ refers to the observed ${}^{15}\text{N}$ atom-% enrichment. A correction coefficient of 1.19 was applied to all zeolite ${}^{15}\text{N}$ 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 ${}^{15}\text{N}$ isotope discrimination by zeolite in filtered (GF/C) surface water samples of widely contrasting ionic content. Surface water samples containing known ambient concentrations of ${}^{14}\text{NH}_4^+$ and augmented with $7.1 \mu\text{M}$ ${}^{15}\text{NH}_4^+$ (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 ($\mu\text{mhos cm}^{-1}$)	Extraction efficiency ($\bar{x} \pm \text{SE}$)	Isotopic discrimination ($\bar{x} \pm \text{SE}$)
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

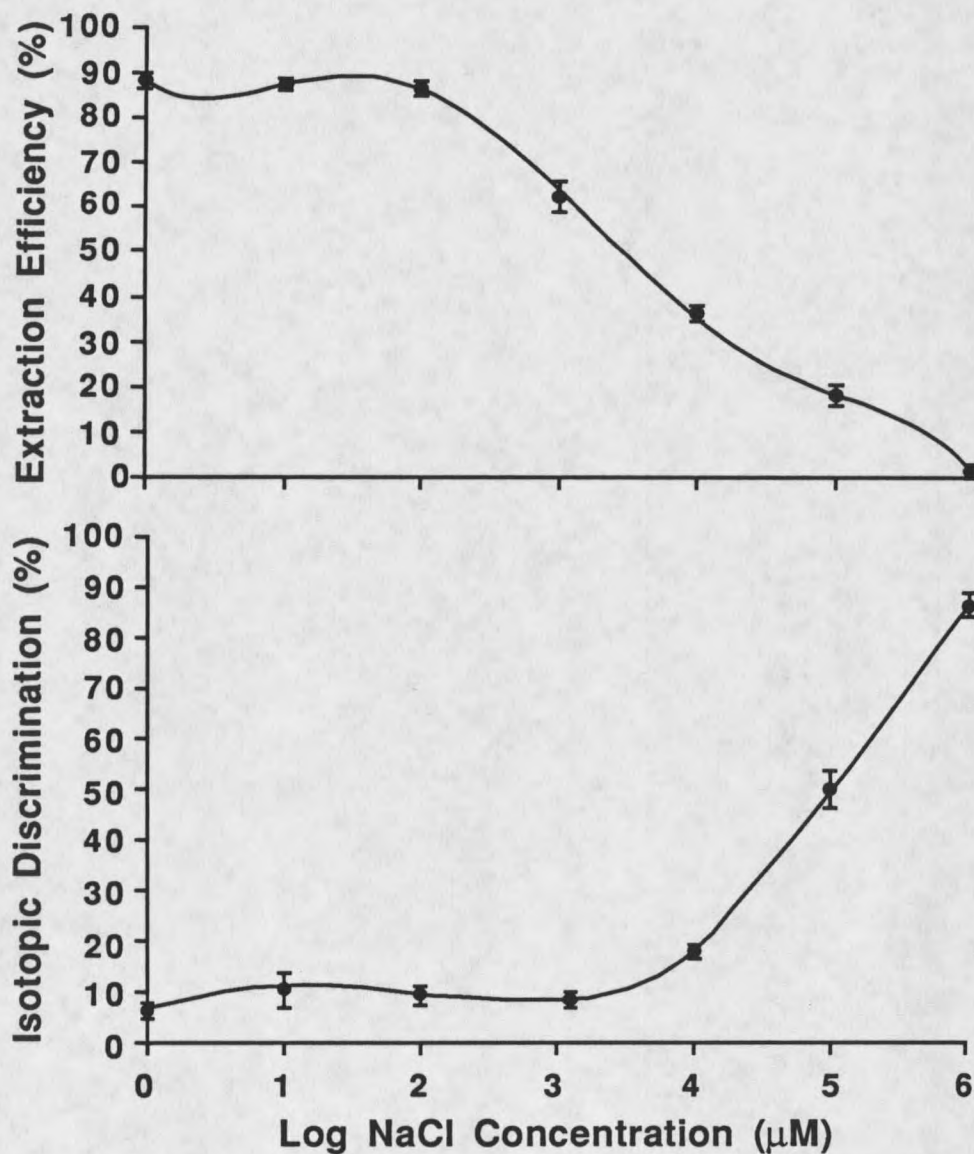


Figure 4. Ammonium extraction efficiency and isotopic discrimination by zeolite as functions of NaCl concentration. Values shown represent treatment means $\pm$ standard error and are corrected for $^{14}\text{NH}_4^+$ contamination of added NaCl. Curves are based on fifth-order polynomial fitting of experimental data.

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

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

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

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

$$P_c = \frac{\rho \bar{P}\text{N}}{\bar{R} t} \quad (10)$$

$$\bar{R} = \frac{R_o S_o - R_t S_t}{u t} \quad (11)$$

$$u = r - [(S_t - S_o)/t] \quad (12)$$

- where P_c = dilution-corrected NH_4^+ uptake rate ($\mu\text{M hr}^{-1}$), based on incorporation of ^{15}N into particulate matter
- $\bar{P}_N$ = 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 ($\mu\text{M hr}^{-1}$), based on change in NH_4^+ concentration and $^{15}\text{NH}_4^+$ 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_i 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 $\mu\text{E m}^{-2} \text{s}^{-1}$ and the prevailing lake water temperature, samples were inoculated with 20 atom-% $^{15}\text{NH}_4^+$ for an added concentration of 3.6 $\mu\text{M NH}_4^+$. 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-% $^{15}\text{NH}_4^+$ 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 $^{15}\text{NH}_4^+$ 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-% $^{15}\text{NH}_4^+$ ($3.3 \mu\text{M}$ added concentration); the other 2 samples were inoculated with 99 atom-% $^{15}\text{NO}_3^-$ ($3.3 \mu\text{M}$ 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 $300 \mu\text{E m}^{-2} \text{s}^{-1}$ 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 $^{15}\text{NH}_4^+$ -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 $[\text{H}^+]$ 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 $^{15}\text{NH}_4^+$ (99 atom-%; $6.7 \mu\text{M}$ added concentration); the remaining samples were inoculated with $^{15}\text{NO}_3^-$ (99 atom-%; $6.7 \mu\text{M}$ added concentration). Following incubation at $185 \mu\text{E m}^{-2} \text{s}^{-1}$ 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 $^{15}\text{NH}_4^+$ (99 atom-%; $6.7 \mu\text{M}$ added concentration), and the remaining 3 samples in each category were inoculated with $^{15}\text{NO}_3^-$ (99 atom-%; $6.7 \mu\text{M}$ 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 $^{15}\text{NO}_3^-$ and HNO_3 , uptake competition between $^{15}\text{NO}_3^-$ and $^{14}\text{NO}_3^-$ required that corrections be made for reduced aqueous $^{15}\text{NO}_3^-$ specific activity. Given the likely saturation of NO_3^- uptake mechanisms at $^{15}\text{NO}_3^- + ^{14}\text{NO}_3^-$ concentrations exceeding $6.7 \mu\text{M}$ (see *Kinetics of NH_4^+ and NO_3^- Uptake*, Chapter 4), reductions in measured rates of specific uptake (i.e., V , where $V = P \div \text{PN}$; see equation 2) resulting from the dissociation of HNO_3 were deemed

proportional to the decrease in $^{15}\text{NO}_3^- : ^{15}\text{NO}_3^- + ^{14}\text{NO}_3^-$ ratio and were corrected for using the following equation:

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

where $a = ^{15}\text{NO}_3^- : \text{total } \text{NO}_3^-$ ratio, given complete dissociation of added HNO_3

$b = ^{15}\text{NO}_3^- : \text{total } \text{NO}_3^-$ 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 $10 \mu\text{Ci ml}^{-1}$ ^{14}C - NaHCO_3 solution. Incubations were conducted at the prevailing lake temperature and $185 \mu\text{E m}^{-2} \text{ s}^{-1}$ 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):

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

where

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

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

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

$$K = \frac{[H_2CO_3]}{[CO_{2,aq}]} \approx 10^{-2.80} \quad (18)$$

$$K_{a1} = \frac{[H^+][HCO_3^-]}{[CO_{2,aq}]} \approx 10^{-6.35} \quad (19)$$

$$K_{a2} = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \approx 10^{-10.25} \quad (20)$$

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:

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

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 $^{14}\text{CO}_2$ 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 N 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 $300 \mu\text{E m}^2 \text{ s}^{-1}$ 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 Scintiverse 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 Scintiverse 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 Scintiverse 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 Scintiverse 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 $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ 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 $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ (99 atom-%) for a final concentration of $6.7 \mu\text{M}$ added NH_4^+ or NO_3^- . Incubations were conducted at $300 \mu\text{E m}^{-2} \text{ s}^{-1}$ 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 $[\text{H}^+]$ 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 μ Ci 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 μ m 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 Scintiverse E scintillation cocktail was added. The ^3H 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.

Parameter	Sampling date							
	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 ($\mu\text{mhos cm}^{-1}$)	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 ($\mu\text{eq l}^{-1}$ 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 ($\mu\text{g l}^{-1}$)	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

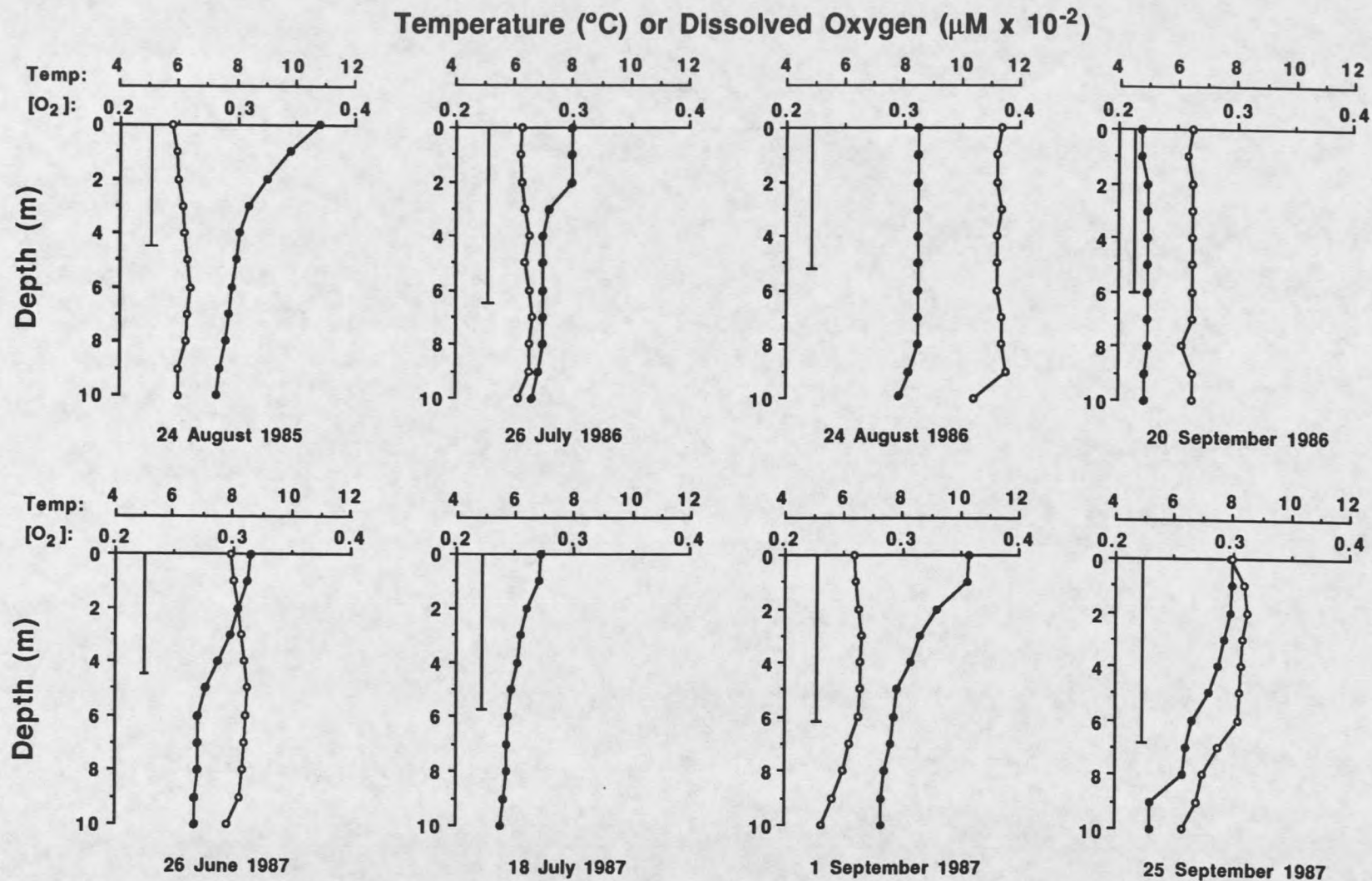


Figure 5. Vertical profiles of temperature ($\bullet$) and dissolved O₂ ($\circ$), and Secchi depth ($\perp$), 1985-87 ice-free seasons. An equipment malfunction precluded the collection of O₂ data on 18 July 1987.

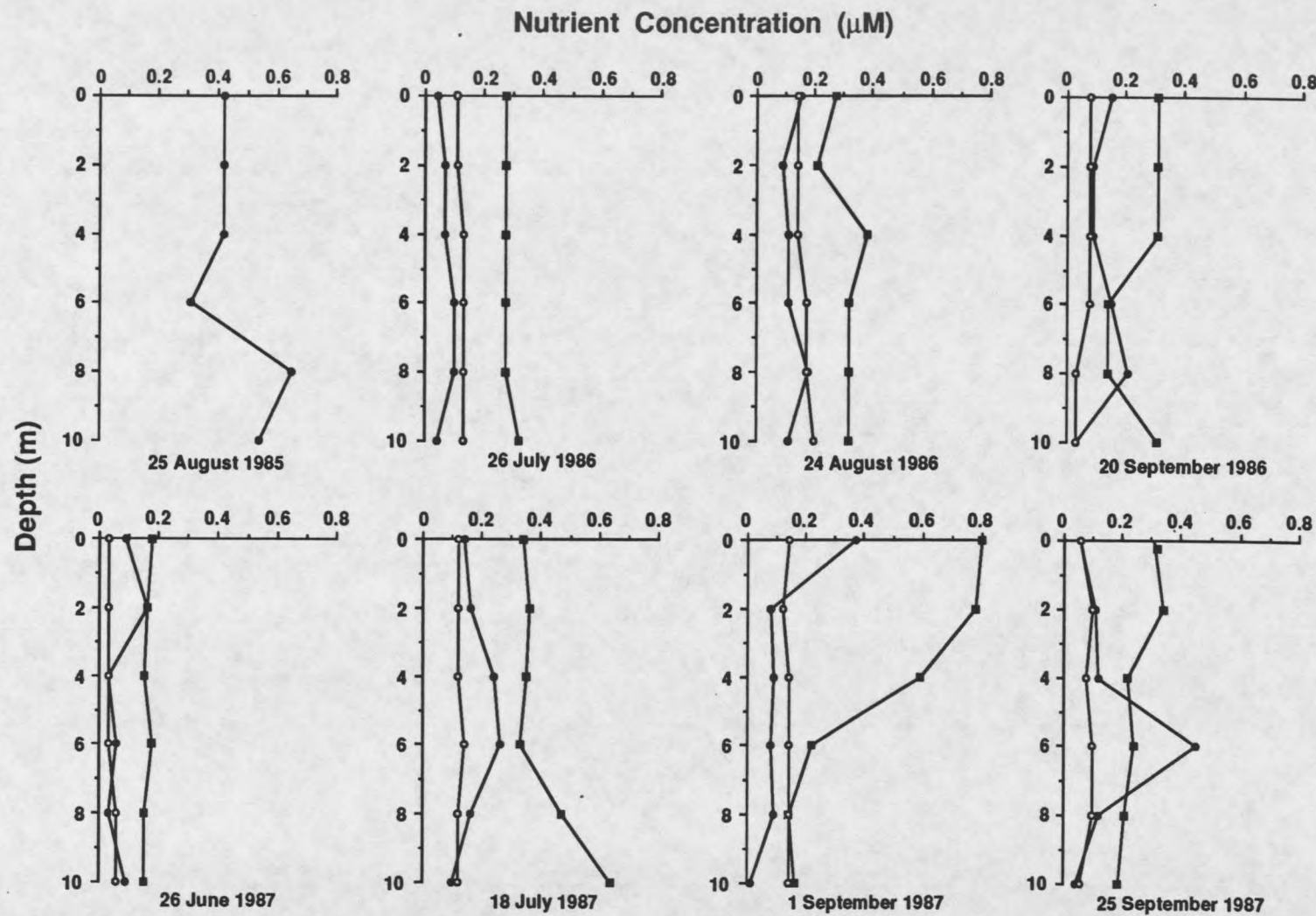


Figure 6. Depth profiles of NH_4^+ ($\bullet$), NO_3^- ($\circ$) and SRP ($\square$) concentrations, 1985-87 ice-free seasons. Nitrate and SRP samples from 25 August 1985 were inadvertently destroyed during processing.

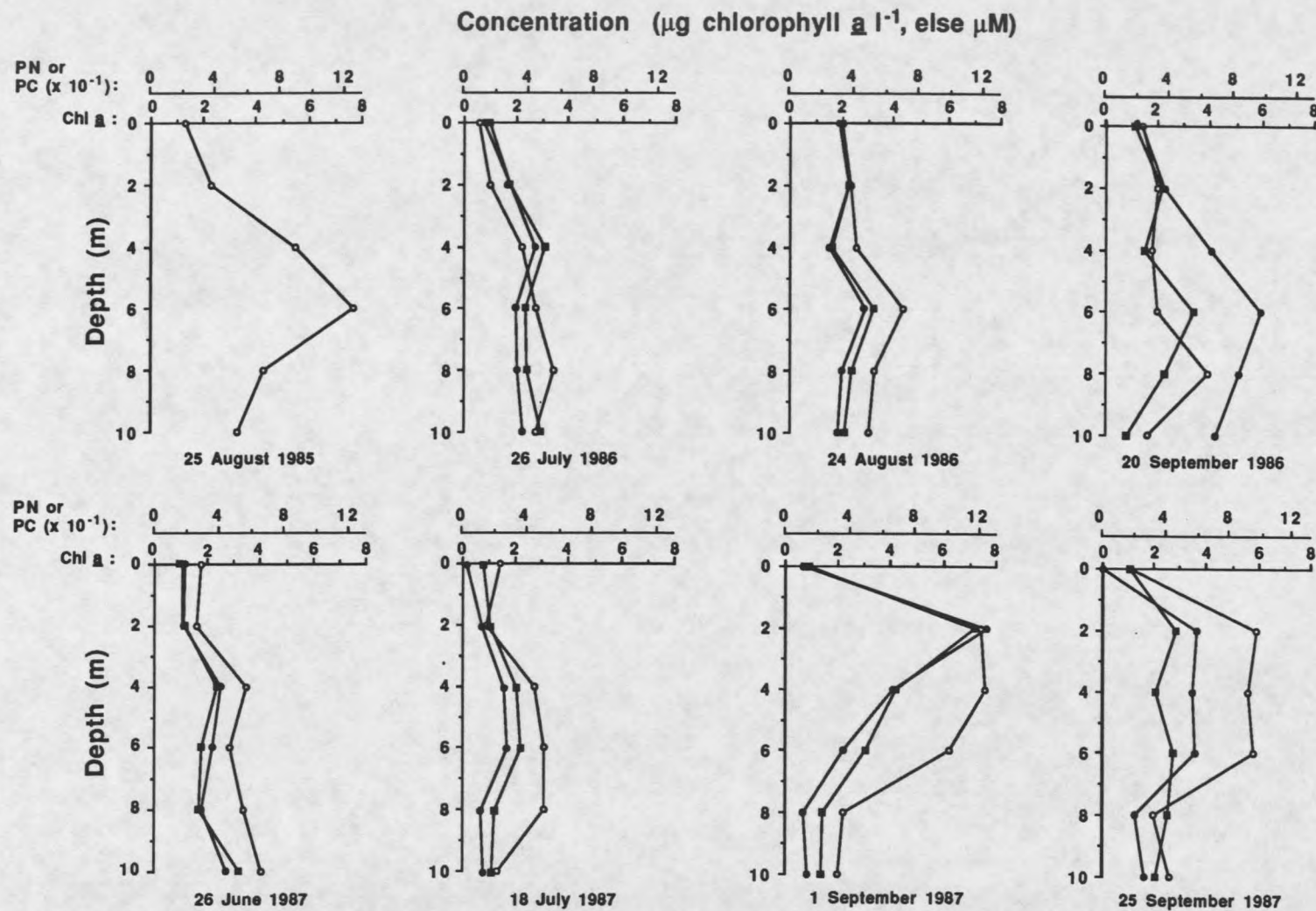


Figure 7. Depth profiles of chlorophyll a ($\circ$), particulate nitrogen ($\bullet$) and particulate carbon ($\blacksquare$) concentrations, 1985-87 ice-free seasons. Note differences in scale between parameters. Samples for particulate carbon and nitrogen were not collected on 25 August 1985.

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 $\mu eq\ liter^{-1}$ 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 ($\mu\text{eq l}^{-1}$ as CaCO_3)	Conductance ($\mu\text{mhos cm}^{-1}$, 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 $\pm$ 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

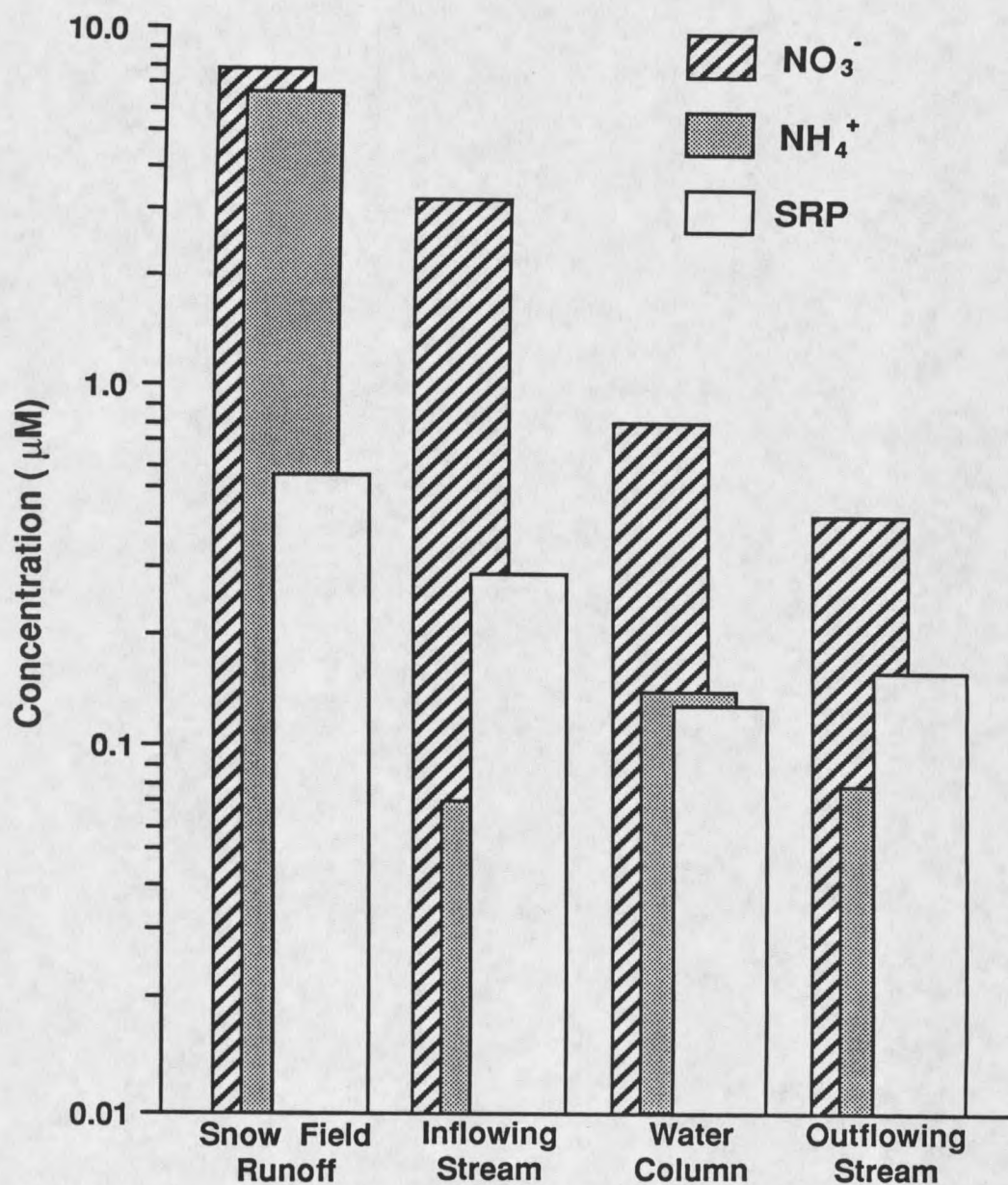


Figure 8. Comparison of dissolved inorganic nutrient concentrations in snow field runoff, lake water column, and inflowing and outflowing streams, 1 September 1987. Water-column values represent depth-integrated, volume-weighted mean concentrations. Note logarithmic scale of vertical axis.

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 $\mu\text{g l}^{-1}$)	y variable (as $\mu\text{g l}^{-1}$)	r	r ²	Slope	Intercept (as $\mu\text{g l}^{-1}/\mu\text{M}$)
Experimental depth:	Particulate N	Particulate C	0.98	0.96	7.60	78.07/6.51
	Chlorophyll <i>a</i>	Particulate N	0.78	0.61	16.22	7.70/0.55
	Chlorophyll <i>a</i>	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 <i>a</i>	Particulate N	0.39	0.15	6.78	27.06/1.93
	Chlorophyll <i>a</i>	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 *Staurostrum* 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).

Lake productivity designation	Productivity index			
	Change in alkalinity in epilimnion over summer (meq l ⁻¹)	Inorganic N (μg l ⁻¹)	Mean chlorophyll (μg l ⁻¹)	Peak chlorophyll (μg 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)

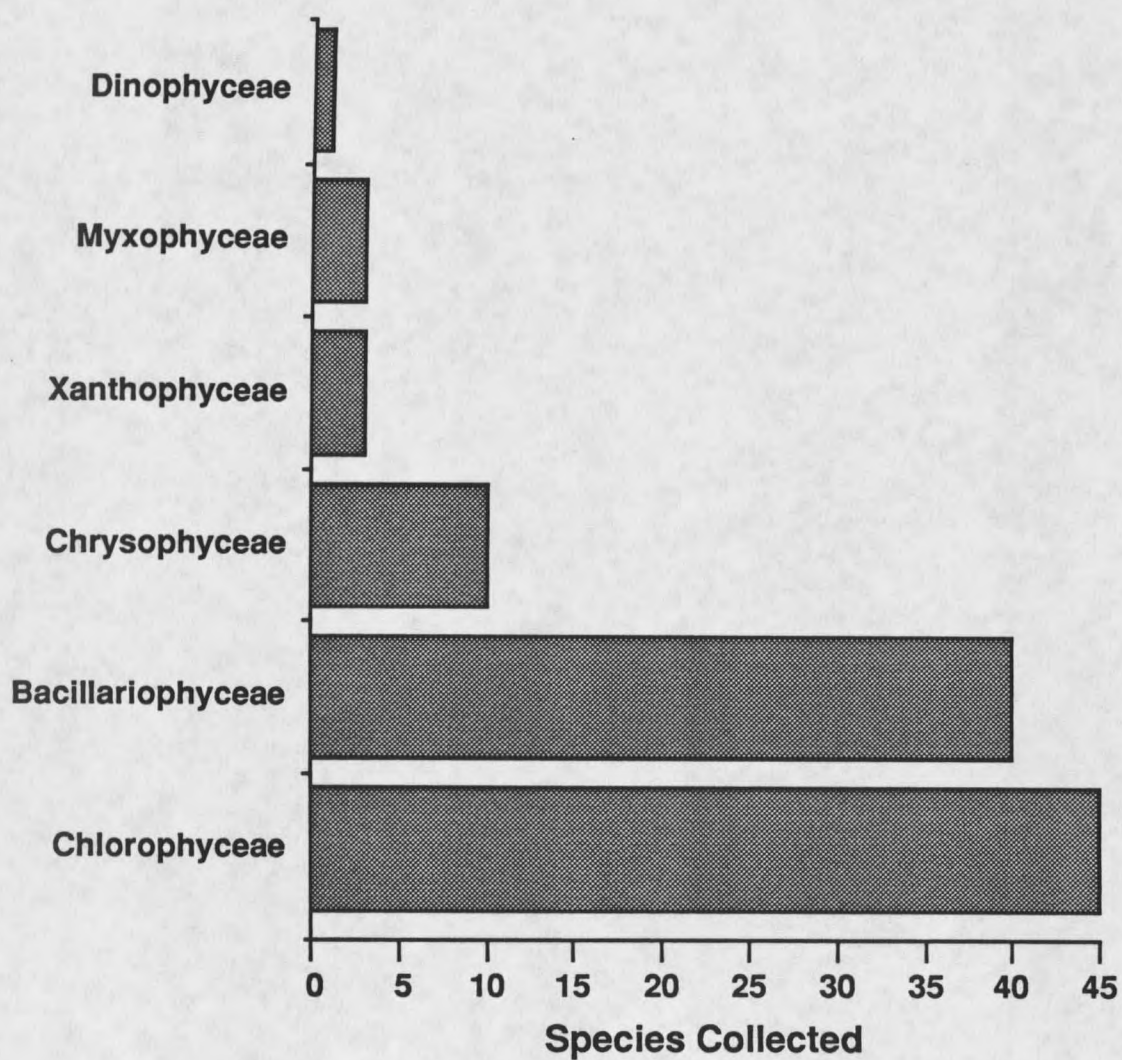


Figure 9. Phytoplankton community composition relativized to numbers of species collected during the period 1985-87. A complete listing of phytoplankton taxa collected from Snowbank Lake is provided in the appendix (Table 30).

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 $\geq 10^4 \mu\text{m}^3 \text{ ml}^{-1}$): the chlorophyceans *Volvox tertius* Meyer, *Schroederia setigera* Lemmermann, *Sphaerocystis schroeteri* Chodat, *Botryococcus* sp., *Cosmarium tinctum* var. *subretusum* Messik and *Staurostrum 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*,

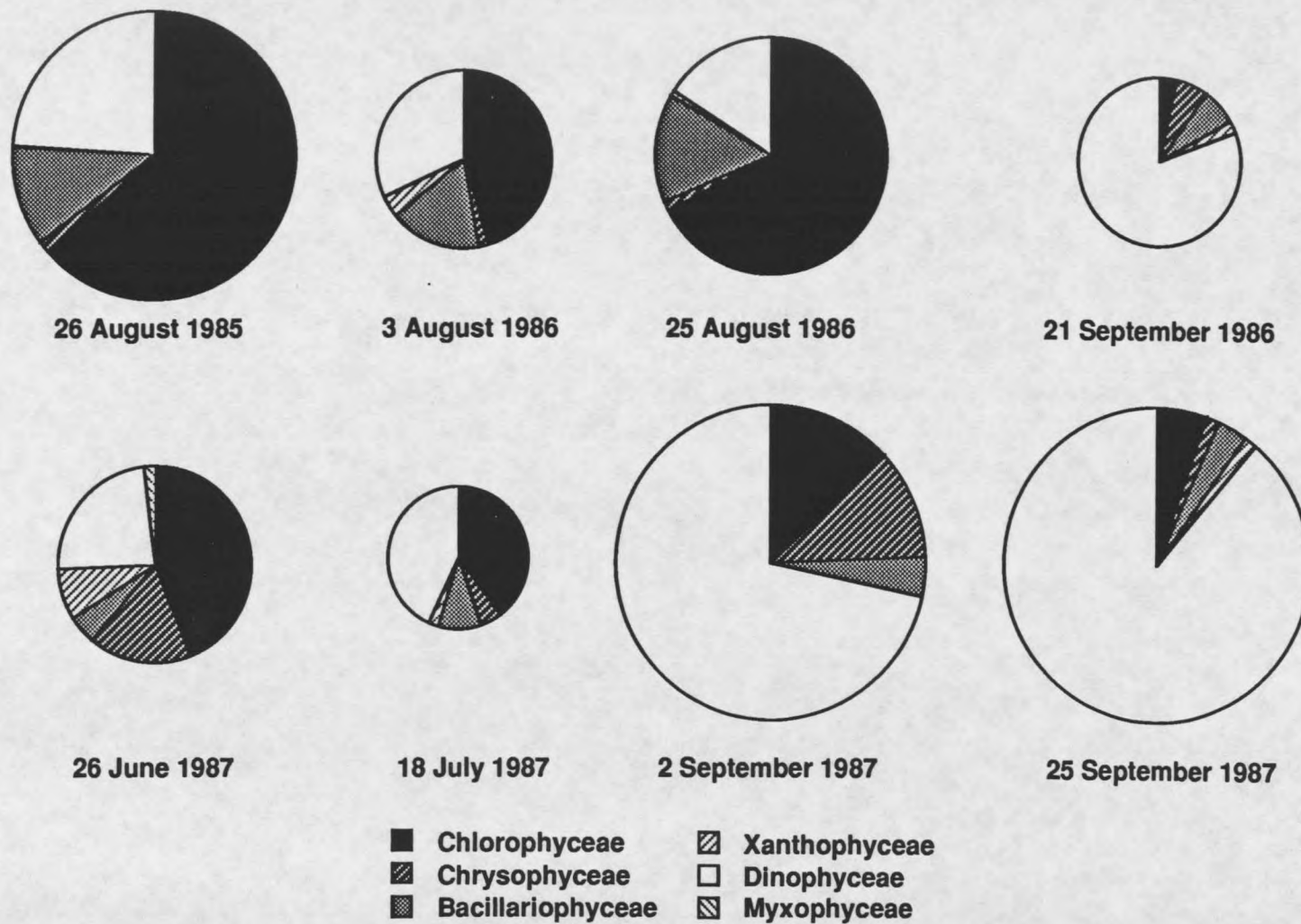


Figure 10. Phytoplankton community composition and total biomass relativized to biovolume, 1985-87. Total (relative) biovolume is indicated by diameter of subfigures.

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 ¹⁴CO₂ was increased significantly ($p < 0.05$, ANOVA/LSD) by additions of NH₄⁺ or NH₄⁺ + PO₄³⁻ but not by the addition of PO₄³⁻ alone (figures 11 and 12). The exclusion of *Volvox tertius* (which represented >97% of the algal biomass removed via size fractionation) from NH₄⁺- and NH₄⁺ + PO₄³⁻-enriched treatments dampened this stimulatory effect. On 26 June 1987, uptake of ¹⁴CO₂ 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 NH₄⁺ + PO₄³⁻ treatment (see Figure 12), when *V. tertius* accounted for 55% of the increase in ¹⁴CO₂ uptake while comprising only 7% of the phytoplankton biovolume. In samples containing *V. tertius*, simultaneous additions of NH₄⁺ and PO₄³⁻ stimulated greater increases in ¹⁴CO₂ uptake than did additions of NH₄⁺ alone ($p < 0.05$; see Figure 12).

No enhancement of ¹⁴CO₂ uptake was observed over a 24-hr period in samples augmented with forms of nitrogen other than NH₄⁺ (Figure 13). Additions of NH₄⁺ increased ¹⁴CO₂ uptake by approximately 20% ($p < 0.05$), whereas uptake was reduced 14% in urea treatments ($p < 0.05$) and was statistically unchanged in NO₃⁻ treatments ($p > 0.05$).

Table 10. Enhancement of algal $^{14}\text{CO}_2$ uptake via NH_4^+ addition in samples with and without *Volvox tertius*.

Sampling date	Increase in $^{14}\text{CO}_2$ uptake (%)		Percent community uptake due to <i>V. tertius</i> (100[a-b]/a)	<i>V. tertius</i> as percent community biomass
	(a) all plankton	(b) <i>V. tertius</i> excluded		
24 August 1986	65	20	69	62
26 June 1987	193	143	26	11
2 September 1987	56	53	5	7

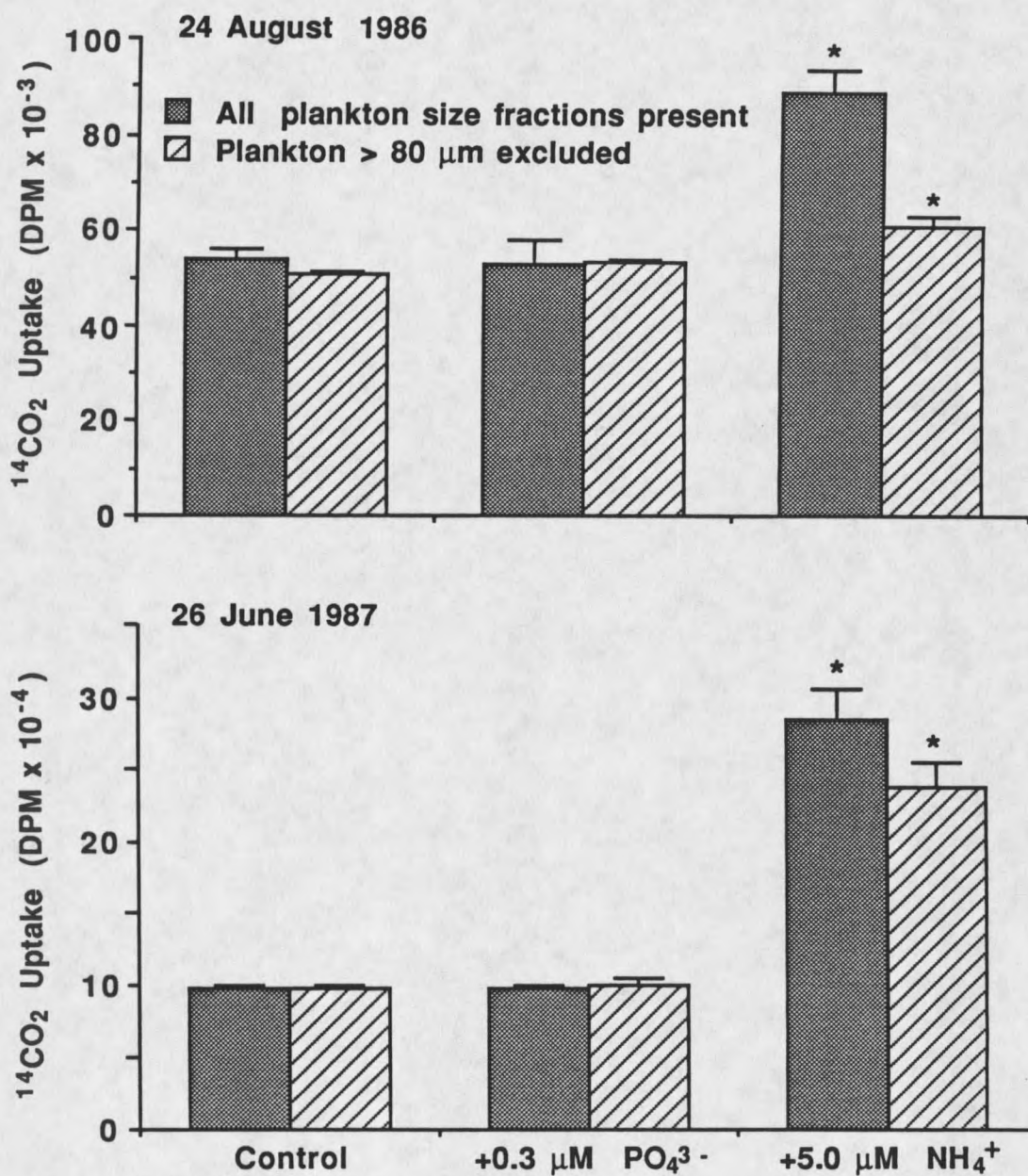


Figure 11. Influence of NH_4^+ or PO_4^{3-} enrichment on phytoplankton uptake of $^{14}\text{CO}_2$, 24 August 1986 and 26 June 1987. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

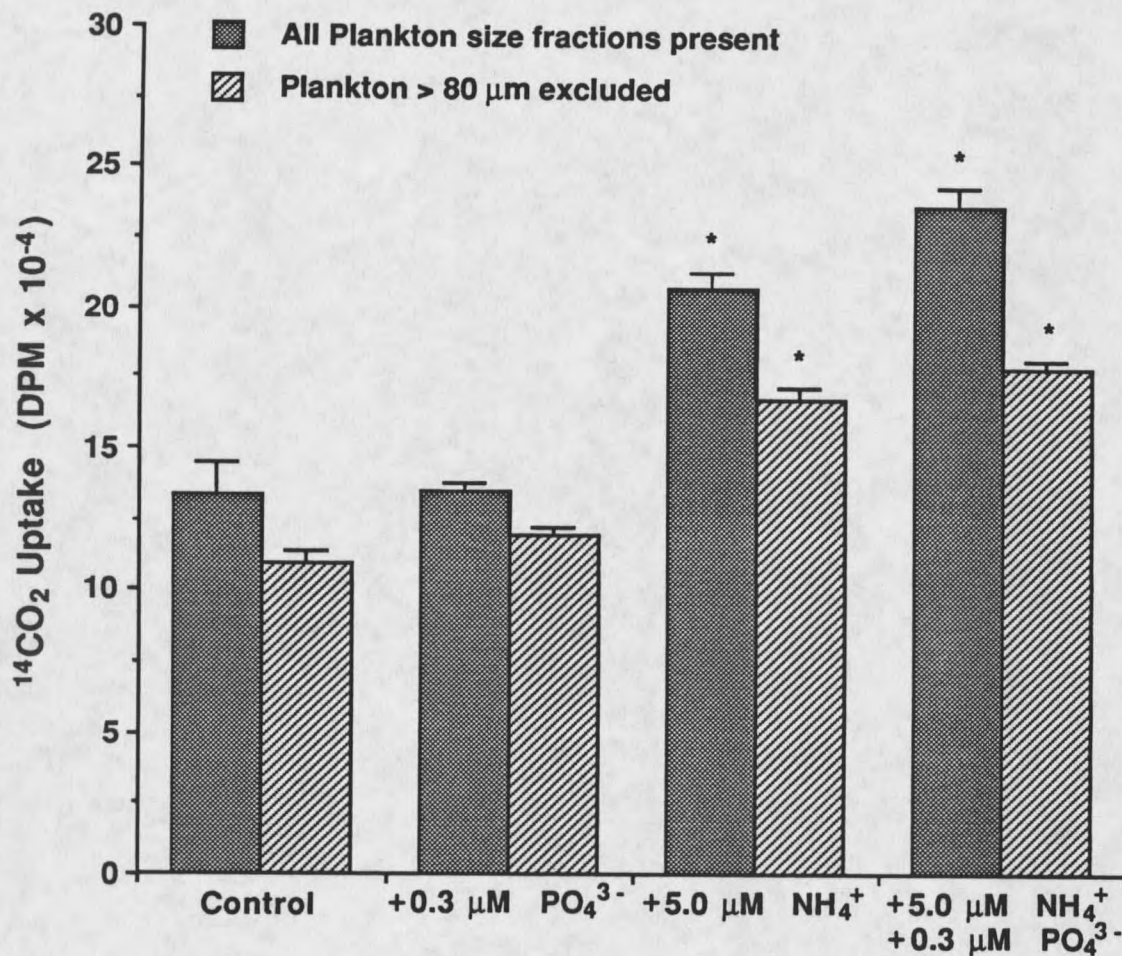


Figure 12. Influence of NH_4^+ and/or PO_4^{3-} enrichment on phytoplankton uptake of $^{14}\text{CO}_2$, 2 September 1987. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

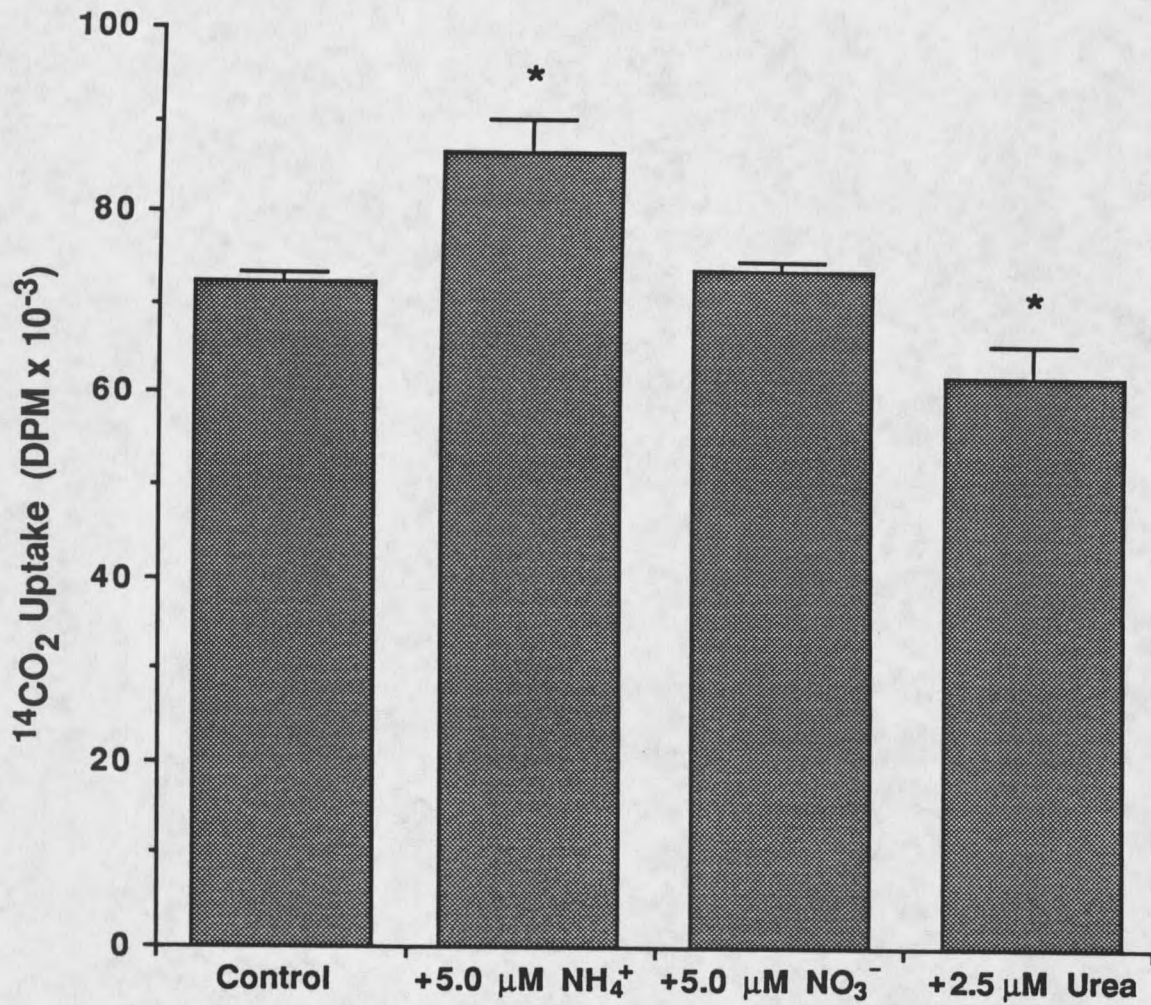


Figure 13. Comparison of effects of NH_4^+ , NO_3^- and urea enrichments on phytoplankton uptake of $^{14}\text{CO}_2$, 2 September 1987. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

Influence of Meltwater Additions

Dilution of lake water with meltwater from snow fields or from freshly fallen snow significantly increased algal cellular incorporation of $^{14}\text{CO}_2$ ($p < 0.05$, 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_s , 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_s and P_{max}

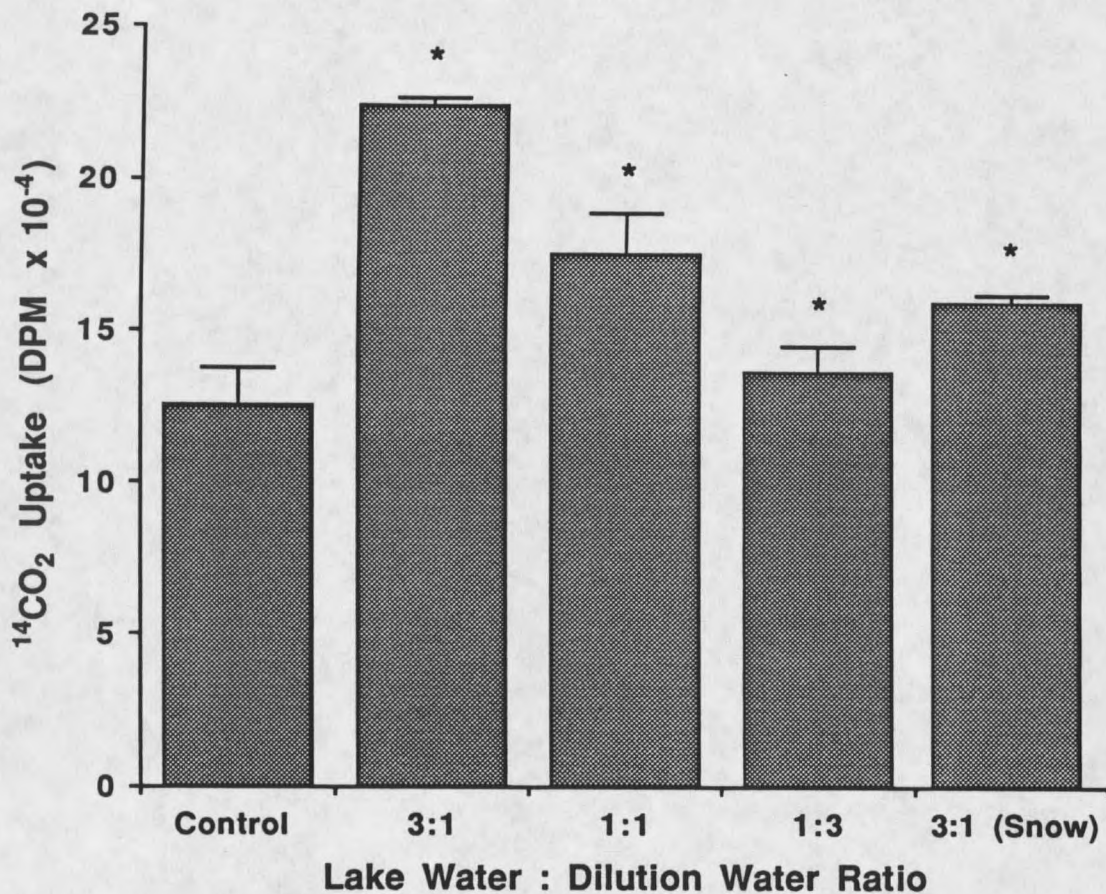


Figure 14. Influence of snowmelt and precipitation additions on phytoplankton uptake of $^{14}\text{CO}_2$, 2 September 1987. This experiment included lake water controls, a lake water:snow field runoff dilution series, and a lake water:snowmelt treatment (far right column). DPM values (treatment means and associated standard errors) have been corrected for differences in phytoplankton density and $^{14}\text{CO}_2$ 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.

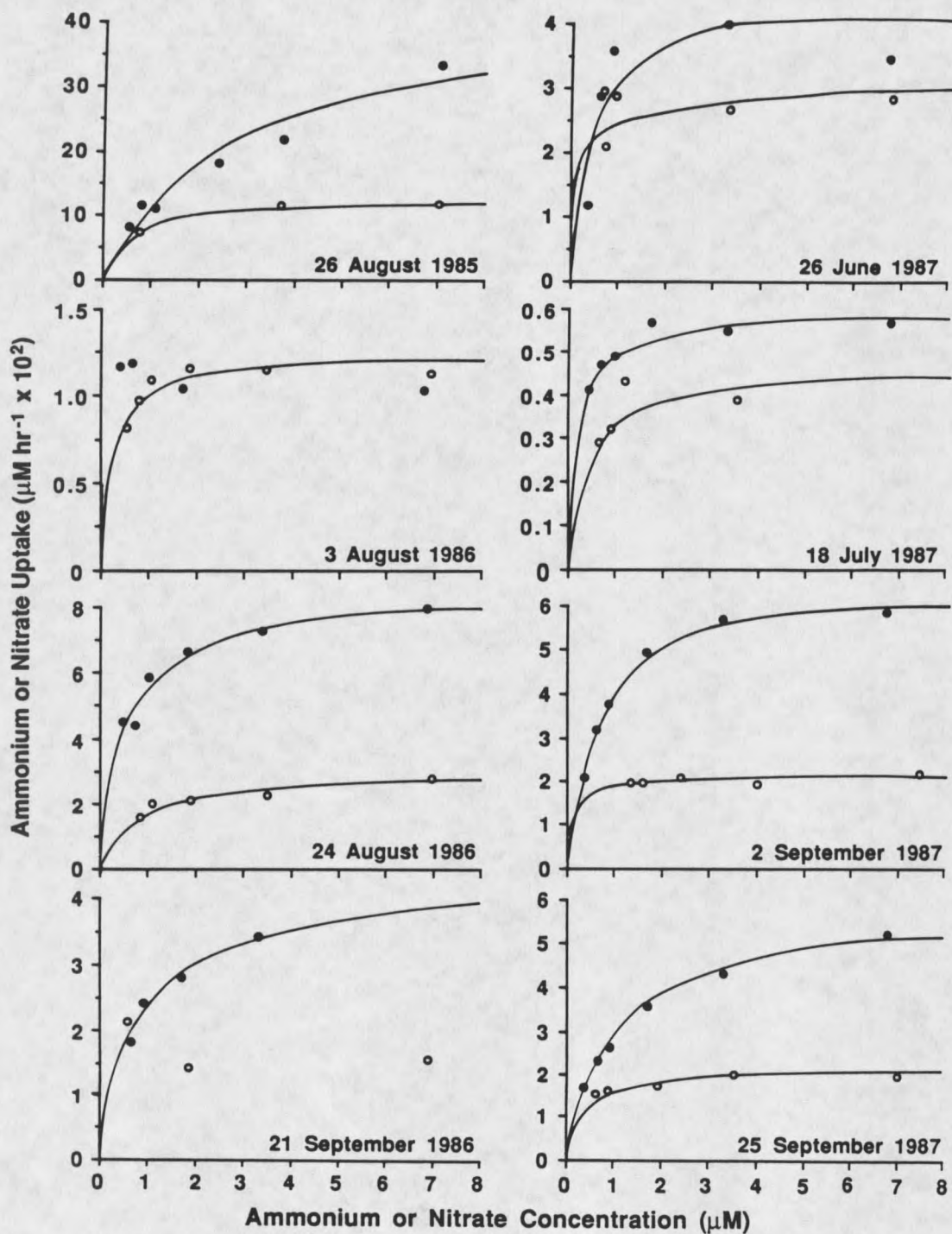


Figure 15. Phytoplankton uptake of NH_4^+ ($\bullet$) and NO_3^- ($\circ$) versus substrate concentration. All NH_4^+ uptake data are corrected for isotope dilution. Regression lines are based on equation 5 (Chapter 3), fitted with Marquart's algorithm. Regression lines are not depicted for 3 August 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 ($\pm$ 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	$P_{\max}$ ($\mu\text{M hr}^{-1}$)	$V_{\max}$ (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^-	$0.0208 \pm 1.14\text{E}8$	$0.0071 \pm 3.88\text{E}7$	$0.2521 \pm 6.08\text{E}9$

(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 $P_{\max, \text{NH}_4^+} : P_{\max, \text{NO}_3^-}$ 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: $\text{NO}_3^- + \text{NH}_4^+$ 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 $P_{\text{NH}_4^+} / (P_{\text{NH}_4^+} + P_{\text{NO}_3^-}) : [\text{NH}_4^+] / ([\text{NH}_4^+] + [\text{NO}_3^-])$ (the Relative Preference Index; see McCarthy et al. 1977) generally was near unity (mean $\pm$ 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_i and $P_{\max}$ (Table 11) to correct for the effect of substrate enrichment on algal NO_3^- uptake. Method 2 assumes $P_{\text{NO}_3^-}$ 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 ($\pm$ 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

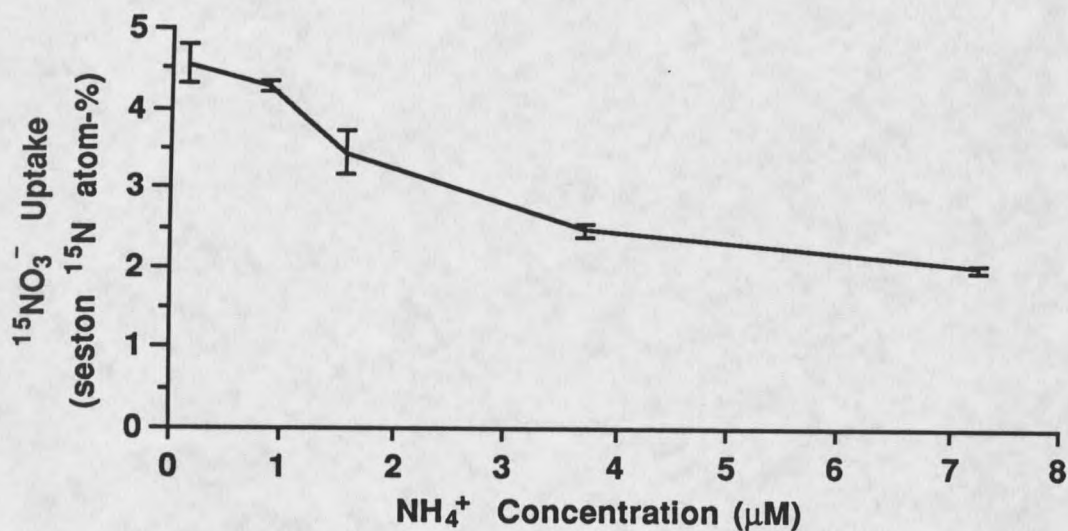


Figure 16. Influence of NH_4^+ concentration on phytoplankton uptake of $^{15}\text{NO}_3^-$. Data represent ^{15}N enrichments (mean $\pm$ 1 standard error) of the seston 30 hr after the addition of $^{15}\text{NO}_3^-$ inoculant.

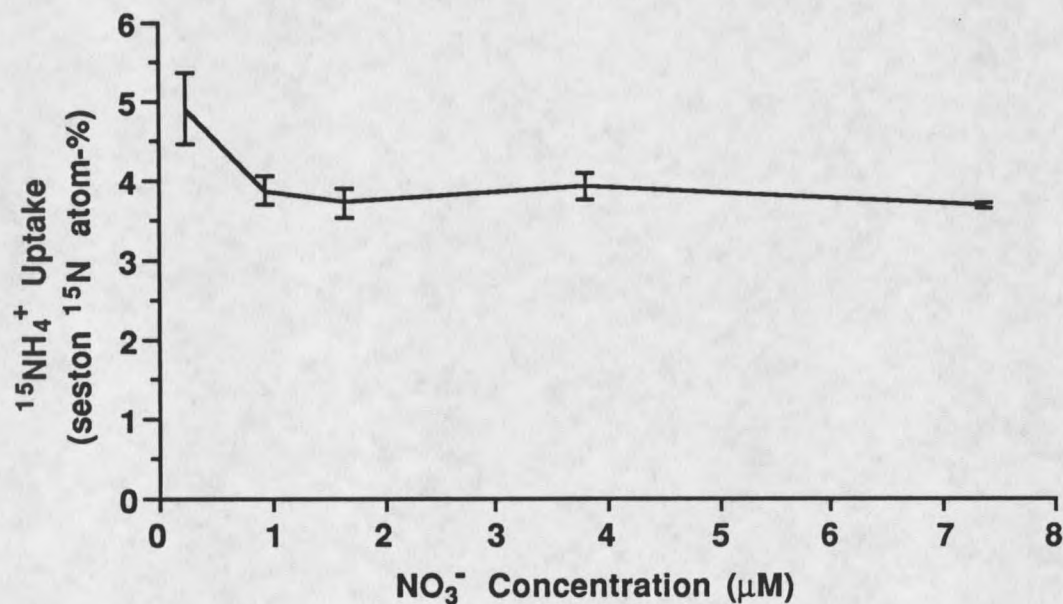


Figure 17. Influence of NO_3^- concentration on phytoplankton uptake of $^{15}\text{NH}_4^+$. Data represent ^{15}N enrichments (mean $\pm$ 1 standard error) of the seston 30 hr after the addition of $^{15}\text{NH}_4^+$ inoculant.

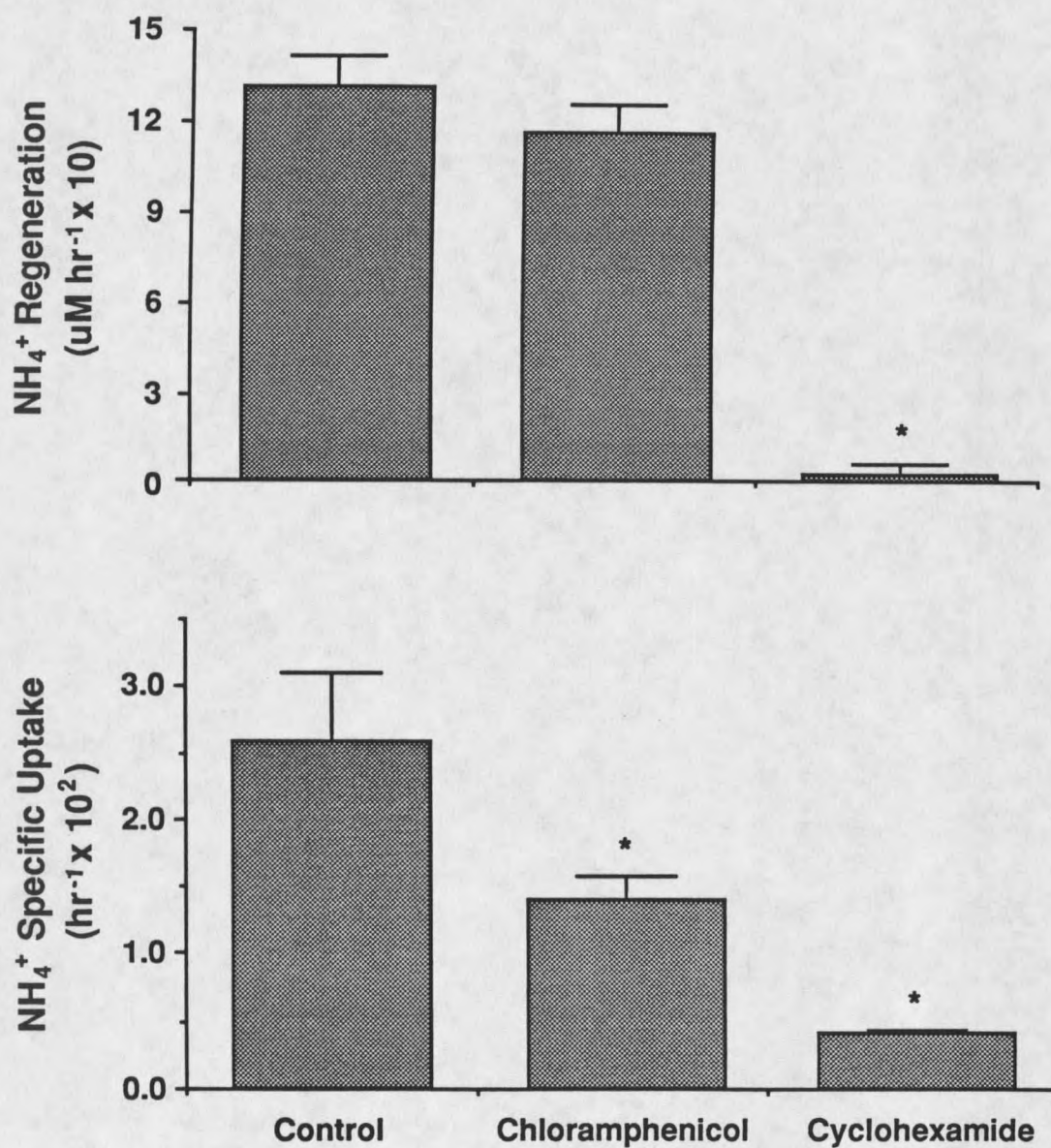


Figure 18. Effects on chloramphenicol and cyclohexamide additions on the regeneration and specific uptake of NH_4^+ by Snowbank Lake plankton. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

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 $^{15}\text{NH}_4^+$ specific activity, and seston ^{15}N atom-% excess occurring over a 6.2-hr *in situ* incubation (26 August 1985). Ammonium depletion, $^{15}\text{NH}_4^+$ 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 ($<63 \mu\text{m}$) 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 ($<63 \mu\text{m}$) size fraction.

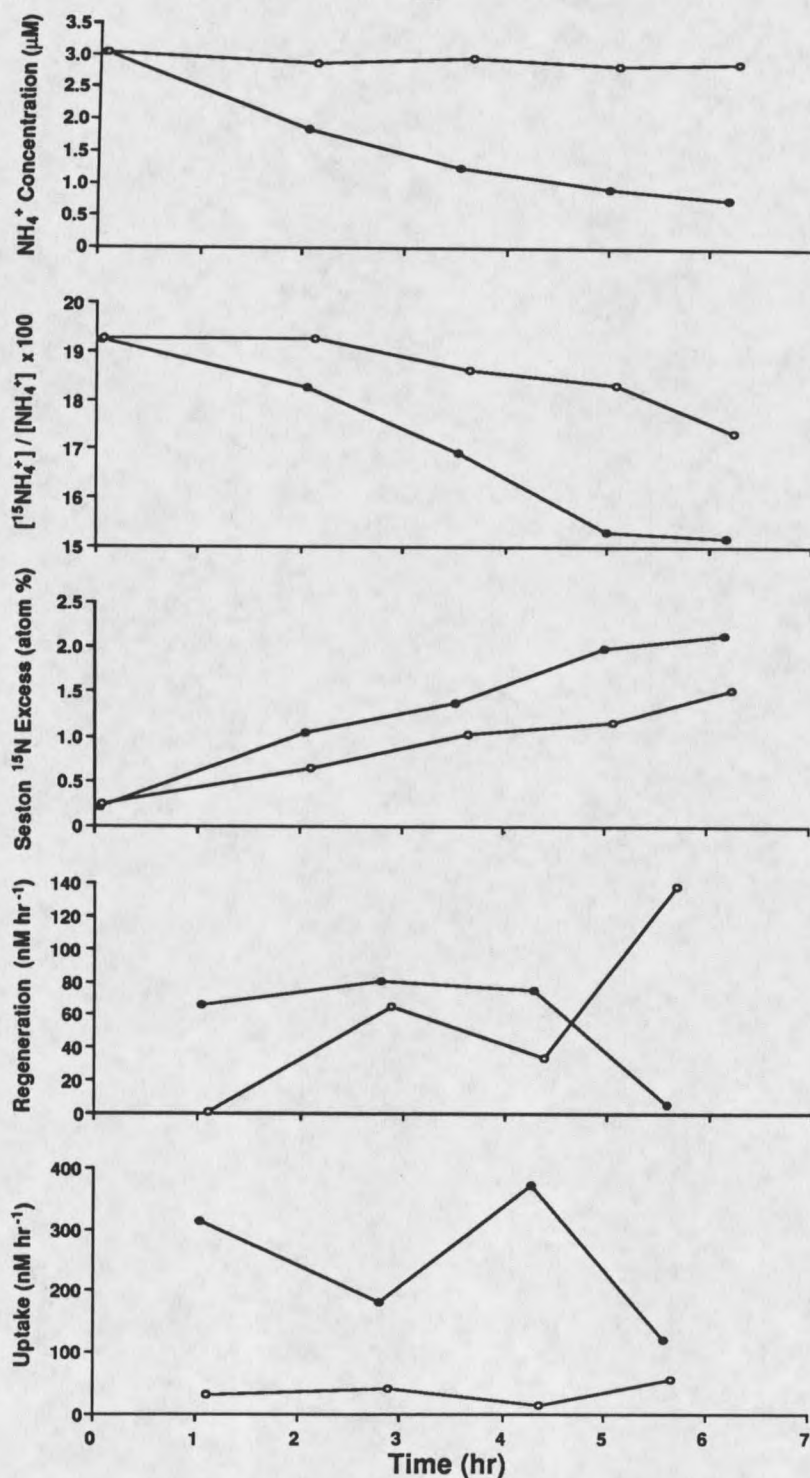


Figure 19. Size-fractionated time course of NH_4^+ regeneration, uptake, aqueous concentration and ^{15}N specific activity and particulate ^{15}N atom-% excess, 26 August 1985 field experiment. Fractions include all plankton (•) and plankton smaller than $63 \mu\text{m}$ (○).

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 $0.0066 \mu\text{M hr}^{-1}$. (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 $^{15}\text{NH}_4^+$ 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).

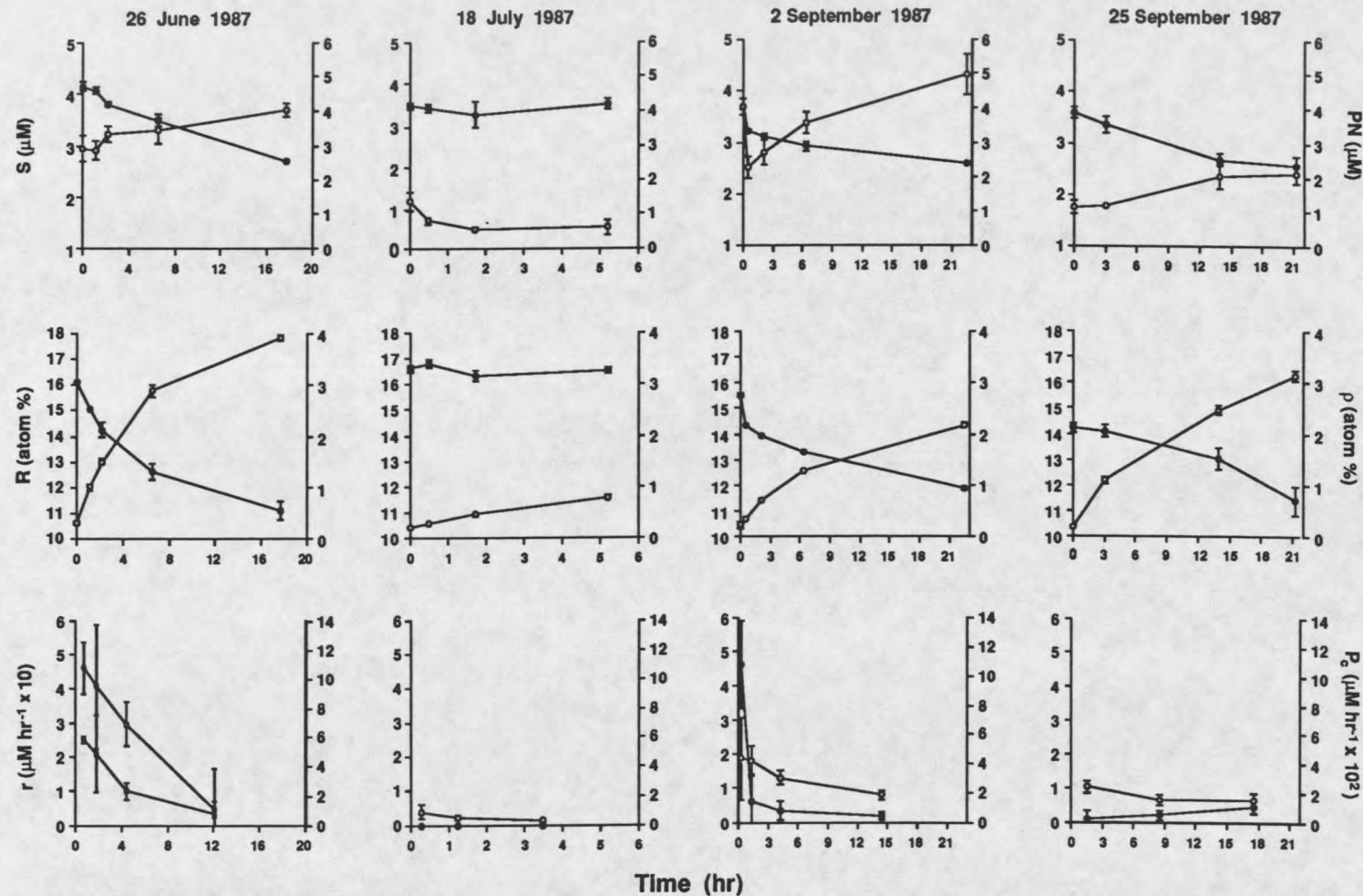


Figure 20. Time course of NH_4^+ regeneration (r), uptake (P_c), aqueous concentration (S) and ^{15}N specific activity (R), and particulate nitrogen concentration (PN) and ^{15}N atom-% excess (ρ), 1987 field experiments. Data (treatment means ± 1 standard error) denoted by darkened circles ($\bullet$) pertain to left axes; data denoted by open circles ($\circ$) 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 $^{15}\text{NH}_4^+$ 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 $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	4.15 $\pm$ 0.08	2.91 $\pm$ 0.38	16.12 $\pm$ 0.00	0.32 $\pm$ 0.03					
0.57					0.2500 $\pm$ 0.0115	0.1123 $\pm$ 0.0153	2.23	1.04	2.85
1.14	4.07 $\pm$ 0.06	2.86 $\pm$ 0.28	15.04 $\pm$ 0.00	1.01 $\pm$ 0.06					
1.65					0.2086 $\pm$ 0.1113	0.1013 $\pm$ 0.0365	2.06	1.03	4.58
2.16	3.81 $\pm$ 0.03	3.32 $\pm$ 0.27	14.25 $\pm$ 0.29	1.50 $\pm$ 0.07					
4.37					0.0996 $\pm$ 0.0274	0.0791 $\pm$ 0.0130	1.26	1.06	2.17
6.58	3.49 $\pm$ 0.12	3.44 $\pm$ 0.37	12.63 $\pm$ 0.32	2.89 $\pm$ 0.11					
12.16					0.0359 $\pm$ 0.0133	0.0292 $\pm$ 0.0048	1.23	1.07	3.62
17.74	2.71 $\pm$ 0.02	4.05 $\pm$ 0.19	11.09 $\pm$ 0.35	3.92 $\pm$ 0.06					
Overall ($t_0 - t_{17.74}$):					0.0712 $\pm$ 0.0079	0.0519 $\pm$ 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 $^{15}\text{NH}_4^+$ 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 $\mu\text{M}\cdot\text{hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	3.45 $\pm$ 0.08	1.37 $\pm$ 0.26	16.62 $\pm$ 0.13	0.22 $\pm$ 0.01					
0.27					not detected	0.0092 $\pm$ 0.0051	--	1.00	--
0.48	3.40 $\pm$ 0.10	0.81 $\pm$ 0.11	16.78 $\pm$ 0.14	0.28 $\pm$ 0.03					
1.25					not detected	0.0058 $\pm$ 0.0019	--	1.00	--
1.74	3.24 $\pm$ 0.31	0.54 $\pm$ 0.08	16.33 $\pm$ 0.23	0.46 $\pm$ 0.03					
3.47					not detected	0.0034 $\pm$ 0.0014	--	1.00	--
<u>5.20</u>	<u>3.49$\pm$0.14</u>	<u>0.62$\pm$0.21</u>	<u>16.52$\pm$0.10</u>	<u>0.79$\pm$0.04</u>					
Overall (t_0 - $t_{5.20}$):					not detected	0.0066 $\pm$ 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 $^{15}\text{NH}_4^+$ 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 $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

t	S	PN	R	ρ	r	P_c	$r:P_c$	$P_c:P$	$u:P_c$
0	3.68 $\pm$ 0.03	4.04 $\pm$ 0.24	15.51 $\pm$ 0.20	0.23 $\pm$ 0.07					
0.29					0.4617 $\pm$ 0.1295	0.0437 $\pm$ 0.0279	10.57	1.04	28.71
0.58	3.22 $\pm$ 0.03	2.26 $\pm$ 0.31	14.35 $\pm$ 0.25	0.35 $\pm$ 0.03					
1.33					0.0618 $\pm$ 0.0742	0.0423 $\pm$ 0.0098	1.46	1.01	2.89
2.07	3.13 $\pm$ 0.03	2.71 $\pm$ 0.35	13.94 $\pm$ 0.42	0.71 $\pm$ 0.03					
4.24					0.0339 $\pm$ 0.0275	0.0304 $\pm$ 0.0052	1.12	1.02	2.63
6.40	2.93 $\pm$ 0.09	3.56 $\pm$ 0.31	13.28 $\pm$ 0.33	1.28 $\pm$ 0.02					
14.41					0.0193 $\pm$ 0.0113	0.0189 $\pm$ 0.0033	1.02	1.06	2.14
22.42	2.59 $\pm$ 0.03	4.99 $\pm$ 0.58	11.87 $\pm$ 0.52	2.17 $\pm$ 0.06					
Overall (t_0 - $t_{22.42}$):					0.0370 $\pm$ 0.0067	0.0286 $\pm$ 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 $^{15}\text{NH}_4^+$ 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 $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

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

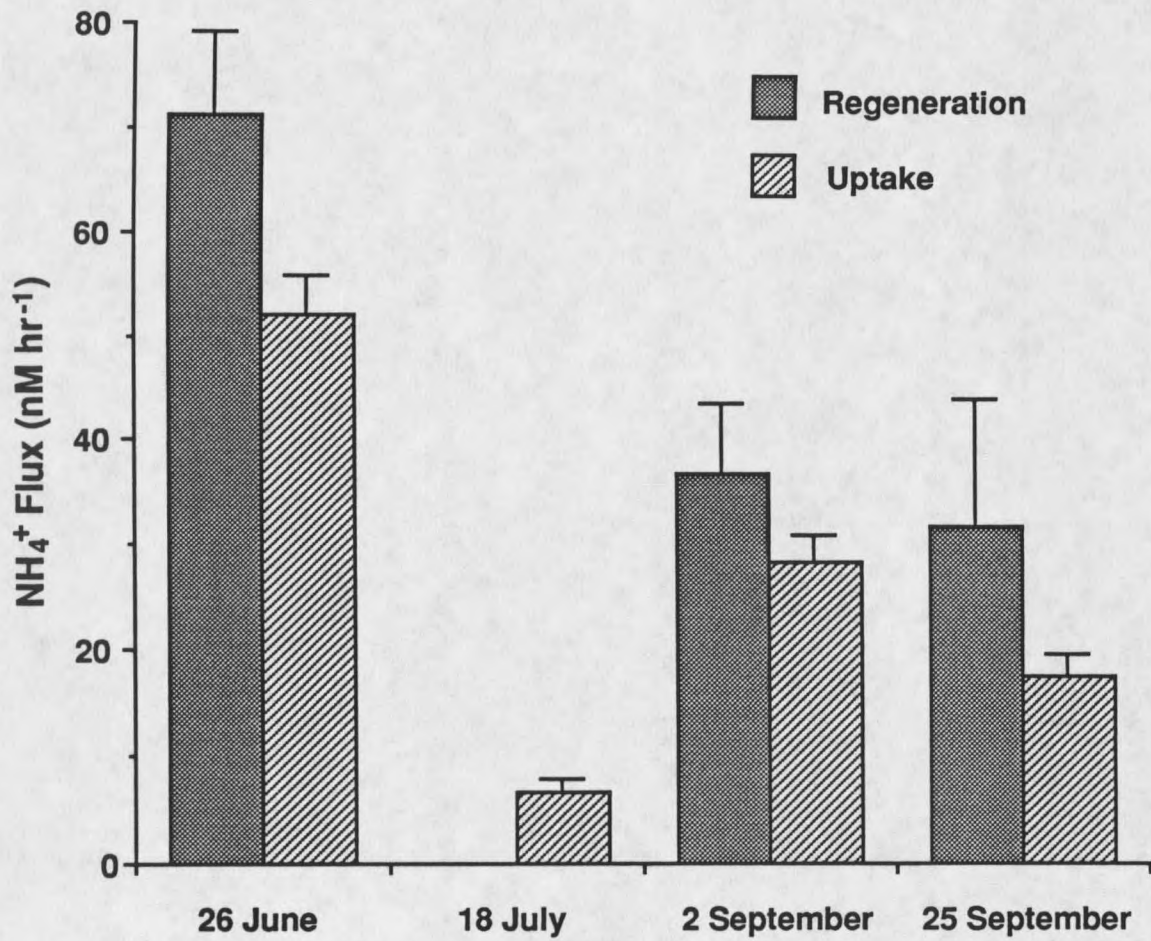


Figure 21. Comparison of NH_4^+ regeneration and uptake rates, 1987 field experiments. Values shown represent treatment means and associated standard errors. Regeneration of NH_4^+ was not detected on 18 July.

Effects of Acidification on Uptake and Regeneration of Nitrogen

Size-Fractionated Uptake and Regeneration of NH_4^+

Acidification of lake water samples from pH 7 to pH 5 markedly reduced NH_4^+ regeneration and uptake rates (Figure 22; tables 17-21). When averaged ($\pm$ standard error) over 5 experiments, regeneration activity was reduced $51.7 \pm 20.2\%$ in acidified samples containing all size fractions and $48.9 \pm 9.7\%$ in acidified samples from which larger ($>63 \mu\text{m}$) plankton were excluded. Similarly, NH_4^+ uptake rates were reduced $51.6 \pm 13.9\%$ in acidified, nonscreened treatments and $36.3 \pm 25.0\%$ in acidified, screened treatments relative to pH 7 controls. Exclusion of larger plankton increased NH_4^+ regeneration rates by an average of $60.7 \pm 14.1\%$ at pH 7 and $321.2 \pm 201.3\%$ at pH 5, whereas volumetric uptake rates were reduced $58.3 \pm 9.5\%$ and $50.1 \pm 15.7\%$, respectively. Each of the above-mentioned effects was statistically significant at $\alpha = 0.05$ (ANOVA/LSD). The discrepancy between $^{15}\text{NH}_4^+$ lost from the aqueous fraction and ^{15}N gained by the particulate fraction generally was increased by the removal of larger plankton; pH exerted no consistent effect on this discrepancy (see u:P_e ratios, tables 17-21).

Time Course of Nitrogen Uptake and Regeneration

Data from the 27 September 1987 laboratory time-course/acidification experiment are summarized in tables 22-23 and in figures 23-24. Changes in aqueous NH_4^+ concentration (S) during the 23-hr incubation were statistically equivalent in pH 7.4 and pH 5.4 treatments ($p > 0.05$,

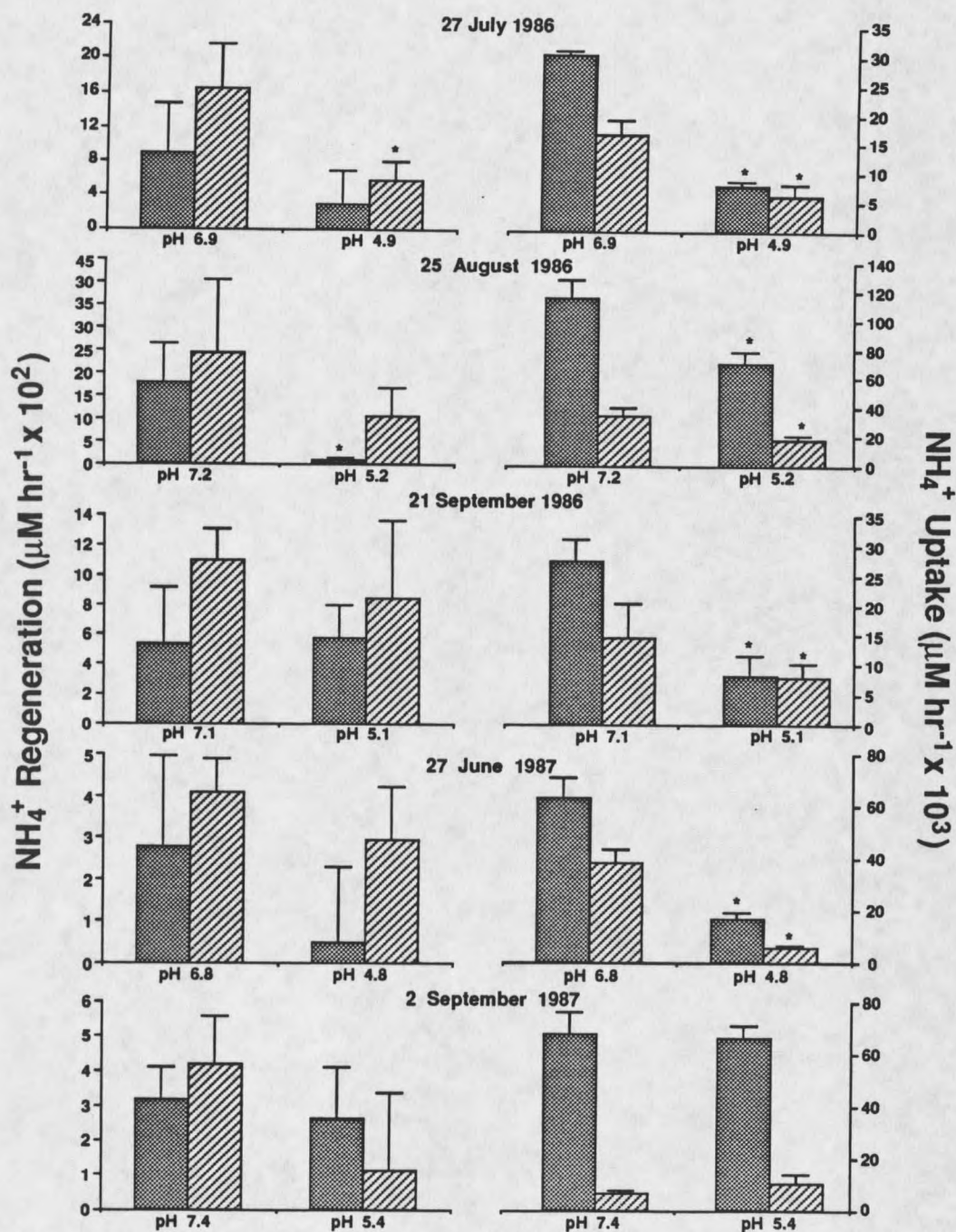


Figure 22. Effects of pH reduction on size-fractionated uptake and regeneration of NH_4^+ , 1986-87 field experiments. Open columns represent total uptake or regeneration (all plankton size classes present); hatched columns denote treatments from which plankton $> 63 \mu\text{M}$ were excluded. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

Table 17. Effects of pH reduction on size-fractionated NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}\text{NH}_4^+$ specific activity (R), 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), 3 August 1986. Ammonium fluxes are in units of $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

Size class	pH	t	S	R	ρ	r	P_c	r: P_c	P_c :P	u: P_c
All plankton										
pH 6.9	{	0	3.62±0.03	14.58±0.47	0	0.0897±0.0574	0.0303±0.0063	2.96	1.07	2.96
		6.08	3.62±0.03	12.54±1.11	0.93±0.14					
pH 4.9	{	0	3.73±0.02	14.20±0.70	0	0.0298±0.0399	0.0080±0.0007	3.73	1.03	5.96
		6.15	3.62±0.02	13.51±0.55	0.25±0.00					
Small plankton ($<63 \mu\text{m}$)										
pH 6.9	{	0	3.77±0.06	14.99±0.22	0	0.1647±0.0522	0.0166±0.0027	9.92	1.05	9.52
		5.98	3.81±0.01	11.56±0.94	0.52±0.06					
pH 4.9	{	0	3.76±0.06	14.52±0.31	0	0.0578±0.0215	0.0062±0.0020	9.32	1.13	7.18
		6.02	3.84±0.04	13.25±0.35	0.20±0.06					

Table 18. Effects of pH reduction on size-fractionated NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}\text{NH}_4^+$ specific activity (R), 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 August 1986. Ammonium fluxes are in units of $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

Size class	pH	t	S	R	ρ	r	P_c	r: P_c	P_c :P	u: P_c
All plankton										
pH 7.2	{	0	3.61±0.05	14.74±0.87	0	0.1783±0.0638	0.1155±0.0130	1.54	1.09	1.87
		3.42	3.48±0.10	12.41±0.20	1.85±0.05					
pH 5.2	{	0	3.62±0.03	13.52 (no rep.)	0	0.0093	0.0701±0.0085	0.13	1.00	1.43
		3.31	3.32±0.09	13.40 (no rep.)	1.12±0.10					
Small plankton ($<63 \mu\text{m}$)										
pH 7.2	{	0	3.48±0.17	14.53±0.19	0	0.2434±0.1625	0.0349±0.0057	6.97	1.10	2.46
		3.24	3.99±0.03	11.76±1.06	0.93±0.03					
pH 5.2	{	0	3.46±0.06	14.37±0.14	0	0.1058±0.0622	0.0181±0.0028	5.85	1.05	3.80
		3.21	4.02±0.08	13.12±0.62	0.39±0.05					

Table 19. Effects of pH reduction on size-fractionated NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}\text{NH}_4^+$ specific activity (R), 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), 21 September 1986. Ammonium fluxes are in units of $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

Size class	pH	t	S	R	ρ	r	P_c	r: P_c	P_c :P	u: P_c
All plankton										
pH 7.1	{	0	3.47±0.08	11.96±0.25	0	0.0539±0.0378	0.0274±0.0037	1.97	1.03	2.36
		3.75	3.43±0.08	11.28±0.40	0.48±0.02					
pH 5.1	{	0	3.40±0.02	11.90±0.02	0	0.0580±0.0218	0.0083±0.0034	6.99	1.04	9.54
		3.77	3.32±0.06	11.15±0.27	0.16±0.05					
Small plankton (<63 μm)										
pH 7.1	{	0	3.07±0.03	11.84±0.28	0	0.1099±0.0207	0.0147±0.0057	7.48	1.07	5.64
		3.71	3.17±0.12	10.39±0.02	0.27±0.09					
pH 5.1	{	0	3.12±0.06	11.61±0.17	0	0.0838±0.0520	0.0080±0.0022	10.48	1.05	1.81
		3.75	3.38±0.06	10.54±0.39	0.14±0.02					

Table 20. Effects of pH reduction on size-fractionated NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}\text{NH}_4^+$ specific activity (R), 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), 27 June 1987. Ammonium fluxes are in units of $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

Size class	pH	t	S	R	ρ	r	P_c	r:P _c	P _c :P	u:P _c
All plankton										
pH 6.8	{	0	3.56±0.11	15.66±0.73	0	0.0279±0.0219	0.0631±0.0078	0.44	1.05	1.97
		10.08	2.59±0.05	14.28±0.76	2.94±0.21					
pH 4.8	{	0	3.52±0.08	16.19±0.79	0	0.0050±0.0180	0.0169±0.0025	0.30	1.01	2.00
		10.06	3.23±0.08	15.95±0.35	0.72±0.06					
Small plankton ($<63 \mu\text{m}$)										
pH 6.8	{	0	3.90±0.02	16.12±0.18	0	0.0410±0.0079	0.0380±0.0052	1.08	1.06	2.80
		10.10	3.24±0.03	14.35±0.25	2.61±0.04					
pH 4.8	{	0	3.89±0.07	17.08±0.21	0	0.0293±0.0136	0.0057±0.0011	5.14	1.04	8.63
		10.07	3.69±0.08	15.80±0.54	0.35±0.06					

Table 21. Effects of pH reduction on size-fractionated NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (P_c), aqueous NH_4^+ concentration (S), aqueous $^{15}\text{NH}_4^+$ specific activity (R), 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 $\mu\text{M hr}^{-1}$, concentrations in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error.

Size class	pH	t	S	R	ρ	r	P_c	r: P_c	P_c :P	u: P_c
All plankton										
pH 7.4	{	0	3.68±0.03	15.51±0.20	0	0.0316±0.0094	0.0678±0.0083	0.47	1.03	1.91
		6.22	3.07±0.05	14.63±0.14	1.45±0.11					
pH 5.4	{	0	3.60±0.09	14.79±0.27	0	0.0261±0.0151	0.0662±0.0050	0.39	1.02	1.76
		6.32	3.03±0.03	14.07±0.27	1.35±0.03					
Small plankton ($<63 \mu\text{m}$)										
pH 7.4	{	0	3.72±0.02	15.43±0.17	0	0.0418±0.0139	0.0067±0.0014	6.24	1.03	14.24
		6.16	3.39±0.03	14.35±0.25	0.96±0.08					
pH 5.4	{	0	3.64±0.05	15.11±0.33	0	0.0115±0.0221	0.0107±0.0032	1.07	1.01	3.49
		6.19	3.48±0.03	14.81±0.47	0.90±0.03					

Table 22. Effects of pH reduction on time course of NH_4^+ regeneration (r), isotope dilution-corrected NH_4^+ uptake (V_c), aqueous NH_4^+ concentration (S), aqueous $^{15}\text{NH}_4^+$ specific activity (R), and particulate ^{15}N atom-% excess (ρ), 26 September 1987. Regeneration rates are in units of $\mu\text{M hr}^{-1}$, specific uptake in hr^{-1} , concentration in μM , ^{15}N enrichments in atom-%, time (t) in hr, and error terms in $\pm$ standard error. Values without parentheses pertain to pH 7.4 controls; values within parentheses pertain to pH 5.4 treatments.

t	S	R	ρ	r	V_c
0	3.20 $\pm$ 0.14 (3.20 $\pm$ 0.14)	80.72 $\pm$ 0.44 (81.16 $\pm$ 0.44)	1.30 $\pm$ 0.15 (0.79 $\pm$ 0.08)		
0.5				0.1166 $\pm$ 0.0178 (0.0675 $\pm$ 0.0730)	0.0507 $\pm$ 0.0040 (0.0344 $\pm$ 0.0036)
1.0	3.23 $\pm$ 0.01 (3.10 $\pm$ 0.12)	77.76 $\pm$ 0.00 (79.49 $\pm$ 1.73)	5.44 $\pm$ 0.28 (3.48 $\pm$ 0.27)		
2.0				0.0404 $\pm$ 0.0148 (0.0250 $\pm$ 0.0446)	0.0418 $\pm$ 0.0046 (0.0306 $\pm$ 0.0044)
2.9	2.77 $\pm$ 0.14 (2.96 $\pm$ 0.04)	75.78 $\pm$ 0.39 (78.22 $\pm$ 1.47)	11.56 $\pm$ 0.61 (8.18 $\pm$ 0.61)		
5.0				0.0627 $\pm$ 0.0375 (0.1007 $\pm$ 0.0600)	0.0285 $\pm$ 0.0035 (0.0288 $\pm$ 0.0032)
7.1	2.32 $\pm$ 0.02 (2.44 $\pm$ 0.02)	68.38 $\pm$ 2.97 (67.07 $\pm$ 5.88)	20.13 $\pm$ 0.79 (16.79 $\pm$ 0.17)		
11.0				0.1317 $\pm$ 0.0544 (0.0233 $\pm$ 0.0234)	0.0184 $\pm$ 0.0033 (0.0163 $\pm$ 0.0027)
14.8	1.81 $\pm$ 0.08 (1.70 $\pm$ 0.05)	41.79 $\pm$ 6.65 (61.38 $\pm$ 0.88)	27.90 $\pm$ 0.55 (24.93 $\pm$ 1.11)		
18.9				R at equilibrium? (0.0436 $\pm$ 0.0176)	0.0081 $\pm$ 0.0024 (0.0047 $\pm$ 0.0062)
23.0	1.31 $\pm$ 0.06 (1.33 $\pm$ 0.06)	42.27 $\pm$ 4.34 (48.47 $\pm$ 3.09)	30.70 $\pm$ 0.33 (27.04 $\pm$ 2.54)		
Overall (t_0 - $t_{14.8}$)				0.1087 $\pm$ 0.0315 (0.0447 $\pm$ 0.0062)	0.0294 $\pm$ 0.0017 (0.0229 $\pm$ 0.0010)

Table 23. Effects of pH reduction on time course of NO_3^- uptake (V) and particulate ^{15}N atom-% excess (ρ), 26 September 1987. Specific uptake rates are in units of hr^{-1} , ^{15}N enrichments in atom-%, and error terms in $\pm$ standard error. Values without parentheses pertain to pH 7.4 controls; those within parentheses pertain to pH 5.4 treatments.

t	ρ	V
0	0.37 ± 0.00 (0.35 ± 0.05)	
0.5		0.0029 ± 0.0006 (0.0024 ± 0.0006)
1.0	0.64 ± 0.06 (0.57 ± 0.05)	
2.0		0.0059 ± 0.0017 (0.0059 ± 0.0009)
2.9	1.72 ± 0.16 (1.65 ± 0.08)	
5.0		0.0069 ± 0.0005 (0.0094 ± 0.0009)
7.1	4.43 ± 0.13 (5.25 ± 0.03)	
11.0		0.0060 ± 0.0004 (0.0083 ± 0.0008)
14.8	8.74 ± 0.26 (11.31 ± 0.55)	
18.9		0.0058 ± 0.0010 (0.0056 ± 0.0024)
23.0	13.23 ± 0.74 (15.59 ± 1.70)	
Overall $t_0-t_{23.0}$		0.0060 ± 0.0003 (0.0071 ± 0.0008)

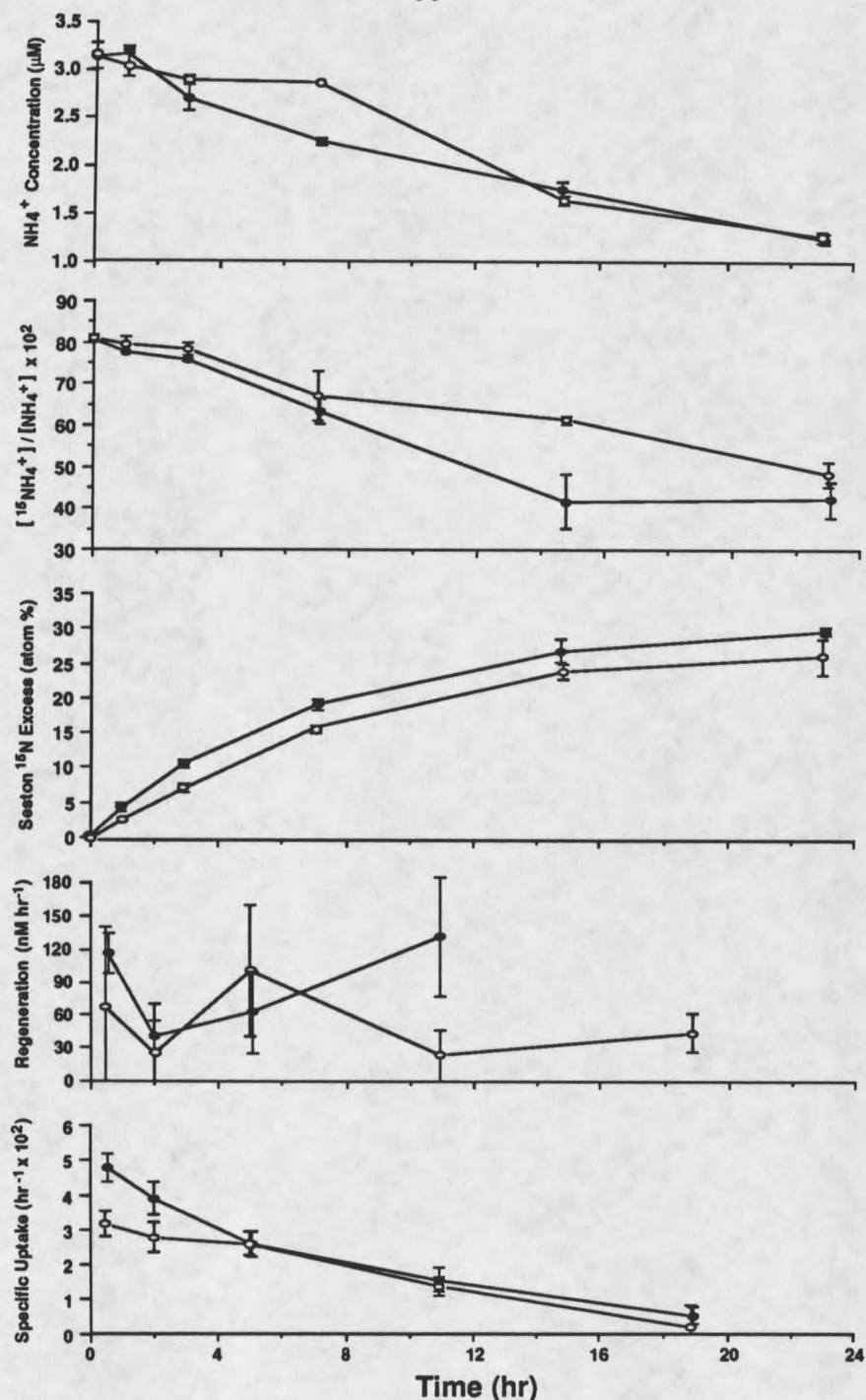


Figure 23. Effects of pH reduction on time course of NH_4^+ regeneration, uptake, aqueous concentration and ^{15}N specific activity, and particulate ^{15}N atom-% excess, 26 September 1987. Values for pH 7 controls are denoted by darkened circles (●); those for pH 5 treatments are represented by open circles (○). All values represent treatment means ± 1 standard error. No value is reported for regeneration rate in controls at 19 hr; see text for explanation.

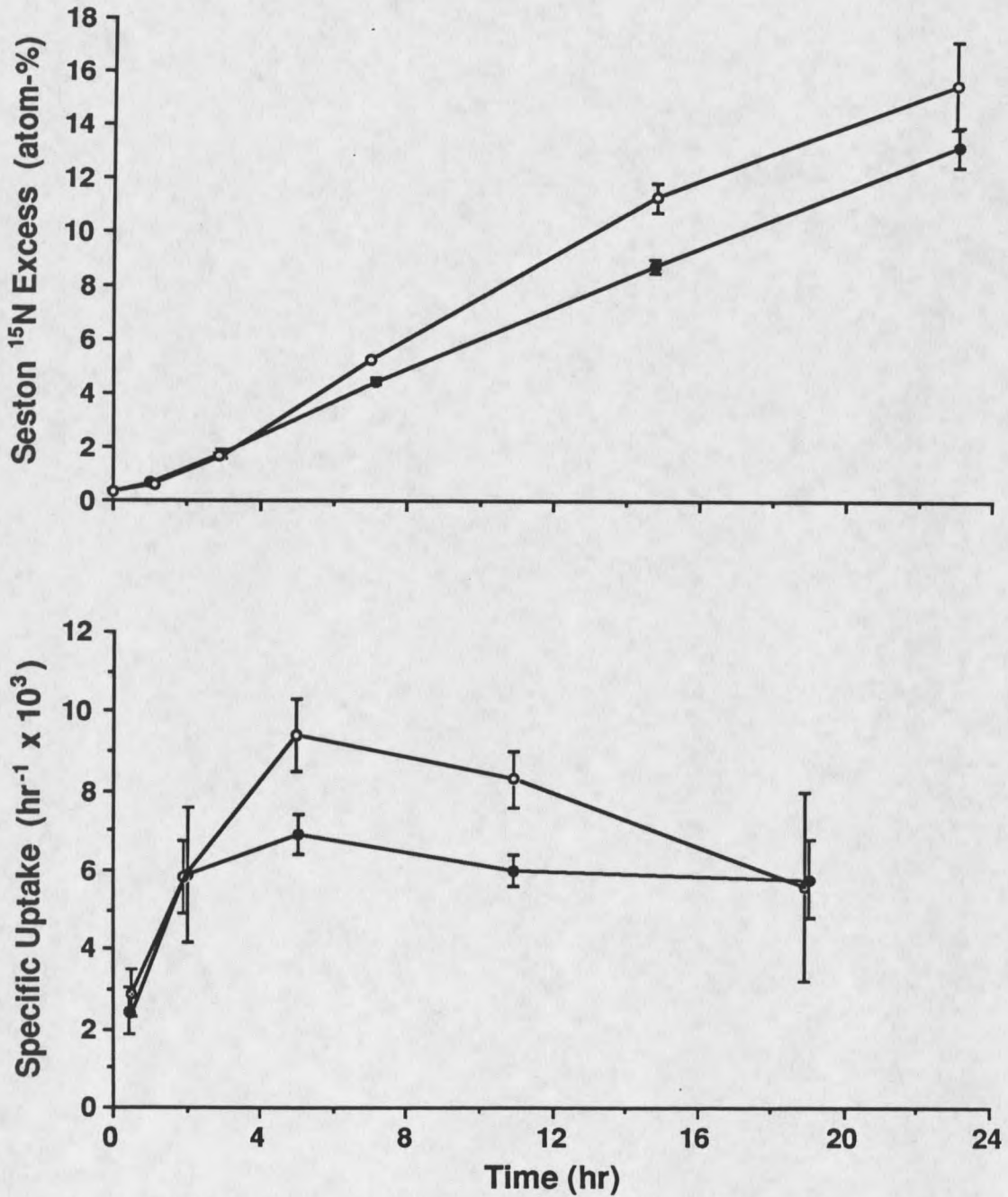


Figure 24. Effects of pH reduction on time course of NO_3^- specific uptake and particulate ^{15}N atom-% excess, 26 September 1987. Values (means ± 1 standard error) for pH 7 controls are denoted by darkened circles ($\bullet$); those for pH 5 treatments are represented by open circles ($\circ$).

point-by-point t-test comparison). Decreases in $^{15}\text{NH}_4^+$ specific activity (R) were comparable among pH treatments for the first 7 hr of incubation ($p > 0.05$); however, isotope dilution proceeded more rapidly in the pH 7.4 treatment during the 7-15 hr time interval ($p < 0.05$). No further decrease in R occurred in the pH 7.4 treatment after approximately 15 hr, indicating that NH_4^+ regeneration had terminated abruptly or, alternatively, that recycling of $^{15}\text{NH}_4^+$ via heterotrophic excretion had dampened further changes in the $^{15}\text{NH}_4^+$:total NH_4^+ ratio (cf., Glibert et al. 1982; see also *Limitations of Nutrient Regeneration Models*, Chapter 5, this report).

Throughout the incubation, seston accumulation of ^{15}N from $^{15}\text{NH}_4^+$ (i.e., ρ) was significantly greater at pH 7.4 than at pH 5.4 ($p < 0.05$). Differences in NH_4^+ uptake between treatments were greatest in the initial stages of the incubation. During the approximately 3-min interval between isotope addition and the filtration of the first ("time zero") sample aliquot, seston ^{15}N enrichments (corrected for ambient ^{15}N and for nonbiological adsorption) increased from zero to 1.30 atom-% at pH 7.4 and from zero to 0.79 atom-% at pH 5.4. These enrichments represented specific uptake rates of 0.3286 and 0.1988 hr^{-1} , respectively, and were approximately 6 times greater than rates reported for the initial 1-hr time interval. (In the pH 7.4 treatment, correction of NH_4^+ uptake rates for isotope dilution was complicated by the apparent heterotrophic recycling of $^{15}\text{NH}_4^+$ during the 15-23 hr time interval. Uptake rates measured during this interval were multiplied by a correction factor of 1.069, based on the average isotope-dilution effect noted during the earlier phases of incubation.)

In $^{15}\text{NO}_3^-$ -enriched samples, seston accumulation of ^{15}N was comparable between pH treatments during the first 3 hr of incubation ($p > 0.05$), then increased disproportionately in the pH 5.4 treatment ($p < 0.05$). Rates of NO_3^- -specific uptake increased markedly during the 0-5 hr time interval in both pH treatments. Maximum values for $V_{\text{NO}_3^-}$ measured during the experiment were 0.0094 hr^{-1} at pH 5.4 and 0.0069 hr^{-1} at pH 7.4. $V_{\text{NO}_3^-}$ decreased rapidly in the pH 5.4 treatment during the latter half of the incubation; specific uptake rates of $0.0056\text{-}0.0058 \text{ hr}^{-1}$ were measured during the final (11-19 hr) time interval, irrespective of pH ($p > 0.05$; see Figure 24).

Threshold of pH Effects on NH_4^+ and NO_3^- Uptake

Acidification from pH 7.4 to pH 6.0 had no statistically significant effect ($p > 0.05$; ANOVA/LSD) on algal incorporation of $^{15}\text{NH}_4^+$ -derived ^{15}N , although a slight decrease in ^{15}N accumulation with declining pH was suggested by the experimental data (see Figure 25). Contrastingly, algal incorporation of $^{15}\text{NO}_3^-$ -derived ^{15}N was enhanced by acidification from pH 7.4 to pH 6.0 ($p < 0.05$). Significant decreases ($p < 10^{-5}$) were noted in the "uptake" of both NH_4^+ and NO_3^- as pH declined from 6.0 to 5.0; further significant decreases ($p < 10^{-5}$) were documented at pH 4.0 and pH 3.0 (Figure 25).

These results, together with those presented in the preceding subsection, suggested that acidification first inhibited NO_3^- uptake within the relatively narrow pH range of 5.4-5.0 (cf., *Comparative Effects of Mineral Acids on Uptake of NH_4^+ and NO_3^-* , below). Ammonium

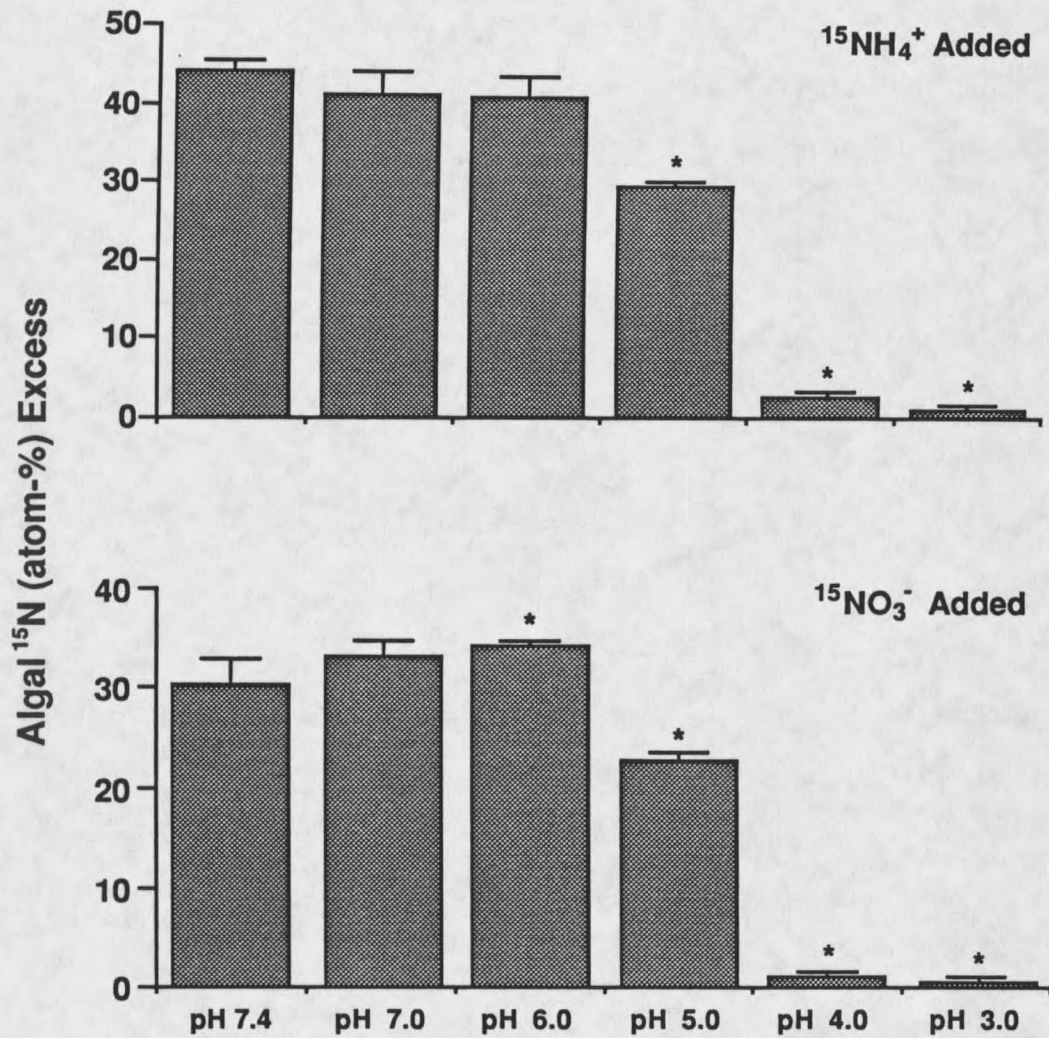


Figure 25. Effects of progressively decreasing pH on phytoplankton utilization of NH_4^+ and NO_3^- . Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

uptake apparently was first strongly affected by increasing $[H^+]$ within the pH range 6.0-5.0 (see below).

Comparative Effects of Mineral Acids
on Uptake of NH_4^+ and NO_3^-

The mineral acid chosen as an acidifying agent had little bearing on the extent of inhibition of NH_4^+ uptake. Seston ^{15}N accumulation in $^{15}NH_4^+$ -enriched samples was comparable among HCl, HNO_3 , H_2SO_4 , and HNO_3/H_2SO_4 treatments ($p > 0.05$, ANOVA/LSD; coefficients of variation among acid treatments were 10.3% on 27 June, 12.1% on 2 September, and 5.2% on 26 September 1987; see Figure 26). Contrastingly, NO_3^- uptake demonstrated consistent differences among acid treatments ($p < 0.05$, ANOVA/LSD). In the 27 June experiment, reduction of sample pH from 6.8 to 4.8 significantly reduced NO_3^- uptake in all acid treatments; however, this decline was significantly less ($p < 0.05$) in HNO_3 and HNO_3/H_2SO_4 treatments. Acidification to pH 5.0 and 5.4 induced significant increases in NO_3^- uptake on 2 and 26 September, respectively; in these instances, HNO_3 and HNO_3/H_2SO_4 stimulated significantly greater increases than did HCl and H_2SO_4 ($p < 0.05$). In the 2 September experiment, $^{15}NO_3^-$ uptake in experimental treatments actually exceeded $^{15}NH_4^+$ uptake in counterpart treatments. Moreover, NO_3^- uptake in samples acidified to pH 5.0 with HNO_3 or HNO_3/H_2SO_4 exceeded NH_4^+ uptake in pH 7.0 controls. Reasons which may account for differences in NO_3^- uptake among experimental treatments (each containing presumably saturating levels of NO_3^-) are considered in detail in Chapter 5.

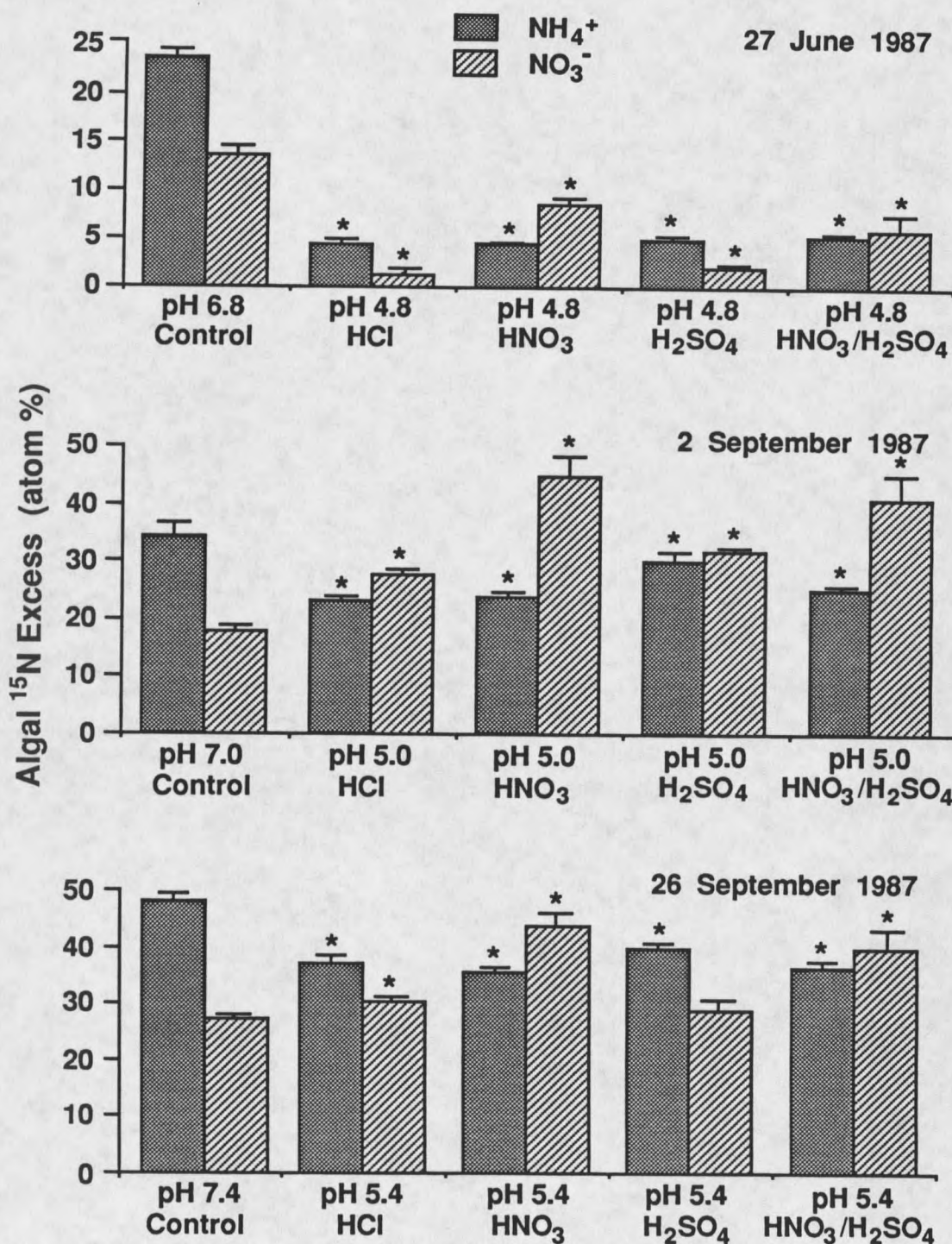


Figure 26. Comparative effects of mineral acids on phytoplankton utilization of NH_4^+ and NO_3^- . Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

Inorganic Nitrogen Incorporation into Protein

Acidification of samples from pH 7.4 to pH 5.4 via HCl addition reduced $^{15}\text{NH}_4^+$ incorporation into algal protein by approximately 8.2% and increased $^{15}\text{NO}_3^-$ incorporation into algal protein by about 7.2%, on average (Figure 27). Although neither finding was statistically significant at $\alpha = 0.05$, each was consistent with the effects of acidification on seston ^{15}N enrichment mentioned previously; specifically, NH_4^+ uptake appeared to be impeded and NO_3^- uptake enhanced by 2-unit reductions in extracellular pH. A comparison of the results from this experiment with those presented in Figure 26 for 26 September 1987 suggested that acidification had less effect on protein metabolism than it did on total nitrogen uptake. (Ammonium incorporation into protein was reduced 8.2%, whereas NH_4^+ uptake was reduced 22.7%; NO_3^- incorporation into protein was increased 7.2%, whereas NO_3^- uptake was increased 10.9%. Recall that the experiments involved in this comparison were conducted concurrently under identical incubation conditions using water dispensed from the same lake water sample.)

Effects of pH Reduction on Photosynthesis and Bacterial Production

Comparative Effects of Mineral Acids on Algal Photosynthesis

Results from the 2 and 26 September 1987 ^{14}C experiments suggested that a 70-80% decrease in algal CO_2 uptake occurred in lake water samples following acidification (Figure 28). Meaningful comparisons of CO_2 assimilation between circumneutral controls and acid treatments were dependent, however, on the accuracy of inorganic carbon equilibrium

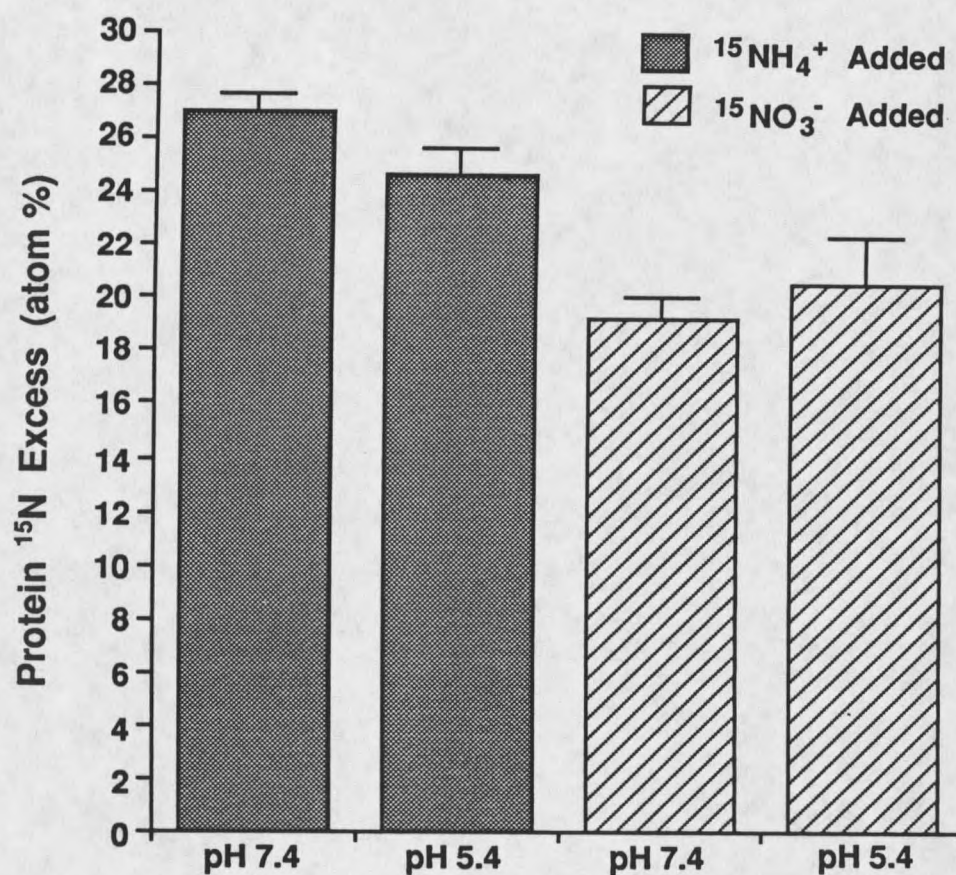


Figure 27. Effects of pH reduction on incorporation of NH_4^+ and NO_3^- into phytoplankton protein, 26 September 1987. Values shown represent treatment means and associated standard errors.

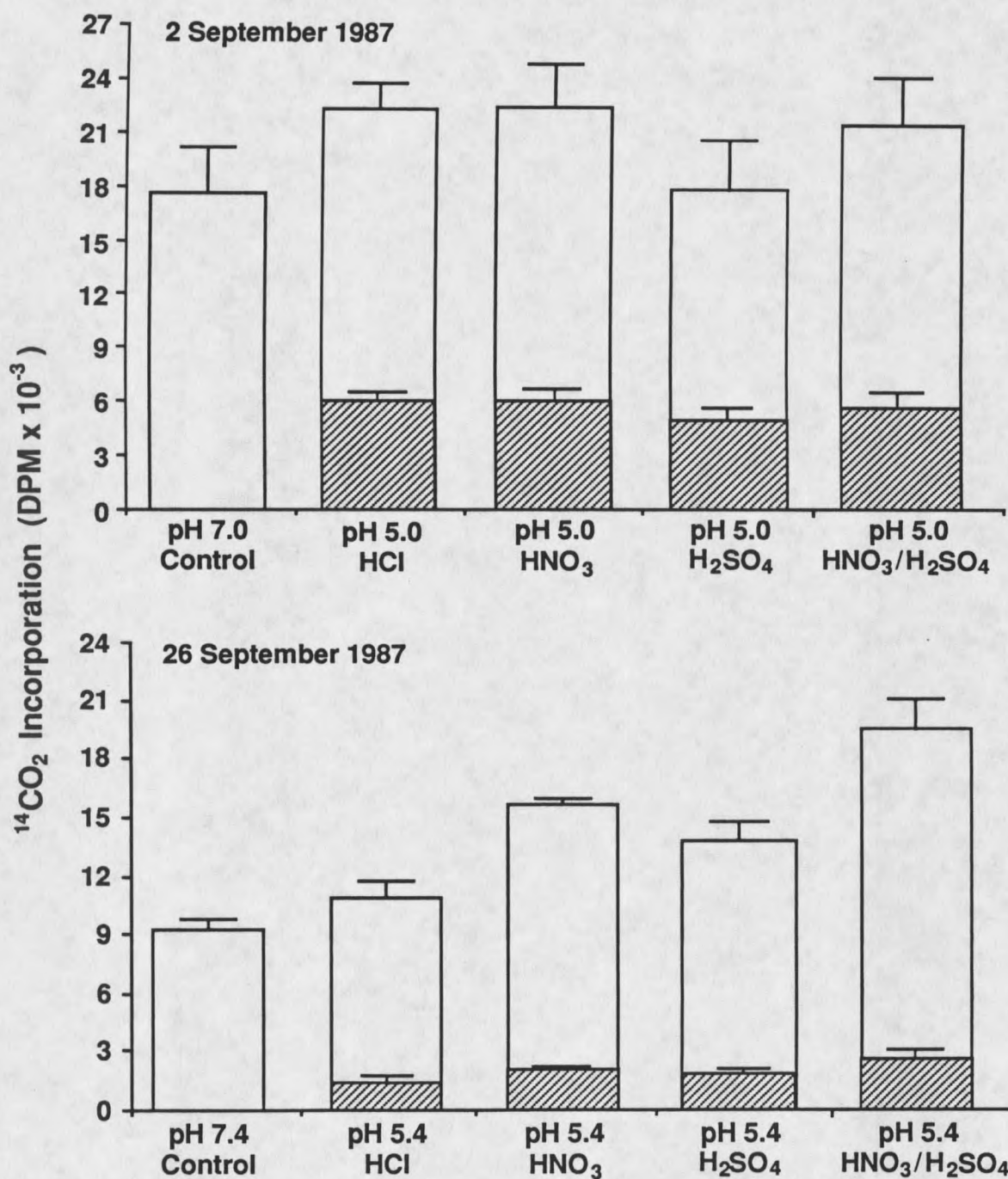


Figure 28. Comparative effects of mineral acids on phytoplankton incorporation of $^{14}\text{CO}_2$. Hatched columns represent $^{14}\text{CO}_2$ incorporation "corrected" for increased $^{14}\text{CO}_2$ specific activity in experimental treatments relative to controls (see text for details). Values shown represent treatment means and associated standard errors.

calculations described in Chapter 3. Given the low values for C_T in both the controls and the experimental treatments, considerable uncertainty was associated with estimates of sample $^{14}\text{CO}_2$ specific activity and with ^{14}C -based inter-sample (control versus experimental) comparisons of carbon fixation rate. Contrastingly, intercomparisons of CO_2 fixation among acid treatments were unambiguous ($^{14}\text{CO}_2$ specific activity was invariant among acid treatments). Seston incorporation of ^{15}N isotope on 2 September was not influenced by the mineral acid or mineral acid combination chosen as an acidifying agent ($p > 0.05$, ANOVA/LSD). On 26 September, samples augmented with HNO_3 or with $\text{HNO}_3/\text{H}_2\text{SO}_4$ demonstrated significantly greater photosynthetic activities than did those augmented with HCl ($p < 0.05$, ANOVA/LSD), suggesting that the availability of NO_3^- (and possibly the combined availability of NO_3^- and SO_4^{2-}) at high concentration (60-117 μM) enhanced rates of algal CO_2 fixation under acidic conditions. Significant differences in chlorophyll *a* concentration were not observed among acid treatments or between controls and acid treatments in either experiment ($p > 0.05$, ANOVA/LSD).

End Products of Algal Photosynthesis

Photosynthetic incorporation of $^{14}\text{CO}_2$ at circumneutral pH was directed primarily towards protein synthesis (44.9%), followed by the synthesis of polysaccharide (25.9%), lipid (16.2%) and LMWM (13.0%; see Figure 29). In acid treatments (pH 5.4), $^{14}\text{CO}_2$ incorporation again was dominated by protein (52.6%), followed by polysaccharides (25.9%), lipid (15.6%) and LMWM (5.9%). Differences in the proportion of assimilated ^{14}C directed into the LMWM fraction at pH 7.4 versus pH 5.4 were statistically

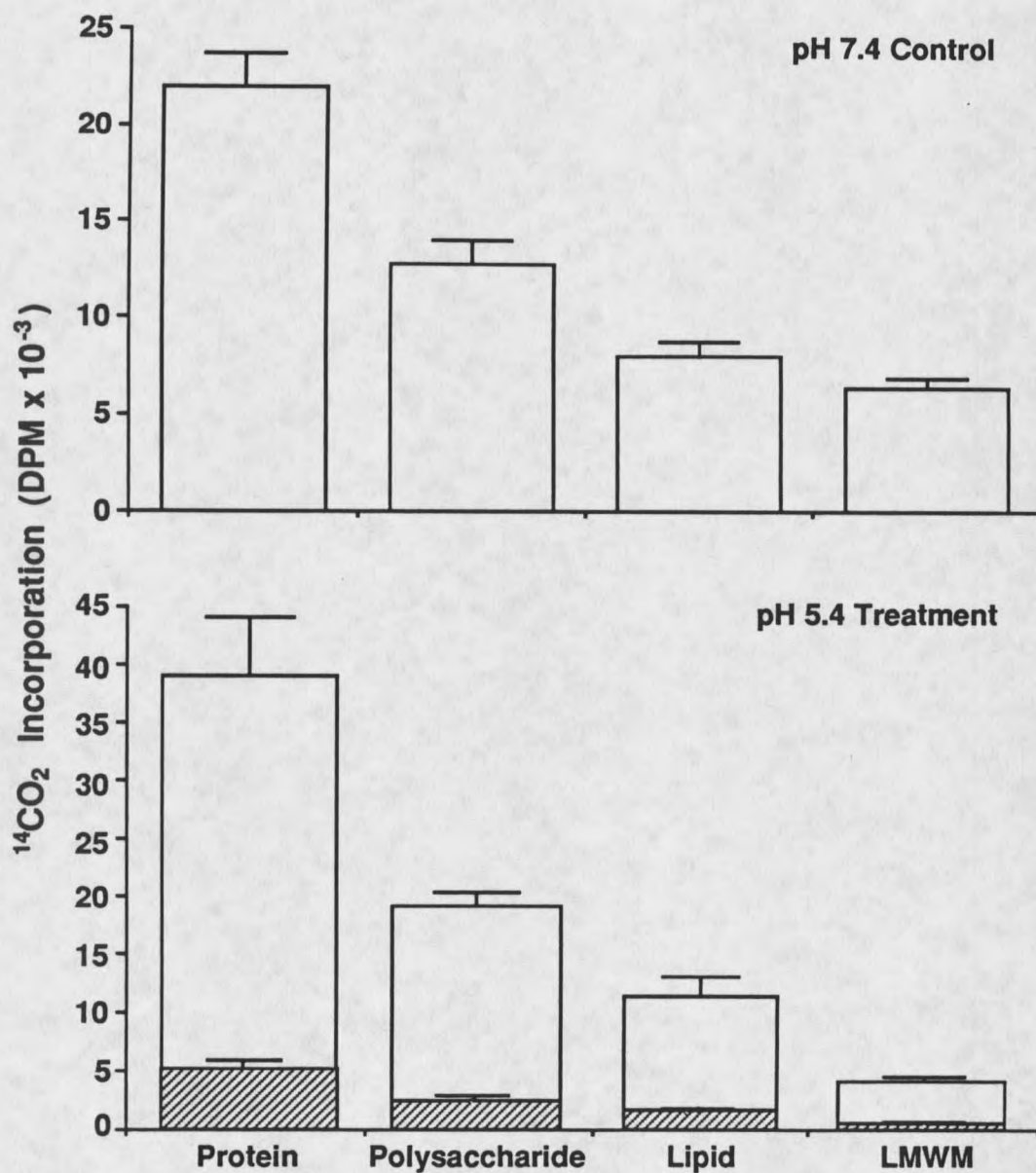


Figure 29. Effects of pH reduction on $^{14}\text{CO}_2$ incorporation into phytoplankton protein, polysaccharide, lipid, and low molecular weight metabolites, 26 September 1987. Hatched columns represent $^{14}\text{CO}_2$ incorporation "corrected" for increased $^{14}\text{CO}_2$ specific activity in experimental treatments relative to controls (see text for details). Values shown represent treatment means and associated standard errors.

significant at ($p < 0.05$; t-test). An approximate 80% decrease in total CO_2 fixation appeared to accompany the acidification of samples from pH 7.4 to pH 5.4. The significance of this finding was obscured, however, owing to the questionable accuracy of the correction factors used to account for differences in $^{14}\text{CO}_2$ specific activity between pH treatments (see preceding subsection).

Bacterial Secondary Production

Acidification of circumneutral lake water samples to pH 5.0 (2 September 1987) or pH 5.4 (26 September 1987) reduced bacterioplankton ^3H -thymidine incorporation 28.2% and 27.2%, respectively ($p < 0.05$, ANOVA/LSD; data corrected for abiotic adsorption of ^3H ; see Figure 30).

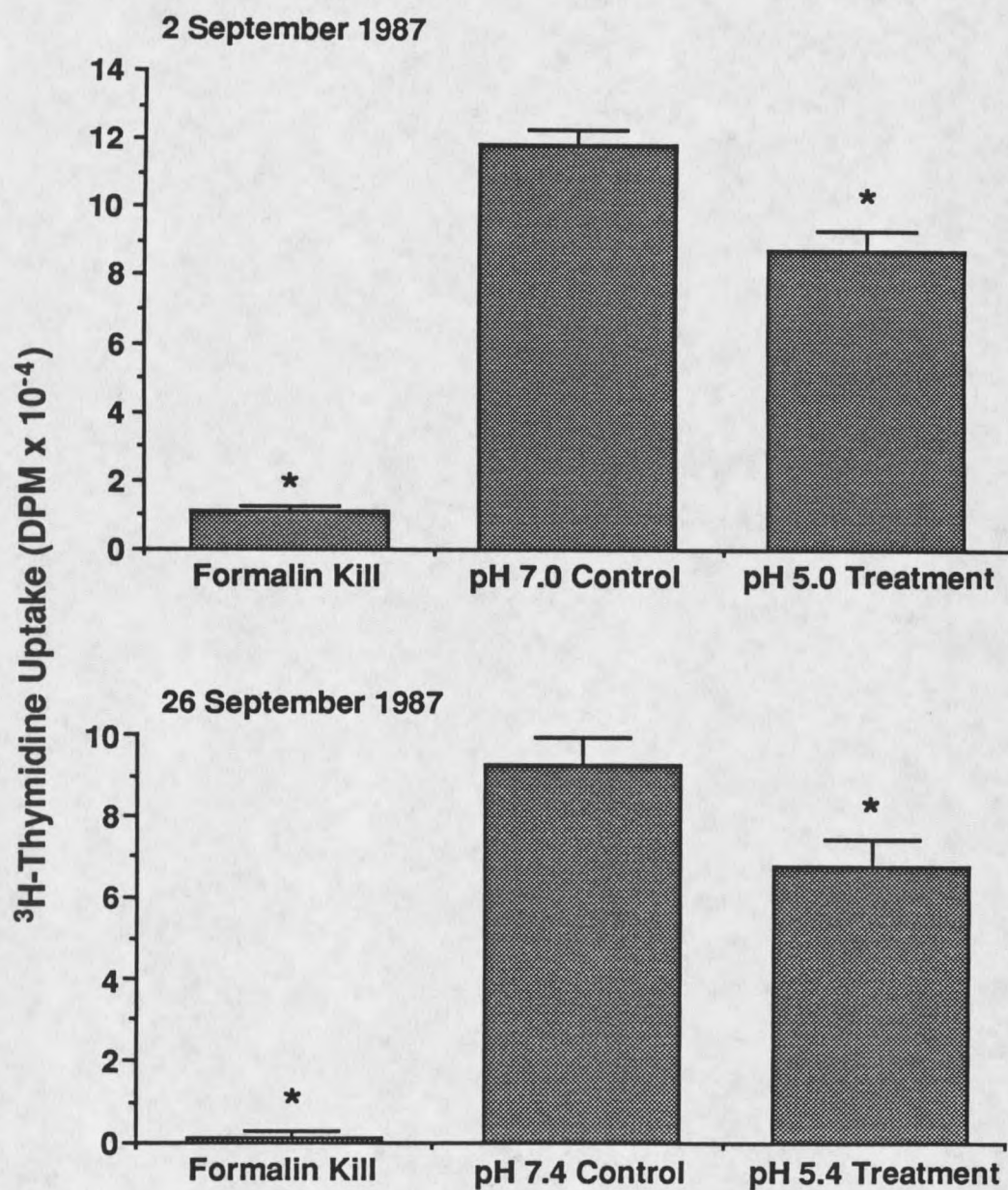


Figure 30. Effect of pH reduction on bacterioplankton uptake of ^3H -methyl thymidine. Values shown represent treatment means and associated standard errors. Asterisks designate treatment means differing significantly ($p < 0.05$) from controls.

CHAPTER 5

DISCUSSION

Ecological Significance of NH_4^+ Regeneration in Snowbank Lake

The importance of NH_4^+ regeneration as a source of nitrogen for phytoplankton growth and metabolism in Snowbank Lake may be evaluated by considering (1) the extent to which nitrogen availability regulates water-column primary production, (2) the ability of the phytoplankton community to selectively exploit regenerated NH_4^+ in the presence of other, more abundant nitrogenous nutrients, (3) the rate of NH_4^+ regeneration relative to the rate of NH_4^+ uptake within the lake's limnetic waters, and (4) the degree to which water-column regenerative and assimilative fluxes of NH_4^+ are temporally and spatially coupled. This section considers each of these factors individually, then concludes by presenting a preliminary conceptual model of water-column NH_4^+ dynamics in Snowbank Lake.

Evidence for Nitrogen Limitation of
Water-Column Primary Production

Algal productivity in freshwater ecosystems is influenced by a diverse and interacting array of environmental factors. Some of these factors (e.g., nutrient supply, radiant energy, temperature) operate directly on cellular metabolism and replication, whereas others (e.g., predation, sedimentation, advective displacement) affect population

density and, therefore, the total production which is possible within a given volume of water over a specified period of time. The perceived importance of any single factor in the regulation of algal growth tends to vary according to the temporal and spatial scale of observation (Carpenter and Kitchell 1988). In some lakes, however, water-column primary production may be enhanced indefinitely by sustained additions of a particular nutritive substance (e.g., phosphorus in many Precambrian Shield lakes of northwestern Ontario; see Schindler *et al.* 1978a), in which case the traditional "limiting resource" concept (Liebig's law of the minimum) appears to have general application. From an operational standpoint, a limiting resource may be defined as the environmental parameter which directly constrains b_t in the equation

$$b_t - d_t = r_t \quad (22)$$

where b_t represents the per capita reproductive ("birth") rate of a given algal population at time t , d_t is the population's instantaneous per capita death rate (the magnitude of which is determined by a myriad of environmental factors), and r_t is the population's instantaneous per capita growth rate (roughly equivalent to per capita net productivity). Limiting resources may change with time, and they may differ among algal populations at any moment in time (see *Partitioning of Nitrogenous Resources among Phytoplankton*, this chapter). Their influence on whole-lake biological productivity is manifested primarily through effects on algal cellular metabolism and reproduction (b_t), and these effects may be offset or exacerbated by concurrent environmental influences on algal mortality (d_t ; see *Metazoan Agents of NH_4^+ Regeneration*, this chapter).

Accordingly, references made to algal nutrient limitation or nutrient deficiency in the remainder of this report allude to the influences exerted by resource availability on cellular metabolism and reproduction. They do not imply that other environmental parameters lack any appreciable influence on water-column primary production.

Experimental and observational data collected during the present study suggested that phytoplankton growth in Snowbank Lake was limited by the availability of assimilatable nitrogen. Consider the following evidence:

1) Short-term nutrient enrichment bioassays consistently implicated nitrogen (rather than phosphorus) as the element present in growth-limiting proportions (figures 11 and 12).

2) Whole-lake $\text{NH}_4^+ + \text{NO}_3^-$:SRP molar ratios were well below the nitrogen:phosphorus atom ratio of 15-16 traditionally associated with balanced nutrient availability in aquatic environments (Redfield 1958; Redfield *et al.* 1963) and consistently less than the optimal supply ratios reported for freshwater phytoplankton species in culture (Table 24).

3) Seston carbon:nitrogen atomic ratios were high relative to the Redfield proportion of $\text{C}_{106}\text{N}_{16}$ achieved in nutrient-saturated algal cultures and in natural aquatic environments under optimal algal growth conditions (e.g., Goldman *et al.* 1979; Goldman 1984a,b, 1986).

4) Maximum biomass-specific uptake rates for both NH_4^+ and NO_3^- were comparable to those reported previously for nitrogen-deficient surface waters; moreover, extremely rapid rates of NH_4^+ uptake noted in the early

Table 24. Literature values of critical (optimum) nitrogen:phosphorus supply ratios for some common lake phytoplankton (from Suttle and Harrison 1988b).

Species	N:P (atoms)	Original references
<i>Melosira binderana</i>	7	Rhee and Gotham 1980
<i>Microcystis</i> sp.	9	Rhee and Gotham 1980
<i>Synedra ulna</i>	10	Rhee and Gotham 1980
<i>Asterionella formosa</i>	12	Rhee and Gotham 1980
<i>Selanastrum minutum</i>	15-22	Elrifi and Turpin 1985
<i>Ankistrodesmus falcatus</i>	21	Rhee and Gotham 1980
<i>Pavlova lutheri</i>	21-45	Terry 1980
<i>Selanastrum capricornutum</i>	22	Rhee and Gotham 1980
<i>Fragilaria crotonensis</i>	25	Rhee and Gotham 1980
<i>Synechococcus linearis</i>	25-45	Healey 1985
<i>Scenedesmus obliquus</i>	30	Rhee and Gotham 1980

minutes of time-course incubations were suggestive of "surge" uptake, a phenomenon previously documented for nitrogen-starved algal cultures and for nitrogen-limited phytoplankton assemblages in various lacustrine and oceanic environments (see Healey 1973; Rhee 1978; Wheeler et al. 1982; Priscu and Priscu 1984; Harrison and Harris 1986; Priscu 1987; Suttle and Harrison 1988c).

Although the above findings collectively provided strong evidence for the seasonal nitrogen limitation of phytoplankton primary production in Snowbank Lake, further comment is warranted concerning their interpretation and general validity. Regarding the short-term nutrient enrichment bioassays (finding 1, above), it is known that some phosphorus deficient algae respond to PO_4^{3-} addition by directing their metabolism

first to the rapid assimilation of phosphorus, then to the conversion of stored carbon to metabolites supportive of enhanced growth and, finally, to the accelerated photoassimilation of inorganic carbon (Healey 1979; Lean and Pick 1981). Similarly, a transient suppression of photosynthesis is sometimes observed following the addition of NH_4^+ to cultures of nitrogen-starved algae, owing to an initial metabolic emphasis on nitrogen assimilation (reviewed by Elrifi and Turpin 1987). Therefore, the outcome of any ^{14}C -based nutrient enrichment bioassay may depend not only on the nature of the nutrient deficiency, but also on the time scale of the experiment. In the present study, an effort was made to minimize the effect of possible time lags in photosynthetic response by allowing nutrient-enriched samples to preincubate for 24 hr prior to ^{14}C - NaHCO_3 addition (Chapter 3).

Measured $\text{NH}_4^+ + \text{NO}_3^-$:SRP molar ratios provided a questionable basis for estimating the relative availability of assimilatable phosphorus and nitrogen in Snowbank lake (finding 2, above). On one hand, SRP has been shown to underestimate the availability of biologically reactive phosphorus in lakes and streams (Bradford and Peters 1987), suggesting that nitrogen deficiency in Snowbank Lake was more severe than originally indicated (cf., tables 6 and 24). On the other hand, dissolved organic nitrogen (DON) was not considered in the dissolved nitrogen/phosphorus comparisons. The labile DON pool in most aquatic ecosystems consists largely of amino acids, peptides and more complex nitrogenous compounds. Many of these compounds satisfy algal nitrogen requirements in axenic culture (reviewed by Healey 1973; McCarthy 1980), but heterotrophic bacteria are known to have greater affinities for these substances and

to comprise the principal sink for DON in natural waters (Crawford *et al.* 1974; North 1975; Payne 1980; Hollibaugh and Azam 1983; Billen 1984; Hagstrom *et al.* 1984; Wheeler and Kirchman 1986). A more readily assimilatable form of DON for phytoplankton is urea, a waste product of heterotrophic metabolism and an important algal nutrient in many marine ecosystems (e.g., McCarthy 1972; Parsons *et al.* 1977; Sahlsten 1987) and in some inland waters (Wynne 1987). For reasons explained in the following subsection, urea was deemed comparatively unimportant to phytoplankton production in Snowbank Lake.

The elemental composition of seston in Snowbank Lake (finding 3) was influenced by the presence of detritus, which tends to have a greater carbon:nitrogen ratio than algal biomass (see Lorenzen 1961; Bloesch *et al.* 1977; Priscu and Goldman 1983). The extent of this influence was estimated on the basis of the observed temporal relationships between chlorophyll *a*, PC and PN (Table 8; cf., Priscu and Goldman 1983). The ordinal intercept of the linear regression of PN on chlorophyll *a* concentration provided a time-integrated approximation of nonalgal PN; similarly, the intercept of the regression of PC on chlorophyll *a* provided an estimate of nonalgal PC. During the 1986-87 ice-free seasons, at a depth of 2 m, detrital PN averaged ($\pm$ propagated standard error; see Skoog 1985) $13.6 \pm 15.6\%$ of the measured PN, whereas detrital PC averaged $16.6 \pm 11.0\%$ of the measured PC. Correction for the presence of detritus reduced the measured, time-integrated average ($\pm$ propagated standard error) ratio of carbon:nitrogen in phytoplankton cells from 11.4 ± 1.0 to 11.0 ± 2.6 . Relative to the Redfield ratio of 7, the

corrected ratio remained suggestive of a nitrogen-deficient phytoplankton assemblage (cf., Goldman 1986).

Regarding finding (4), above, temporal reductions in $V_{\text{NH}_4^+}$ and $P_{\text{NH}_4^+}$ (figures 20 and 23) may have resulted from a rapid satiation of biological nitrogen demand, from the observed gradually diminishing availability of NH_4^+ due to substrate utilization or loss (i.e., a methodological artifact; see *Limitations of Nutrient Uptake Models*, this chapter), or from a combination of these factors. No method existed for clearly distinguishing among these possibilities *ex post facto*. That added NH_4^+ concentrations of approximately 7 μM in experimental vessels were reduced by as much as 35% in less than 24 hr (Figure 20), however, suggested that the phytoplankton demand for nitrogen greatly exceeded NH_4^+ supply under ambient conditions. Horrigan and McCarthy (1982) have argued that ". . . evidence of enhanced potential for NH_4^+ uptake by natural (algal) assemblages cannot be used to infer nitrogen limitation; rather, it may indicate only that NO_3^- and/or NO_2^- are supplying the bulk of the N ration." Implicit in their argument is the preferential utilization of NH_4^+ over oxidized forms of nitrogen (considered below). However, a stoichiometric substitution of NH_4^+ for NO_3^- in the algal "N ration" could not have accounted for the extraordinarily large increases in NH_4^+ uptake frequently documented in the present study following the addition of ^{15}N -labeled substrate to lake water samples (Figure 15); i.e., NH_4^+ prompted a significant increase in total nitrogen ($\text{NH}_4^+ + \text{NO}_3^-$) uptake, as one would expect given its strong stimulatory effect on algal photosynthesis (figures 11 and 12).

Despite the inevitable uncertainties associated with any measure of algal nutrient status in natural aquatic ecosystems, the evidence considered in this subsection suggested that nitrogen availability exerted a singularly important influence on phytoplankton growth in Snowbank Lake during the 1986-87 ice-free seasons. Nitrogenous compounds which were most important to phytoplankton nutrition during this period are considered in the following subsection.

Evidence for Algal Preferential Uptake of NH_4^+

Many algae utilize NH_4^+ preferentially in culture and will deplete this resource completely before commencing uptake of alternative nitrogen compounds (reviewed by Healey 1973; see also Caperon and Myer 1972; Horrigan and McCarthy 1982). Similarly, many natural phytoplankton assemblages selectively exploit NH_4^+ in the presence of more abundant nitrogenous nutrients, such as NO_3^- (e.g., McCarthy *et al.* 1977; Axler *et al.* 1982; Berman *et al.* 1984; Priscu *et al.* 1985; Sahlsten 1987). Physiological explanations for this phenomenon invariably focus on the inhibitory effects of NH_4^+ on NO_x^- reductases or, alternatively, on the unfavorable expenditures of reductant necessary to convert oxidized forms of nitrogen into cellular components (reviewed by Raven 1980). Of greater interest to this discussion, however, are the environmental conditions which tend to promote the preferential uptake of NH_4^+ over oxidized forms of nitrogen. Horrigan and McCarthy (1982) have proposed that nitrogen availability in euphotic regions of the open ocean is associated predominantly with ephemeral microzones or "patches" of regenerated NH_4^+ , which must be exploited rapidly given the dispersion

dynamics of turbulent waters; hence, a necessary attribute among marine phytoplankton is the capacity to sequester NH_4^+ at a rate many times greater than required for steady-state growth. In contrast, episodes of high NO_3^- availability are associated with infrequent, relatively persistent disruptions of the thermocline, and a continuously maintained capability for rapid NO_3^- uptake would provide no obvious advantage to a marine phytoplankter (*cf.*, Priscu and Priscu 1984a). According to this model (essentially an extension of the Dugdale/Goering paradigm; see Chapter 1), nitrogen deficient marine algae presented with an experimental pulse of both NO_3^- and NH_4^+ would tend to utilize the latter nutrient most effectively, owing to an intrinsic capacity for surge NH_4^+ uptake. Ammonium preferences observed in freshwater algae have been interpreted similarly (Priscu *et al.* 1985).

In Snowbank Lake, maximum specific uptake rates for NH_4^+ and NO_3^- were comparable to those reported for other nitrogen-deficient lakes and for coastal and open oceanic waters (Axler *et al.* 1982; Priscu and Priscu 1984; Sahlsten 1987), suggesting that the evolutionary forces acting upon nutrient uptake have been similar in a wide variety of aquatic ecosystems. Maximum uptake rates were generally much greater for NH_4^+ than for NO_3^- when based on 5-hr field incubations (Table 11). As demonstrated in the 26 September 1987 laboratory time-course experiment, however, perceived differences in uptake rates were largely a function of experimental time frame: field incubations of lesser duration would have emphasized the rapid initial uptake of NH_4^+ and suggested a wide discrepancy between $V_{\text{max},\text{NH}_4^+}$ and $V_{\text{max},\text{NO}_3^-}$, whereas longer incubations would have implied little difference between NH_4^+ and NO_3^- uptake capabilities

(figures 23 and 24). Nevertheless, the observed capacity for immediate utilization of added NH_4^+ was consistent with the aforementioned conceptual model of Horrigan and McCarthy (1982), in that Snowbank Lake phytoplankton appeared physiologically capable of exploiting ephemeral NH_4^+ patches resulting from zooplankton excretion and microbial decomposition activities. Comparatively slow physiological responses to NO_3^- addition suggested that phytoplankton were incapable of exploiting NO_3^- microzones, such as presumably occur in the vicinity of nitrifying bacteria (*cf.*, Priscu 1987; Priscu *et al.* 1989); instead, NO_3^- utilization appeared oriented toward a less variable "background concentration" (hereafter defined as the nutrient level resulting from the uniform mixing of an arbitrarily large volume of lake water).

Relative Preference Index (RPI) values near unity indicated that NO_3^- utilization in Snowbank Lake was roughly proportional to NO_3^- availability and that algal utilization of NO_3^- was not inhibited by the comparatively low ($\approx 0.1 \mu\text{M}$) ambient concentrations of NH_4^+ (*cf.*, McCarthy *et al.* 1977). The design of the substrate competition experiments obscured the actual effect of serial NH_4^+ additions on NO_3^- uptake, in that the effects of unlabeled nitrogen uptake on measured $V_{\text{NO}_3^-}$ were not considered (serial additions of $^{14}\text{NH}_4^+$ undoubtedly increased NH_4^+ uptake asymptotically, thus artificially decreasing seston $^{15}\text{N}:^{14}\text{N} + ^{15}\text{N}$ accumulation ratios (see Collos 1987; *cf.*, Figure 15, this report). Interestingly, it has been shown that phytoplankton grow equally well in NO_3^- - or NH_4^+ -supported cultures under nitrogen-limited conditions, implying that the energetic disadvantages presumably associated with NO_3^- assimilation may be inconsequential under such circumstances (discussed by Raven 1980). Given the

nitrogen-limited rather than light-limited status of Snowbank Lake (at least during summer daylight hours at a depth of 2 m), the utilization of all forms of assimilatable nitrogen to the fullest possible extent would tend to maximize algal growth, regardless of the energetic expenditures involved. Future substrate competition studies (in which unlabeled nitrogen uptake is adequately accounted for) may reveal that uninhibited NO_3^- utilization by phytoplankton is a distinguishing feature of Snowbank Lake and similarly nitrogen-deficient, "energy-rich" aquatic ecosystems.

The ability of Snowbank Lake phytoplankton to rapidly exploit NH_4^+ additions undoubtedly influenced the results of the $\text{NH}_4^+/\text{NO}_3^-/\text{urea}$ enrichment comparison experiment (Figure 13). By quickly alleviating nitrogen deficiency, saturating NH_4^+ additions permitted a significant photosynthetic response within a comparatively short experimental time frame. The gradual increase in NO_3^- uptake noted during the laboratory time course experiment (Figure 24, control treatment) suggested that, given a longer incubation period, NO_3^- additions also would have stimulated inorganic carbon fixation. Indeed, NO_3^- uptake did not differ significantly from NH_4^+ uptake during the final (11-19 hr) interval of the time-course incubation, implying that both nutrients would have served equally well as a nitrogen source if provided continuously over a longer period. The significant reductions noted in photosynthetic activity following 7- μM additions of urea (Figure 13) suggested that said additions had an inhibitory (toxic) effect on algal growth or, alternatively, that metabolic energy had been diverted to the production and/or activation of urea assimilatory enzymes (see Raven 1980; Elrifi and

Turpin 1987). The apparent absence of a high assimilatory potential for urea implied that Snowbank Lake phytoplankton did not regularly encounter microzones of this compound. Given that most aquatic heterotrophs are ammonotelic rather than ureotelic (Campbell 1970a,b) and that sewage inputs are nonexistent in the upper Hell Roaring Creek watershed, urea would not be expected to contribute significantly to phytoplankton primary production in Snowbank lake.

Based on the results of the nutrient enrichment experiments (figures 11 and 12), the rapid photosynthetic enhancements noted in lake water samples following dilution with snowmelt and snow field runoff were attributed primarily to NH_4^+ inputs (see Table 4). However, it is likely that the relatively high concentrations of NO_3^- in meltwater would have contributed to longer-term (days-weeks) enhancements of primary production, as demonstrated for nitrogen-deficient marine waters following episodes of NO_3^- -enriched precipitation (Paerl 1985; cf., Figure 24, control treatment, this study). Ammonium, NO_3^- and SRP concentrations in snow field runoff were reduced markedly en route to Snowbank Lake (Figure 8), presumably owing to uptake by epilithic diatoms which dominated the boulder field aquatic flora. Concentrations of NO_3^- were reduced further within the lake; a comparison of NO_3^- levels in the inflowing stream with those in the lake water column and outflowing stream indicated that Snowbank Lake was a net sink for NO_3^- . Incoming concentrations of NH_4^+ and SRP were not changed significantly in Snowbank Lake, suggesting that regenerative and assimilative fluxes of these nutrients were approximately in balance on an ecosystem scale. This

contention receives more thorough consideration in the following subsection.

Quantitative Comparison of NH_4^+ Regenerative and Assimilative Fluxes

Background concentrations of nutrients in any aquatic ecosystem are determined by incoming and outgoing nutrient fluxes (advective, diffusive and atmospheric) and by the biogeochemical production and consumption of these compounds within the system. A detailed analysis of all factors influencing NH_4^+ availability in Snowbank Lake was far beyond the scope of this study. However, water-column concentrations of NH_4^+ clearly reflected levels in inflowing stream waters, suggesting that the availability of this nutrient was based primarily on advective inputs (which provided a baseline concentration of approximately $0.1 \mu\text{M}$ on which biogeochemical forces within the lake could act) and internal remineralization activities (which precluded NH_4^+ depletion despite a high biological demand for nitrogen). Regarding the latter factor, measured whole-lake concentrations of NH_4^+ were relatively constant during the 1986-87 ice-free seasons (mean = $0.13 \mu\text{M}$; CV = 31%), implying that regenerative and assimilative fluxes of NH_4^+ were approximately in balance on an ecosystem scale. Such a balance was not corroborated by the field experiments conducted during this period (tables 13-16). Instead, regeneration rates were typically greater than uptake rates calculated as P_c (i.e., based on $^{15}\text{NH}_4^+\text{-N}$ gained by the particulate fraction), even though P_c was based on saturating substrate additions; contrastingly, r was consistently less than uptake rates calculated as u (i.e., based on $^{15}\text{NH}_4^+\text{-N}$ lost by the aqueous fraction; see equations 8-12, Chapter 3).

The remainder of this subsection attempts to resolve these discrepancies by examining the limitations of the theoretical models and experimental methods used in this study.

Limitations of Nutrient Uptake Models. Although seldom explicitly stated, the following 6 assumptions underlie most ^{15}N -based models of NH_4^+ uptake: (1) assimilative fluxes of NH_4^+ are constant between successive sampling points in an experimental time series; (2) isotope dilution due to ammonification is negligible; (3) algae do not discriminate between $^{15}\text{NH}_4^+$ and $^{14}\text{NH}_4^+$; (4) algae do not exude ^{15}N -labeled metabolites; (5) no significant change occurs in algal nitrogen content during incubations; and (6) uptake of other, unlabeled nitrogenous compounds is quantitatively unimportant. With the possible exception of (3), above, none of these assumptions is entirely consistent with empirical evidence. Ammonium uptake is often conspicuously nonlinear following experimental additions of ^{15}N tracer (figures 20 and 22; cf., Collos 1980; Glibert et al. 1982; Priscu 1987); isotope dilution is frequently significant and can lead to gross interpretational errors if ignored (Garside 1984); algae exude substantial amounts of DON, and the potential exists for rapid biochemical turnover of labeled nitrogen compounds (see Mague et al. 1980; Søndergaard et al. 1985; Bjørnsen 1988); increases in algal nitrogen content occur during many ^{15}N -tracer studies, especially those involving nitrogen-deficient algal assemblages (e.g., Collos 1980; Collos and Slawyk 1980); and phytoplankton uptake of unlabeled nitrogen (especially NO_3^- -N) is unavoidable in many aquatic environments (Table 12;

see also Horrigan and McCarthy 1982; Berman *et al.* 1984; Priscu *et al.* 1985; Harrison *et al.* 1987; Sahlsten 1987).

Ammonium uptake models formulated by Glibert *et al.* (1982) and Laws (1984) have improved upon previous models by compensating for the effects of isotope dilution. More recently, Collos (1987) has presented a mathematical model which corrects for *increases* in algal nitrogen content and for the simultaneous uptake of labeled and unlabeled nitrogen compounds. Recall that in the present study NH_4^+ uptake rates were calculated via Laws' equation, with one modification: PN concentrations measured at the beginning and end of each incubation (or time step) were averaged, and uptake was normalized to biomass based on this average ($\bar{\text{PN}}$) rather than on the final PN concentration (PN_i ; see equation 10, Chapter 3). The rationale behind this modification was two-fold. First, changes in particulate nitrogen concentration were seldom statistically significant, and it was believed that by averaging 6 observations rather than 3, a more representative estimate of this concentration would be obtained. Secondly, uptake rates based on PN_i did not accommodate *decreases* in PN concentration observed at the beginning of some time-course experiments. Figure 31 illustrates the influence that various models would exert on one's perception of NH_4^+ uptake under such circumstances. The delayed increase in NH_4^+ uptake indicated by models 1-3 was clearly an artifact resulting from the use of PN_i in the numerator of the uptake equations. Model 4 was less sensitive to decreases in particulate nitrogen concentration, but gave lower estimates of NH_4^+ uptake when PN_i exceeded PN_0 (where PN_0 represents the concentration at the

- (1) — · — · — $P_{\text{NH}_4^+} = (\Delta\rho \cdot \text{PN}_t) \div ((R_o - \rho_o)\Delta t)$; Collos (1987)
 (2) — — — — $P_{\text{NH}_4^+} = (\Delta\rho \cdot \text{PN}_t) \div (\bar{R} \cdot \Delta t)$; Laws (1984)
 (3) - - - - - $P_{\text{NH}_4^+} = (\Delta\rho \cdot \text{PN}_t) \div (R_o \cdot \Delta t)$; Paasche and Kristiansen (1982)
 (4) ········ $P_{\text{NH}_4^+} = (\Delta\rho \cdot \bar{\text{PN}}) \div (\bar{R} \cdot \Delta t)$; this study
 (5) - - - - - $P_{\text{NH}_4^+} = (\Delta\rho \cdot \text{PN}_o) \div (R_o \cdot \Delta t)$; Eppley et al. (1977)

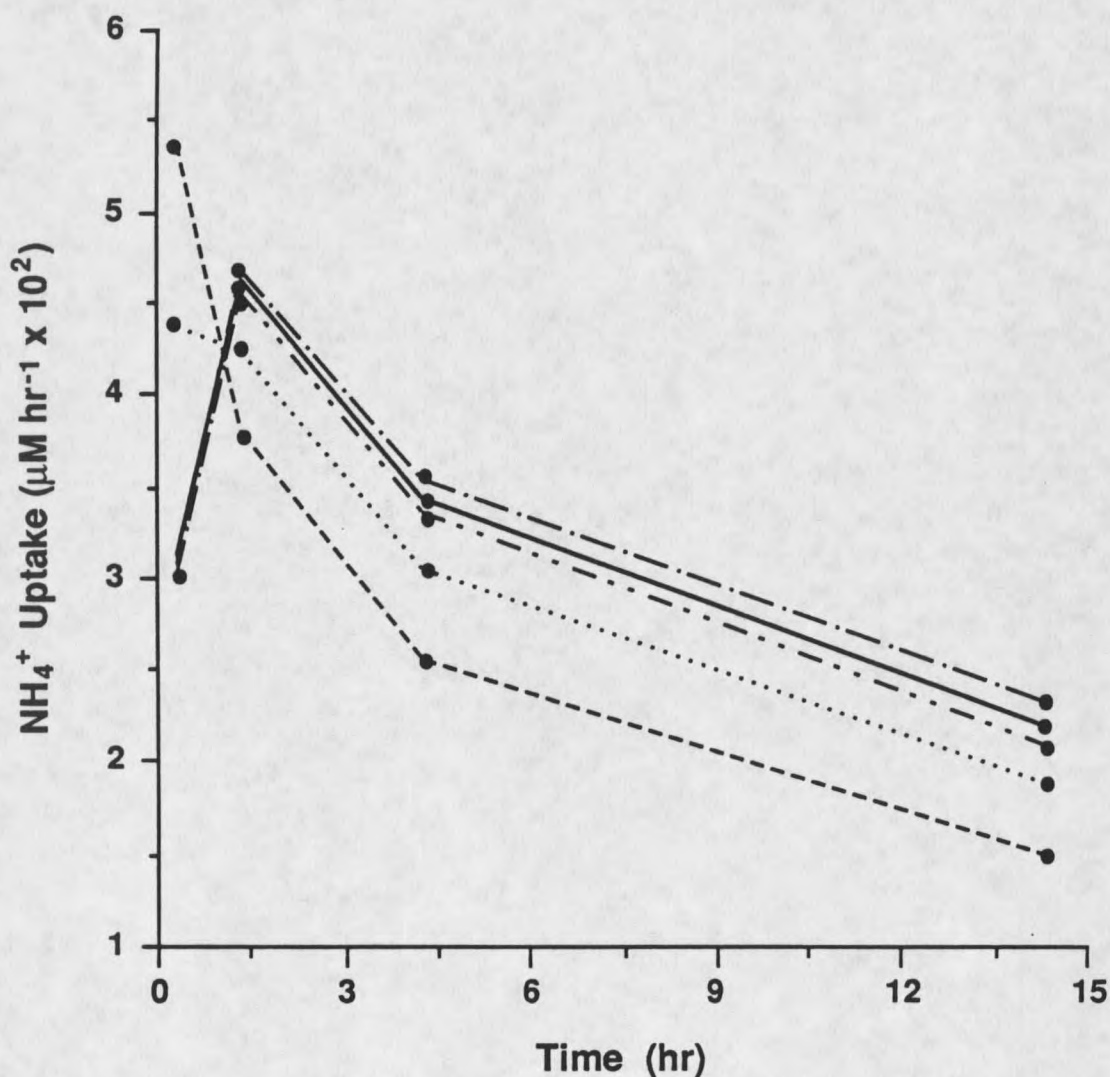


Figure 31. Comparative effects of 5 mathematical models on perceived time-course patterns of NH_4^+ uptake. Data shown are based on field experiments conducted 2 September 1987 (Table 15; Figure 20). Symbols used in original reports have been reexpressed for consistency; see Chapter 3 for definition of terms.

beginning of the time step). Calculated over the entire incubation period, uptake rates assumed the following order: model 2, 32 nM hr⁻¹; model 4, 29 nM hr⁻¹; models 1 and 3, 28 nM hr⁻¹; and model 5, 23 nM hr⁻¹. The higher rates associated with models 2 and 4 reflected the fact that decreases in R were of greater relative magnitude than increases in PN (Table 15); that is, compensating for the effects of isotope dilution was quantitatively more important than correcting for increases in algal biomass (model 1). Interestingly, differences in $P_{NH_4^+}$ values among models appeared relatively minor when compared to the large experimental errors commonly associated with ¹⁵N-based estimates of NH₄⁺ uptake (see Laws 1984; Kokkinakis and Wheeler 1987; cf., tables 13-16, this study).

By comparing $P_{NH_4^+}$ values based on model 3 with those based on models 1 and 2 in Figure 31, it becomes apparent that the uptake of unlabeled nitrogen compounds, the observed increase in PN concentration, and the dilution of the ¹⁵NH₄⁺ pool contributed little to the large discrepancies noted between u- and P_c-based measurements of NH₄⁺ uptake (tables 13-16). However, the initial decrease in PN concentration noted in this and other time-course experiments (Figure 20) suggested that the release of ¹⁵N-labeled DON by phytoplankton may have constituted a more serious source of error. Whether such decreases reflected a normal physiological response to enhanced nutrient availability or were simply a manifestation of handling procedures, bottle confinement or other experimental conditions is uncertain. Regardless, they implied that further nitrogen losses were possible during later time intervals (despite net increases in cellular nitrogen) and emphasized the potential for the release of ¹⁵N-labeled compounds by algae within the time frame of the experiment. As

depicted in Figure 32, discrepancies between u and P_c were greatest at the beginning of the time-course experiment considered in Figure 31, despite the fact that algal uptake of NH_4^+ was most rapid during this period. The concomitant decrease in PN concentration provided strong circumstantial evidence for the release of ^{15}N -labeled DON by phytoplankton. Assuming for a moment that discrepancies between u and P_c in field time-course experiments were due entirely to the loss of labeled DON, 75-82% of the $\text{NH}_4^+\text{-N}$ taken up by phytoplankton subsequently was discharged as organic nitrogen (based on whole-experiment $u:P_c$ ratios; see tables 13-16). These percentages would not be altogether unrealistic in many aquatic ecosystems; for example, Williams (1981) estimated that 50-60% of the carbon fixed by marine phytoplankton was cycled through the dissolved organic carbon (DOC) pool to bacterioplankton, either directly (via algal exudation) or indirectly (through zooplankton grazing and excretion; see *Trophic Interactions of Importance in Planktonic Nitrogen Cycling*, this chapter). Laws (1984) critically evaluated the methods and results of several ^{15}N -based nutrient cycling experiments conducted by Glibert *et al.* (1982) in coastal and open oceanic waters, concluding that algal DON loss was a major factor influencing $u:P_c$ ratios in that study. The results of the present investigation likewise suggested that comparisons between u and P_c were influenced by algal release of ^{15}N -labeled organic compounds.

Bacterial nitrification activities may have constituted an additional sink for $^{15}\text{NH}_4^+$ in this study. Although nitrification rates were not monitored during field experiments, indirect evidence suggested that these rates were negligible on an experimental basis. For example, NO_3^- concentrations were stable during time course incubations despite $3.3\text{-}\mu\text{M}$

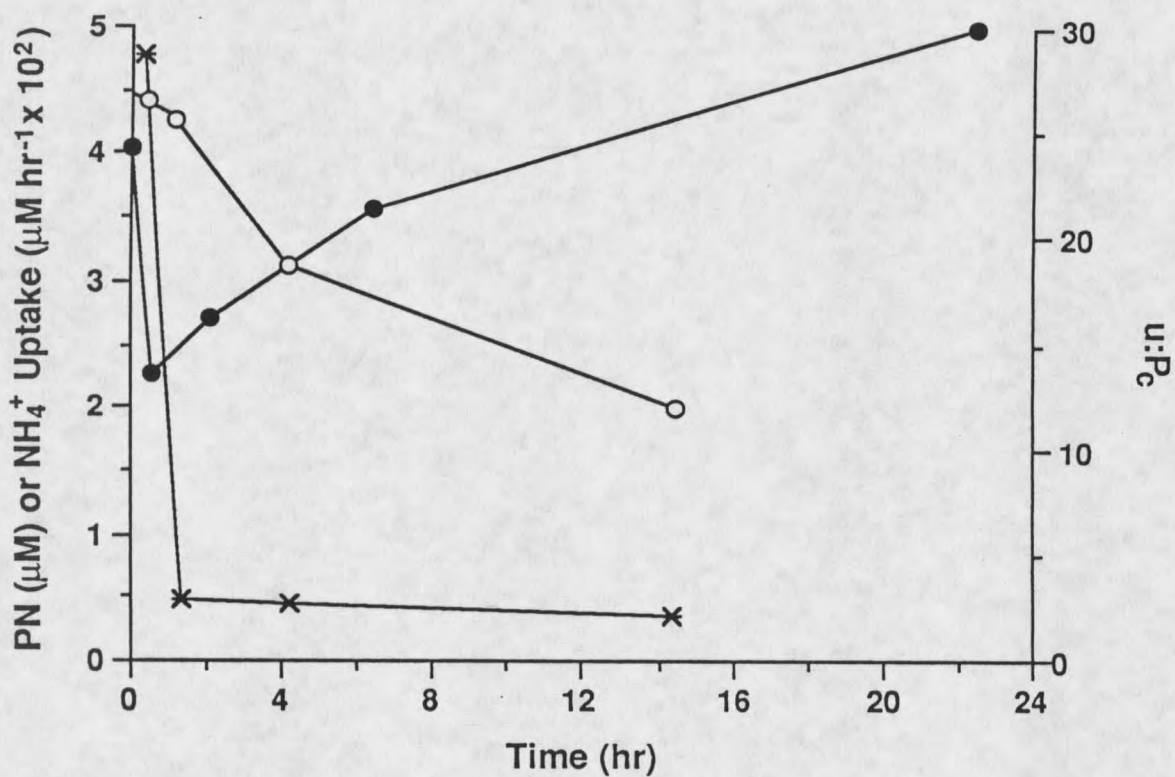


Figure 32. Time course of particulate nitrogen concentration (●) and NH_4^+ uptake (○) and the ratio $u:P_c$ (x). Data are based on the 2 September 1987 field experiment considered in Table 15 and in figures 20 and 31.

additions of NH_4^+ ; therefore, any increase in NO_3^- production would have been offset by a concomitant, stoichiometric increase in NO_3^- consumption, an unlikely scenario given the slow response of phytoplankton to NO_3^- enrichment (Figure 24). Secondly, bacterial nitrification is strongly inhibited by light (Horrigan *et al.* 1981; Olson 1981), and field experiments in the present study were carried out at the well-illuminated depth of 2 m (3 August 1986 only) or at the lake surface under somewhat reduced irradiance conditions (40% ambient irradiance; see Chapter 3). Water-column profiles for NH_4^+ and NO_3^- suggested that nitrification rates were substantial in the deeper (>6 m), more poorly illuminated waters of Snowbank Lake (Figure 6). Hence, NH_4^+ oxidation may have been an important sink for NH_4^+ on a whole-lake, if not an experimental, basis during the 1986-87 ice-free seasons.

Ammonium uptake by cells too small to be retained on GF/C filters (effective retention capacity $\approx 1.2 \mu\text{m}$) also undoubtedly contributed to the large u:P_c ratios reported in this study. Previous investigators have found that 60-70% of all countable bacterioplankton (large enough to be enumerated by fluorescent microscopy) readily pass through GF/C filters (e.g., Hobbie *et al.* 1977). These organisms, together with small (≤ 0.5 - $1.0 \times 1.0 \mu\text{m}$) chroococcoid cyanobacteria may account for a substantial fraction of the total plankton biomass (see Johnson and Sieburth 1979) and for a disproportionately large share of NH_4^+ uptake (Wheeler and Kirchman 1986). Recently, electron microscopic studies have documented a large variety of femto- (0.02 - $0.2 \mu\text{m}$) and pico- (0.2 - $2.0 \mu\text{m}$) sized autotrophic and heterotrophic plankton in oligotrophic, softwater lakes (Stockner and Klut 1988; Stockner and Shortreed 1989). The ecology of

these extremely small organisms is virtually unknown, but they may contribute significantly to biological nitrogen demand in some ecosystems. Furthermore, some adsorption of NH_4^+ to surface-active inorganic colloids, organic macromolecules and organic colloids probably occurs in most natural waters (see Pagenkopf 1978; Stumm and Morgan 1981; Cosović 1985). These adsorption reactions are not readily accounted for in ^{15}N -based studies of NH_4^+ uptake by phytoplankton.

Experimental effects such as NH_4^+ adsorption to container walls, depletion of substrate, or alterations in the physiological activities of phytoplankton due to changes in abiotic conditions or to handling stress also may have influenced the results of time-course experiments performed in this study. Whereas problems related to NH_4^+ /container adsorption reactions traditionally have involved glass, rather than LPE containers (see DeGobbis 1973; Liddicoat *et al.* 1975) and probably have been exaggerated (see Dugdale and Wilkerson 1986), artifacts resulting from NH_4^+ depletion have been demonstrated convincingly in many studies of NH_4^+ utilization by nitrogen-deficient algal assemblages (reviewed by Fisher *et al.* 1981; Dugdale and Wilkerson 1986; see also Goldman *et al.* 1981). In general, an initial period of rapidly declining $P_{\text{NH}_4^+}$ followed by a period of approximately constant $P_{\text{NH}_4^+}$ has been interpreted as evidence of surge uptake rather than as a manifestation of substrate depletion (Goldman *et al.* 1981; Harrison 1983a; cf., Priscu 1987). Although no leveling off of NH_4^+ uptake was observed in one of the time-course experiments performed in this study (see 26 June 1987 entry, Figure 20), the duration of this experiment may have been insufficient to detect such a trend. A leveling off of P_c was evident on 25 September

1987 despite a continued decrease in NH_4^+ concentration; conversely, a slight decrease in P_e occurred on 18 July 1987 despite a relatively constant NH_4^+ concentration. Furthermore, in none of the field time-course experiments did NH_4^+ concentrations decline below presumably saturating substrate levels (i.e., $2 K_s$; see tables 11 and 13-16, Chapter 4). Hence, it appeared that the time-course patterns of NH_4^+ uptake observed in this study were little affected by substrate depletion.

Because time-course experiments were conducted at the water surface, they undoubtedly involved somewhat different temperature and light conditions than those to which phytoplankton collected from a depth of 2 m were accustomed. However, the experimental temperature was consistently within 1°C of the temperature at 2 m, and the use of neutral density screening reduced ambient irradiance to a level comparable to that at 2 m. (This contention is based on the general relation $Z_E = -(2.3^{-1} \ln \gamma) Z_S$, where Z_E refers to the experimental depth, Z_S to the Secchi depth, and γ to the fraction of incident light penetrating to the experimental depth; see Preisendorfer 1986, pg 925. Applying this relationship to the present study, the estimated percentage of surface irradiance penetrating to a depth of 2 m ranged from 36% during the 26 June 1987 time-course experiment to 52% on the 25 September 1987 time-course experiment.) Other experimental effects (e.g., changes in the spectral qualities of light, changes in the grazing activities of zooplankton, handling stress) also may have influenced the absolute rates and time-course patterns of NH_4^+ uptake, but their quantification was not within the scope of this study.

Limitations of Nutrient Regeneration Models. Implicit in the Blackburn/Caperon model for the calculation of NH_4^+ regeneration rates from ^{15}N -tracer data (equation 8, Chapter 3) is that (1) regenerative fluxes of NH_4^+ are constant between successive sampling points in an experimental time series and (2) regeneration of ^{15}N -labeled NH_4^+ does not occur. The first assumption is violated in many ^{15}N -tracer studies (cf., Harrison and Harris 1986; figures 20 and 23, this report), and in practice, changes in r are minimized by collecting data (R , S) at closely spaced time intervals. However, the accuracy of r (and u) is directly related to the uncertainty in the ratio R_t/R_o , and compromises must be made between very brief incubations, in which artifacts associated with temporal changes in r are unlikely, and longer incubations, in which significant changes in R are assured (see Laws 1984). The validity of assumption (2), above, is in doubt in any time series experiment demonstrating a significant decline in regeneration activity. Such a decline might reflect a gradual buildup of ^{15}N in the regenerated NH_4^+ pool, a genuine biological effect, or a combination of these factors. Although it is not possible to clearly distinguish between these possibilities *ex post facto*, an abrupt leveling off of R and ρ at nearly the same ^{15}N atom-% enrichment would suggest that an equilibrium had been attained between $^{15}\text{NH}_4^+$ consumption and production processes.

In the present study, an apparent equilibration between R and ρ was approached toward the end of the 26 September 1987 laboratory time-course experiment (Figure 23, control treatment; note that $R_o = R_t = 42\%$ and $\rho_o = \rho_t = 31\%$ after approximately 11 hr). This phenomenon was not observed in the field time-course experiment conducted one day earlier,

presumably because regeneration activity (*i.e.*, algal nutrient turnover) was less rapid than under laboratory conditions (*cf.*, figures 20 and 23, tables 16 and 22; note that $r_{\text{lab}} \approx r_{\text{field}} \times 6$, whereas $V_{\text{lab}} = V_{\text{field}}$). Indeed, no indication of an equilibration between ρ and R was observed in any of the field time-course experiments, including those wherein an initial reduction in r was observed (see 26 June and 2 September entries, Figure 20). Similar reductions in ammonification rate have been documented in previous laboratory studies employing conventional (nonisotopic) chemical methods; hence, they cannot automatically be regarded as artifacts of ^{15}N -tracer techniques. Ikeda (1977) attributed high initial rates of $^{14}\text{NH}_4^+$ release by zooplankton in his experiments to handling stress. Other factors influencing NH_4^+ regeneration rates in previous studies have included comparatively large changes in temperature (*e.g.*, Caron *et al.* 1986) and in food availability (Mayzaud 1976; Gardner and Scavia 1981). Because neither of the latter factors were deemed descriptive of the field experimental conditions in the present study, the observed initial enhancements (*i.e.*, rapid reductions) in heterotrophic regeneration activity (26 June and 2 September 1987 field experiments) were attributed primarily to sample collection and handling.

Influence of Substrate Enrichment on $r:P_c$ and $u:r$ Ratios. Direct comparisons of NH_4^+ regenerative and assimilative fluxes under ambient nutrient conditions often require that the experimentally determined values for $P_{\text{NH}_4^+}$ be corrected for substrate enrichment. The Michaelis/Menten model (equation 5, Chapter 3) traditionally has been employed for this purpose, even though its application to

nutrient-limited algal assemblages presupposes that all species present possess equal affinities for the nutrient in question or, alternatively, that the dominant taxa have similar K_s values, thereby approximating nutrient utilization by a unialgal population (Dugdale 1967). The robustness of the Michaelis/Menten model with regard to these assumptions and its general validity in investigations of nutrient uptake by natural algal assemblages has been substantiated both empirically (reviewed by Dugdale 1975) and via computer simulation (Williams 1973).

In the present study, corrections based on the Michaelis/Menten model increased the average $r:P_c$ ratio in field time-course experiments from 1.5 to 5.5 (these figures are based on fluxes calculated over entire incubation periods and do not reflect the data from 18 July 1987, when no regeneration activity was detected). Average $u:r$ ratios decreased from 2.3 to 0.6 upon compensating for the effects of substrate enrichment (an average $P_c:u:r$ ratio of 1:3.3:5.5, assuming that the correction coefficients applied to P_c pertained equally well to u). Similar discrepancies between measured rates of NH_4^+ regeneration and NH_4^+ uptake have been reported by many previous researchers (Alexander 1970; Harrison 1978; Liao and Lean 1978; Axler *et al.* 1981; Glibert 1982, as reported by Laws 1984; Harrison *et al.* 1983; Lipschultz *et al.* 1986; Kokkinakis and Wheeler 1987). Several of these investigators assumed that seston ^{15}N incorporation was the most direct and, therefore, the least ambiguous indicator of NH_4^+ uptake; however, Laws (1984) acknowledged problems related to ^{15}N -labeled DON release by algae and to the loss of extremely small cells during filtration and suggested that u , rather than P , was the most reliable indicator of NH_4^+ uptake by phytoplankton.

Despite the limitations of the mathematical models and experimental methods used in the present study, and despite the ambiguities in NH_4^+ production and consumption data arising therefrom, the tendency for nitrogen remineralization rates (calculated as r) to equal or exceed NH_4^+ uptake rates (calculated as u or P_c and corrected for substrate enrichment) suggested that regenerated NH_4^+ constituted an important nutrient resource in Snowbank Lake. The efficiency with which the phytoplankton community utilized this resource during the 1986-87 open-water seasons is considered in the following section.

Coupling of NH_4^+ Regenerative and Assimilative Fluxes

Although ammonification processes comprise a major source of assimilatable nitrogen in many marine and freshwater ecosystems, the efficiency with which phytoplankton utilize regenerated NH_4^+ remains a matter of speculation. Presumably, some fraction of the NH_4^+ produced via heterotrophic metabolism is advectively displaced from the site of production; other fractions may be assimilated or oxidized by heterotrophic bacteria or involved in abiotic adsorption reactions, thereby detracting from the efficiency of the NH_4^+ recycling process. Despite these losses, empirical evidence suggests that a close spatial and temporal coupling exists between the heterotrophic production and the autotrophic utilization of NH_4^+ in nitrogen-limited waters. Laboratory studies have confirmed that zooplankton produce minute nutrient patches and that nutrient-limited phytoplankton benefit from random encounters with such patches (Lehman and Scavia 1982). Also, surge uptake capabilities evident in nitrogen-starved algal cultures and in nitrogen-deficient

algal assemblages suggest that much of the NH_4^+ contained within nutrient patches can be taken up by phytoplankton in a matter of minutes or less (e.g., McCarthy and Goldman 1979; Suttle and Harrison 1988c). Similar considerations have led McCarthy and Goldman (1979) to conclude that

"Variability in the small-scale temporal and spatial patterns in nitrogenous nutrient supply, coupled with an enhanced uptake capability for nitrogenous nutrients induced by nitrogen limitation, make it possible (in some cases) for phytoplankton to maintain nearly maximum rates of growth at media concentrations that cannot be quantified with existing analytical techniques."

In the present study, temporal relationships between NH_4^+ regeneration rates and NH_4^+ uptake rates provided considerable insight into the ecological importance of regenerated nitrogen in Snowbank Lake. Recall that regenerative and assimilative fluxes of NH_4^+ closely paralleled each other during the 1987 ice-free season (Figure 21), whereas no significant relationships were evident between NH_4^+ uptake rates and measured NH_4^+ concentrations (cf., tables 6 and 11). Hence, fluctuations in algal utilization of NH_4^+ apparently were associated more with the production of nutrient microzones than with changes in analytically detectable (background) nutrient levels. The strong correlation noted between $V_{\max}$ and r (Pearson $r = 0.95$, $p < 0.05$) indicated that the capacity for rapid NH_4^+ uptake by phytoplankton was greatest during periods of intense regeneration activity. Interestingly, the lowest values for $V_{\max}$ (0.0086 hr^{-1}) and K_t ($0.1889 \mu\text{M}$) were recorded on 18 July 1987, when regeneration activity was negligible and the background NH_4^+ concentration had attained a seasonal high of $0.16 \mu\text{M}$. Given that this concentration approached the experimentally determined half-saturation level, it appeared that much of the NH_4^+ taken up by phytoplankton on this date was derived from the

background NH_4^+ pool. Moreover, the low V_{max} value suggested that the phytoplankton community did not maintain a physiological capacity for rapid NH_4^+ uptake in the absence of significant regeneration activity, an interpretation which was supported by the results of the 18 July time-course experiment (see Figure 20).

It could be argued that the coupling of NH_4^+ regeneration and uptake rates observed from June through September 1987 was primarily an artifact of bottle confinement. That is, water samples (incubated in tightly sealed containers at the lake surface) might have undergone less vigorous physical mixing than was typical of the lake water column, thereby prolonging the existence of individual nutrient patches and increasing algal access to regenerated NH_4^+ . Although this possibility could not be ruled out entirely, the routine mixing of samples prior to the collection of subsamples for analytical determinations (Chapter 3) and the gentle, wind-induced movement of samples during incubations presumably precluded the development of unrealistically heterogeneous sample environments. Another concern was the representativeness of biological assemblages within the incubation bottles. Because many metazoan zooplankton and various flagellated algae engage in diel vertical migrations extending many meters (reviewed by Wetzel 1983; see also Sommer and Gliwicz 1986), the physiological activities of heterotrophic and autotrophic specimens collected from an arbitrary depth and time of day may not have reflected activities occurring on a whole-lake 24-hr basis. Given the relatively shallow, well mixed and well illuminated character of Snowbank Lake, however, the biological representativeness of the experimental depth (2 m) was probably greater than would have been true in the deeper, more

completely stratified, and/or less transparent lakes traditionally chosen for ^{15}N tracer studies (cf., Alexander 1970; Liao and Lean 1978; Axler et al. 1981; Takahashi and Saijo 1981; Jellison and Melack 1986).

The onset of dawn or dusk had no discernable influence on rates of NH_4^+ regeneration or uptake in this study (Figure 20). In contrast, diel periodicities in regenerative and assimilative fluxes of NH_4^+ have been reported by several researchers. McCarthy et al. (1982), Priscu (1984) and Miyazaki et al. (1987) reported that NH_4^+ uptake by lake phytoplankton was most rapid during daylight hours, presumably due to the immediate cellular availability of both photosynthetically generated ATP (required for active uptake of NH_4^+ ; see *Influence of pH on Nitrogen Uptake: A Preliminary Biochemical Model*, this chapter) and photosynthetically derived carbon skeletons capable of accepting reduced nitrogen (see Miyazaki et al. 1985); conversely, Caperon et al. (1979) and Glibert (1982) reported that NH_4^+ regeneration rates in coastal and open oceanic surface waters were greatest at night, coinciding with the active grazing periods of suspension-feeding zooplankton (cf., Wetzel 1983). In the euphotic waters of many aquatic ecosystems, therefore, NH_4^+ production and consumption processes may be out of phase by several hours rather than closely coupled in time. Interestingly, the apparent nonperiodic nature of NH_4^+ production in Snowbank Lake suggested that the physiological activities of zooplankton (at least those collected from a depth of 2 m during daylight) were not regulated strongly by irradiance levels or by endogenous rhythms (the relatively large errors associated with the regeneration data may have masked the effects of such influences; see tables 13-16 and Figure 20). More importantly, the absence of diurnal

periodicities in NH_4^+ uptake may have reflected the nitrogen-deficient status of Snowbank Lake. Berman *et al.* (1984) noted 3- to 4-fold diurnal variations in NH_4^+ uptake in Lake Kinneret (Israel) during seasons of high NH_4^+ availability (early November through late January: 17-18 $\mu\text{g-atoms N liter}^{-1}$), whereas no periodicity in uptake was detected during seasons of extremely low NH_4^+ availability (late March: $<1 \mu\text{g-atom N liter}^{-1}$). Based on a review of laboratory findings, Raven (1980) concluded ". . . any energetic advantage (to algae) of assimilating nitrogen in light is sacrificed to the need to assimilate nitrogen as fast as possible when it is growth limiting." This conclusion would appear to be applicable to the environmental conditions in Snowbank Lake.

Thus far, this discussion has not addressed the possibility of sediment/water-column nutrient exchanges in Snowbank Lake. However, the magnitude of such exchanges may have been substantial given (1) the large area of the sediment/water interface relative to the lake water volume (Table 1), (2) the freely-mixing character of the lake (*i.e.*, the general absence of water-column thermo-density gradients and diffusional constraints on sediment/deep water/shallow water nutrient transfers; see Figure 5), and (3) the luxuriant growths of epilithic diatoms and filamentous green algae, which may have placed considerable demands on water-column nutrient resources (see Chapter 2). In previous studies of sediment/water-column nutrient exchanges in freshwater lakes, biogeochemical processes in sediments have variously constituted net sources or net sinks for water-column NH_4^+ or, alternatively, they have made little net contribution to water-column NH_4^+ dynamics (see Mortimer 1940/41; Kuznetsov 1968; Keeney 1973; Isirimah *et al.* 1976; Axler *et al.*

1984). In the absence of empirical data on sediment NH_4^+ fluxes, speculation as to which of these possibilities was most descriptive of Snowbank Lake would be superfluous. Suffice it to say that some degree of spatial disequilibrium between the regeneration and the uptake of NH_4^+ was likely in Snowbank Lake, owing to the exchange of this nutrient between water-column and sediment biological communities.

Water-Column NH_4^+ Dynamics in Snowbank Lake:

A Preliminary Conceptual Model

For over two decades, the conceptual model of Dugdale and Goering (1967) has shaped our perception of the factors regulating primary production in the world's oceans (see Chapter 1). More recently, this model has provided a valuable interpretive framework for the study of nitrogen cycling in closed (or seasonally closed) nitrogen-limited lakes (see Axler *et al.* 1981, 1982). Snowbank Lake differs from such ecosystems in that both allochthonously supplied NO_3^- and internally regenerated NH_4^+ regularly constitute major forms of assimilable nitrogen (Table 12). Hence, any realistic assessment of the factors regulating algal productivity in Snowbank lake must consider the availability and utilization of both NH_4^+ and NO_3^- and acknowledge the advectively dominated character of this ecosystem.

Figure 33 depicts a highly simplified conceptual model of nitrogen supply and demand in Snowbank lake. This model is based on the following assumptions: (1) discrepancies between advective additions of dissolved inorganic nitrogen (DIN) to the water column and advective and sedimentation losses of total nitrogen from the water column represent changes (increases or decreases) in plankton biomass (*i.e.*, non-detrital

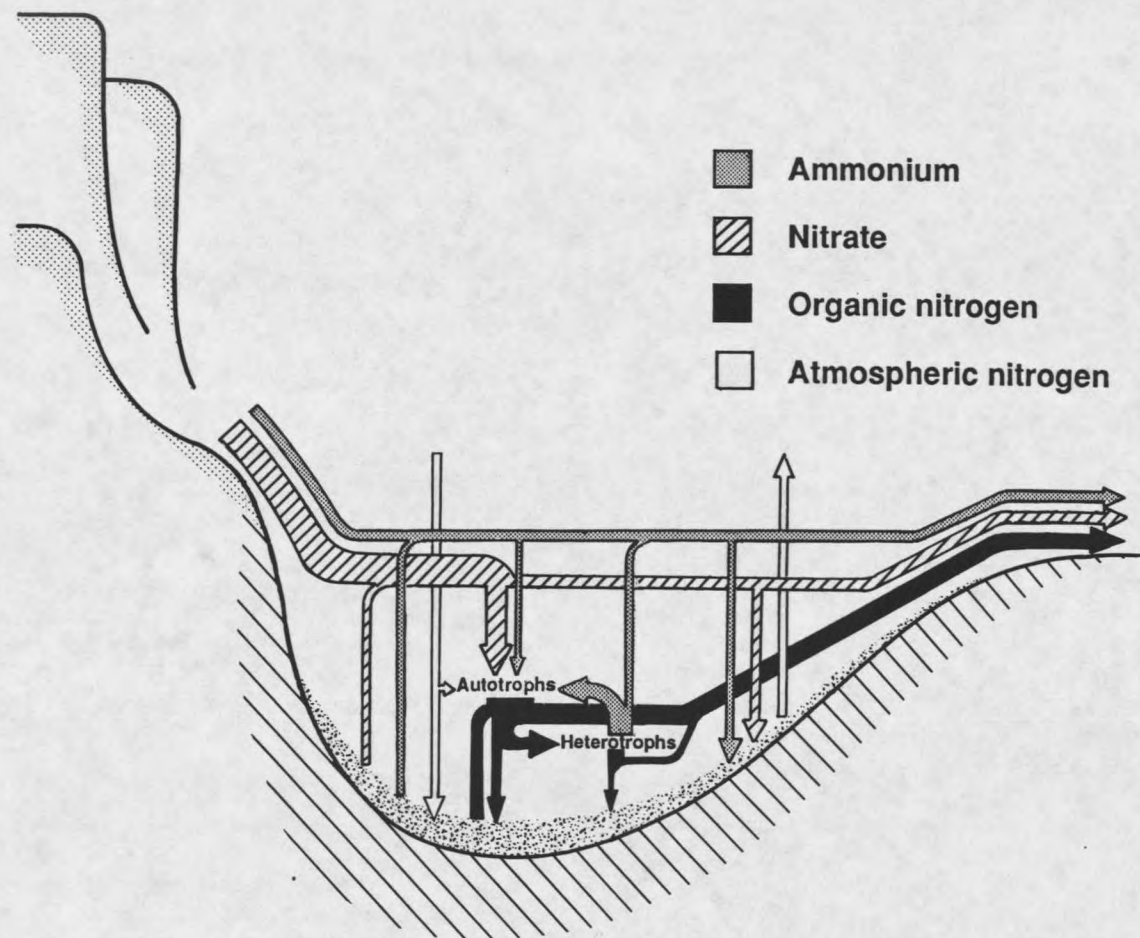


Figure 33. Schematic representation of nitrogen supply and demand in Snowbank Lake. See text for details.

particulate nitrogen); (2) most of the nitrogen assimilated by phytoplankton is derived either from advectively supplied NO_3^- or from microzones of regenerated NH_4^+ ; (3) a close spatial and temporal coupling exists between the heterotrophic production and the autotrophic utilization of NH_4^+ ; (4) the release or "leakage" of NH_4^+ from the regenerative/assimilative cycle compensates for any uptake of NH_4^+ from the background nutrient pool; and (5) nitrification, denitrification and nitrogen-fixation processes, abiotic adsorption reactions, organic nitrogen inputs, and exchanges of DIN and organic nitrogen between the water column and the sediments may be ignored for simplicity's sake without detracting from the general validity of assumptions 1-4, above. Assumption (1) is justifiable from the standpoint of mass balance; however, time lags of varying duration obviously would accompany any equilibration between nitrogen additions to and nitrogen losses from the water column. Assumptions (2) and (3) are supported by empirical evidence collected during the 1986-87 open-water periods (considered previously). Assumption (4) conveniently accounts for the observed stability of the background NH_4^+ concentration; however, interactions between the abiotic and biotic factors mentioned in (5), above, also undoubtedly contribute to this stability.

A conclusion that logically follows from this model is that any change in the availability of NO_3^- or regenerated NH_4^+ , or, alternatively, any fluctuation in the efficiency with which the algal community utilizes these nutrients, would induce a corresponding change in phytoplankton primary production during seasons of nitrogen limitation. Perturbations

which could alter DIN supply and demand in Snowbank Lake and affect the productivity of this water body are considered in the following sections.

Trophic Interactions of Importance in Planktonic Nitrogen Cycling

Thus far, nutrient cycling processes in Snowbank Lake have been alluded to as relatively simple, two-way material transfers between heterotrophic and autotrophic components of the plankton community. Actual pathways of nitrogenous nutrient flow and transformation in Snowbank Lake are probably far more complex, however, especially given the biological diversity of this ecosystem (see appendix) and the high degree of nutritional interaction common among components of aquatic food webs (Lindeman 1942; Azam *et al.* 1983). A more detailed understanding of nitrogen cycling processes in Snowbank Lake may be gained by identifying the specific groups of organisms most active in NH_4^+ regeneration and uptake and by ascertaining the major nutritional/energetic relationships existing among such groups. This section considers (1) the importance of micro- and nanozooplankton and heterotrophic bacterioplankton as agents of NH_4^+ regeneration and as trophic links between the aqueous nutrient pool and the metazoan zooplankton community, (2) the importance of crustacean and rotiferan zooplankton as sources of regenerated nitrogen and as regulators of microbial regeneration activity, (3) the extent to which DIN resources are partitioned among the phytoplankton community, and (4) the ecological implications of NH_4^+ uptake by heterotrophic bacterioplankton. Conclusions reached in this section permit the construction of a revised, more detailed model of nitrogen supply and demand in Snowbank Lake.

Microbial Agents of NH_4^+ Regeneration

Less than two decades ago, nutrient regeneration in aquatic ecosystems was attributed primarily to the decompositional activities of saprophytic organisms such as fungi and heterotrophic bacteria (reviewed by Wetzel 1983). These "decomposers" seldom were considered in early studies of aquatic food webs, which focused instead on the trophic pathway extending from phytoplankton through metazoan zooplankton to larger consumers such as fish. In recent years, it has become increasingly evident that bacteria figure prominently in the nutrition of many protistan and metazoan consumers (discussed below) and that many eucaryotic consumers contribute significantly to nutrient regenerative fluxes in marine and freshwater ecosystems (see *Metazoan Agents of NH_4^+ Regeneration*, this chapter); that is, organisms active in NH_4^+ regeneration are often important components of planktonic food webs, and factors affecting the transfer of energy and organic materials among these components can influence both the magnitude of water-column regenerative fluxes and the efficiency of the limnetic nitrogen cycle. It is therefore difficult to discuss the organisms responsible for NH_4^+ regeneration in a nonsuperficial manner without first considering the trophic dynamics of planktonic communities.

Recall from the previous subsection that phytoplankton may exude large quantities of dissolved organic matter (DOM) and largely support bacterioplankton secondary production in many aquatic ecosystems (see Pomeroy 1974, 1984; Fogg 1977; Aaronson 1978; Williams 1981, 1984; cf., Sverdrup et al. 1942, pg 885). Traditionally, DOM availability has been deemed the principal determinant of bacterioplankton secondary

production; however, recent studies suggest that bacterivory may be a far more important regulator of bacterioplankton biomass and productivity in many aquatic ecosystems (e.g., Scavia and Laird 1987). Moreover, bacterial growth rates and grazing losses may be tightly coupled, implying that biomass produced by bacteria is passed rapidly to higher trophic levels (Scavia *et al.* 1986b). Azam *et al.* (1983) considered heterotrophic nanoflagellates to be the principal bacterivores in pelagic waters and coined the term "microbial loop" to designate the transfer of energy and nutrients contained in algal exudates through bacterioplankton and nanoflagellates to higher consumers (*i.e.*, to the conventional planktonic food chain; see also Turner 1984; Fenchel 1986; Rassoulzadegan and Sheldon 1986; Sheldon *et al.* 1986; Sherr *et al.* 1986a,b; Stockner and Antia 1986). Recent evidence also points to the potential importance of chlorophyll-containing mixotrophic flagellates (Estep *et al.* 1986; Bird and Kalff 1987) and crustacean zooplankton (Porter *et al.* 1983; Knoechel and Holtby 1986) as bacterivores in both marine and freshwater environments. Furthermore, many mixotrophic and heterotrophic protists undoubtedly compete directly with heterotrophic bacteria for algal organic exudates, as suggested by Hentschel (1936) over half a century ago and subsequently confirmed by Vincent and Goldman (1980) and others.

The assimilation of large quantities of nitrogen-containing organic material by heterotrophic bacteria seemingly would favor their role as agents of NH_4^+ regeneration in natural waters. However, Goldman *et al.* (1987) have shown that the efficiency with which bacteria regenerate NH_4^+ is influenced strongly by the nitrogen content of available organic substrates. In laboratory experiments involving marine bacterioplankton

assemblages maintained in an exponential state of growth, regeneration efficiencies varied from approximately zero percent when substrate carbon:nitrogen ratios were 10:1 up to 86% when carbon:nitrogen ratios equaled 1.5:1. Noting that carbohydrates were frequently the major carbon sources available to bacterioplankton, Goldman *et al.* concluded that substrate carbon:nitrogen ratios often exceeded 10:1 in nature and that actively growing bacteria were inefficient remineralizers of nitrogen in many aquatic ecosystems (*cf.*, Johannes 1964, 1965; Fenchel and Blackburn 1979; Mann 1982; Billen 1984). This contention was supported by the results of metabolic inhibitor experiments performed by Wheeler and Kirchman (1986) in which NH_4^+ regeneration rates in marine waters were correlated with eucaryotic rather than procaryotic activity.

Given that microbial biota less than 20-35 μm in size purportedly account for the bulk of the nitrogen remineralization activity in many marine and freshwater ecosystems (*e.g.*, Glibert 1982; Harrison *et al.* 1983; Park *et al.* 1986) and that bacterioplankton contribute little to this activity in many instances (discussed above), micro- and nanoplanktonic protistans appear to be major participants in aquatic NH_4^+ regeneration and nitrogen cycling. This view has been corroborated experimentally by Goldman and Caron (1985), who found that rates of NH_4^+ regeneration were negligible in nitrogen-limited laboratory cultures containing only phytoplankton, only bacteria, or both phytoplankton and bacteria, whereas the addition of the phagotrophic marine flagellate *Paraphysomonas imperforata* (Lucas) stimulated rapid regeneration of NH_4^+ in all cultures. The widespread presence of similar bacterivorous, planktonic flagellates in freshwater ecosystems has been acknowledged

only recently (e.g., Kalff and Watson 1986; Bird and Kalff 1987; Dokulil 1988; Rott 1988; Salonen and Jokinen 1988). Kalff and Watson (1986) noted that phagotrophic chrysomonads achieved greatest abundance in oligotrophic lakes and argued that the impact of bacterivory on energy flow and nutrient cycling was greatest in such ecosystems. Bird and Kalff (1987) found that 30% of the "phytoplankton cells" (i.e., the mixotrophic "algal" or protistan component of the autotrophic community) in an oligotrophic Canadian Shield lake engaged in phagotrophy. These organisms, rather than the less numerous metazoan zooplankton, were reportedly the major planktonic bacterivores in the lake.

Results from the metabolic inhibitor experiments performed in this study suggested that procaryotes made little direct contribution to NH_4^+ regeneration rates in Snowbank Lake (Figure 18). The absence of any detectable regeneration activity in the $<1\text{-}\mu\text{m}$ size fraction likewise suggested that bacterioplankton were not important sources of NH_4^+ in this ecosystem. Instead, NH_4^+ regeneration by the microbial ($<63\text{-}\mu\text{m}$) community appeared to be associated primarily with eucaryotic biota. Several taxa of mixotrophic and heterotrophic protistans were present in Snowbank Lake during the 1986-87 ice-free seasons and likely were responsible for much of the measured regeneration activity (Table 25; cf., Ishida and Kimura 1986; Bird and Kalff 1987). Phagotrophic forms included the heterotrophic chrysomonads *Chromulina pascheri* and *Chrysococcus* sp., the mixotrophic chrysomonads *Dinobryon sertularia* and *Ochromonas* sp. and the mixotrophic cryptomonad *Cryptomonas* sp. Previous studies have shown that at least some of the mixotrophic forms depend heavily on bacterivory as an energetic supplement to photosynthesis. For example, Bird and Kalff

Table 25. Flagellates potentially active in NH_4^+ regeneration in Snowbank Lake.

<i>Chromulina pascheri</i> Hofeneeder	<i>Mallomonas</i> sp. #3
<i>Chrysococcus</i> sp.	<i>Ochromonas</i> (<i>Chlorochromonas</i>) sp.
<i>Cryptomonas</i> sp.	<i>Rhizochrysis limnetica</i> G.M. Smith
<i>Dinobryon sertularia</i> Ehrenberg	<i>Rhodomonas lacustris</i> Pascher et Ruttner
<i>Mallomonas</i> sp. #1	<i>Synura uvella</i> Ehrenberg
<i>Mallomonas</i> sp. #2	<i>Synura</i> sp. #2

(1987) reported that phagocytotic consumption of bacterioplankton by *Dinobryon* sp. proceeded at a similar rate both day and night and accounted for at least 50% of the carbon assimilated by this organism. In a study of flagellate/bacterioplankton grazing interactions in a dystrophic Finnish lake, Salonen and Jokinen (1988) reported that *Ochromonas* sp. and *Chromulina* spp. ingested the equivalent of 75-203% of their body carbon mass per day by bacterivory. Interestingly, Aaronson and Baker (1959) found that *Ochromonas*, a genus capable of phototrophy, heterotrophy and phagotrophy, was most actively phagotrophic under conditions of nutrient deficiency, suggesting that the need to procure nitrogen and phosphorus (in addition to energy) provided a major impetus for algal bacterivory.

The degree to which nonphagotrophic, mixotrophic flagellates (e.g., *Rhizochrysis*, *Rhodomonas*, *Synura*) rely on algal exudates in oligotrophic aquatic environments is not well known. Although competition with bacterioplankton for organic substrates is undoubtedly intense,

heterotrophy probably plays some role in the nutrition of these organisms (see Vincent and Goldman 1980). The presence of apochlorotic nonphagotrophic protozoans such as *Mallomonas* in Snowbank Lake lends credence to the claim that some eucaryotic heterotrophs can compete successfully with bacteria for nitrogen containing exudates. It is likely that a fraction of the NH_4^+ produced via microbial metabolism in Snowbank Lake is generated by these nonbacterivorous forms.

Metazoan Agents of NH_4^+ Regeneration

Metazoan zooplankton constitute major sources of regenerated NH_4^+ in many marine and inland waters (Harris 1959; Bidigare et al. 1982; Harrison et al. 1983; Jellison and Melack 1986; Park et al. 1986; King et al. 1987). Moreover, small quantities of free amino acids and other organic compounds are released by actively growing zooplankton (Gardner and Miller 1981) and conceivably play a role in the nutrition of the microbial heterotrophic community. Zooplankton also contribute indirectly to aquatic nutrient supply and demand through the regulation of prey abundance, community composition and nutrient cycling activity. Many filter-feeding forms (e.g., *Daphnia*, *Oikopleura*) consume a diverse array of organisms, including algae, bacteria, protozoans, and small metazoans, whereas predation by larger forms (e.g., *Chaoborus*, *Sagitta*) is often highly selective (see Sverdrup et al. 1942; Porter 1973, 1983; Williams 1980; Alldredge 1984; Burns and Gilbert 1986a,b; Knoechel and Holtby 1986; Turner et al. 1988). Prey populations may be maintained at relatively low levels during periods of intense grazing activity, thereby reducing interspecific competition for nutrient resources and

precluding the competitive exclusion of less vigorous prey species (Porter 1973; McCauley and Briand 1979). Alternatively, selective feeding by zooplankton may lead to the extirpation of the more vulnerable prey species and to a concomitant increase in the abundance of less edible and/or more elusive forms (Brooks and Dodson 1965; Hall *et al.* 1976; and many others). Such influences on prey abundance and community composition undoubtedly contribute to the overall pattern of nutrient exchange and energy flow in aquatic ecosystems.

Size-fractionation experiments performed in the present study demonstrated that zooplankton larger than 63 μm imposed strong constraints on microbial remineralization activity in Snowbank Lake (figures 19 and 22). These results were consistent with previous research which has shown that both nano- and microprotozooplankton may be consumed by metazooplankton (Porter *et al.* 1985; Turner *et al.* 1988) and that protozoan abundance and remineralization activity in lakes may be regulated by predation rather than by resource availability (see Hamilton and Taylor 1987). More importantly, *total* community regeneration consistently increased following the elimination of the larger fauna (Figure 22), suggesting that a decline in metazooplankton abundance in Snowbank Lake would effectively enhance NH_4^+ production, at least over the short term. These results supported Henry's (1985) contention that:

"The introduction of a size selective planktivore to a lake may, in addition to influencing zooplankton community structure, have a large scale ecosystem impact mediated through alterations in nutrient dynamics. (Specifically) . . . higher trophic level interactions, such as size selective predation, may have a significant impact upon nutrient regeneration rates and, hence, primary production."

Because nitrogen:phosphorus regenerative ratios are grazer specific (Ikeda 1977), zooplankton community composition may influence not only the rate of nutrient cycling, but also the form of nutrient limitation imposed on phytoplankton growth (see Lehman 1980, 1984; Bartell 1981; Stenson 1982). Transitions between nitrogen and phosphorus limitation in lake mesocosm experiments sometimes accompany major changes in zooplankton community structure (Elser *et al.* 1988). Similar transitions have resulted from whole-lake experimental manipulations of fish populations or, more precisely, from the attendant effects of these manipulations on zooplankton communities (Shapiro and Wright 1984; Carpenter *et al.* 1987; Carpenter and Kitchell 1988). It is possible that the initial stocking of Snowbank Lake and other formerly barren alpine lakes in the Beartooth Mountains (Chapter 2) resulted in the elimination of key macrozooplankton taxa (especially large and darkly pigmented species such as *Daphnia middendorffiana* Fisher; *cf.*, Brooks and Dodson 1965; Walters and Vincent 1973), thereby affecting microbial regeneration activities and altering nutrient constraints on algal growth. However, the advectively dominated character of Snowbank Lake and the large discrepancy between assimilatable phosphorus and nitrogen availability in inflowing waters (relative to the Redfield ratio; see *Evidence for Nitrogen Limitation of Water-Column Primary Production*, this chapter) may have prevented any shift in the prevailing form of nutrient limitation. (The recent discontinuation of restocking efforts in some wilderness lakes of the Beartooth Mountains presumably will result in the gradual disappearance of nonviable fish populations; these losses could provide an opportunity to monitor, on a natural scale, the changes in

aquatic nutrient cycling and primary production which accompany the reestablishment of larger zooplankton species; see Marcuson 1980a-g.)

Contrary to the impression given above, regulatory interactions among fish and metazoan zooplankton are not strictly unilateral. Rather, the composition of the macrozooplankton community, though largely shaped by fish predation, may determine the capacity of the aquatic ecosystem to support fish and higher trophic components. Consider the macrozooplankton communities of two hypothetical freshwater ecosystems, the first dominated by *Daphnia* and the second dominated by *Diaptomus*. In the first ecosystem, daphnids consume phytoplankton, protozoans and bacterioplankton and are themselves directly consumed by fish; hence, the transfer of energy and nutrients from microbial biota to higher trophic components is relatively direct. *Diaptomus* is much less efficient in its utilization of bacteria and smaller (pico- and nano-sized) algae and protistans; in the second ecosystem, therefore, the transfer of energy and nutrients contained in algal organic exudates (which may represent >50% of the algal primary production) proceeds from bacteria through nanoflagellates, microflagellates, ciliates and rotifers to *Diaptomus* and, finally, to fish. Because no trophic transfer is completely efficient (i.e., nutrients are lost via respiration, remineralization, defecation and sedimentation, and energy is lost as heat along each step of the trophic pathway) the second ecosystem would be expected to support less fish production than the first. Interestingly, the importance ascribed to *Daphnia* in this hypothetical example is justified on the basis of recent research. Riemann (1985) reported that intense zooplanktivory markedly reduced populations of large-bodied *Daphnia* and

significantly decreased carbon flow from bacteria to fish in large-scale enclosure manipulations. Stockner and Shortreed (1985) found that carbon flow in ultraoligotrophic coastal lakes of British Columbia proceeded from picoplankton through protozoa and rotifers to copepods and characteristically resulted in small-bodied fish populations and low fish production. Contrastingly, the presence of *Daphnia* in oligotrophic interior lakes of British Columbia was associated with increased carbon flow, larger fish and higher fish production.

Daphnia generally contribute little to fish production in shallow alpine lakes and ponds. Instead, more plentiful food resources (e.g., insects, oligochaetes) often support fish populations capable of rapidly eliminating these and other macroplankton (Walters and Vincent 1973). Zooplankton are more important prey for fish in larger, deeper alpine lakes, wherein access to benthic organisms is limited and the availability of emergent aquatic insects is sporadic (see Wells 1986). Within such lakes, the tendency for macrozooplankton to migrate to deeper, more dimly lit waters during daylight probably confers some degree of protection from fish and other visual predators; in some instances, this behavior may preclude the extirpation of the larger or more darkly pigmented zooplankton species (reviewed by Wetzel 1983). In Snowbank Lake, the relatively large cladoceran, *D. pulex*, appears restricted in distribution to deeper waters (i.e., collected only in 10-m vertical tows), whereas the smaller species, *D. schodleri* and *D. rosea*, are more uniformly distributed throughout the water column. (The large-bodied, darkly pigmented form, *D. middendorffiana*, though abundant in barren alpine lakes of the region, apparently is not present in Snowbank Lake.)

Although these observations are suggestive of responses by large-bodied *Daphnia* to fish predation, the extent to which the resident population of *S. fontinalis* is dependent on these organisms, and on the microbial nutrients and energy channeled through them, is uncertain. Studies conducted in nearby lakes by Wells (1986) suggest that the copepod *D. shoshone*, which is relatively abundant in Snowbank Lake, comprises an important, perhaps dominant component of the trout diet in the latter portion of the open water season. Thus, it is unlikely that daphnids represent the only significant link between planktonic microorganisms and fish in Snowbank Lake.

Assuming for a moment that daphnids are primarily responsible for the regulation of microbial prey densities and community metabolism in Snowbank Lake, and that they provide the principal trophic link between the microbial and fish communities, then the appellation of "keystone" genus previously given to *Daphnia* by Porter et al. (1988) seems appropriate to this ecosystem. However, another consideration is involved: the efficient transfer of microbial nutrients through *Daphnia* to higher trophic components would require that nutrient regenerative fluxes be correspondingly low; conversely, less direct and highly inefficient trophic pathways would tend to maximize nutrient remineralization activity. From this perspective, a macrozooplankton community dominated by *Daphnia* theoretically would encourage the efficient channeling of microbial nutrients to fish and higher trophic components, whereas a community dominated by *Diaptomus* would promote the regeneration of NH_4^+ , PO_4^{3-} and other assimilatable compounds and favor the growth of nutrient-limited phytoplankton. Future comparisons of remineralization activity

between *Daphnia*- and *Diaptomus*-dominated communities could provide considerable insight into the regulation of nutrient cycling rates in freshwater ecosystems.

Thus far, no attempt has been made to directly compare nitrogen regeneration rates between the metazoan ($>63\text{-}\mu\text{m}$) and microbial ($\leq 63\text{-}\mu\text{m}$) components of the Snowbank Lake plankton community. The reason for this becomes apparent when one considers the potential stimulatory effect of size fractionation on microbial remineralization activity. For example, the time-course data depicted in Figure 19 show that NH_4^+ regeneration by the isolated ($<63\text{-}\mu\text{m}$) size fraction increased markedly over the course of a 6.2-hr field incubation, whereas regeneration associated with the "intact" plankton community decreased rapidly towards the end of the experiment. The interpretation of such data is difficult. While it is tempting to assume that calculations based on the initial time interval are most representative of *in situ* metabolic activity, this assumption ignores the large potential for handling artifacts discussed previously. Comparisons based on later time intervals are probably less subject to such artifacts; however, the biological and chemical representativeness of lake samples progressively diminishes with time, and such changes in experimental conditions almost certainly are accompanied by variations in sample community metabolism (cf., Ikeda 1977; Caron et al. 1986). Ultimately, time-series measurements such as those illustrated in Figure 19 may provide insight into the changes in regeneration activity which occur during ^{15}N -isotope experiments, but their usefulness in quantitative comparisons of NH_4^+ regeneration among size fractions is questionable.

Comparisons of NH_4^+ regeneration activity among size fractions are even more prone to misinterpretation when based on only one time interval. For example, had the experiment considered in Figure 19 involved two sets of measurements, one at the beginning and one at the end of the incubation period, perceived differences in remineralization activity between size fractions would have depended entirely on the duration of the experiment (i.e., briefer incubations (<1 hr) would suggest that microbial organisms contribute negligibly and metazoans overwhelmingly to NH_4^+ production; longer incubations (>6 hr) would suggest the converse). Similar ambiguities undoubtedly have affected the results of previous research. A case in point is the study by Glibert (1982), in which size-fractionated, single-interval measurements of NH_4^+ regeneration were conducted repeatedly over a one year period in Vineyard Sound, Massachusetts. Glibert concluded that "the smallest size fraction was usually the most important in remineralizing NH_4^+ ." On closer inspection of her data (specifically, Figure 10A-C, pg 217), however, it is evident that NH_4^+ regeneration by the smaller size fractions (<35 μm and <101 μm) often greatly exceeded that of the largest size fraction (<202 μm). These results are similar to those of the present study (Figure 22) and suggest that the elimination of grazing constraints on microbial community metabolism may have stimulated atypically high rates of nutrient regeneration.

Recent laboratory and shipboard studies of nitrogen remineralization based on rapid spectrophotometric assays of NAD^+ -linked glutamate dehydrogenase activity (see *Influence of pH on NH_4^+ Regeneration: Physiological Implications*, this chapter) suggest that nano-, micro- and

macrozooplankton are each important sources of regenerated NH_4^+ in coastal and open oceanic waters (Park *et al.* 1986; King *et al.* 1987). Similarly, Goldman and Caron (1985) have found that the NH_4^+ regeneration efficiencies of heterotrophic flagellates account for only a fraction of the overall remineralization efficiency in pelagic ecosystems. They conclude that "to achieve regeneration efficiencies in pelagic surface waters of 80-90%, as is generally believed to occur, requires that the microbial food web be exceedingly complex with a hierarchy of at least several grazing steps." Based on the results of the present study, it would appear that nutrient remineralization processes in oligotrophic alpine lakes likewise involve a diverse and interacting array of heterotrophic forms.

Partitioning of Nitrogenous Resources among Phytoplankton

The proportion of phytoplankton species exhibiting nitrogen-limited growth in any aquatic ecosystem would be expected to increase as the availability of assimilatable nitrogen declined in relation to the availability of other potentially limiting growth factors (see Table 25). Competition for regenerated NH_4^+ and other nitrogenous compounds presumably would intensify under such circumstances. On theoretical grounds, the persistence or perenniality of multiple nitrogen-limited populations would imply that the ecosystem in question was maintained in a state of competitive disequilibrium, wherein fluctuating environmental conditions precluded or greatly retarded the competitive exclusion process (see Levin 1970; Harris 1986). Alternatively, each phytoplankton population might obtain nitrogen from its own unique source, thereby

ameliorating resource competition within the algal community and permitting the long-term coexistence of numerous nitrogen-limited taxa. Several forms of nitrogen resource partitioning conceivably occur in aquatic ecosystems and could provide an important basis for algal niche differentiation. In the discussion which follows, *chemical partitioning* refers to the monopolization of a given nutrient species by a particular subset of the phytoplankton community; *temporal partitioning* entails the noncontemporaneous sharing (e.g., diurnal versus nocturnal utilization) of a given nutrient compound between two or more algal populations; *spatial partitioning* designates nutrient uptake within a particular region of the water column, accessible only to a select group of phytoplankton; and *kinetic partitioning* refers to the effective utilization of a given nutrient compound by a given algal taxon only under specific conditions of nutrient patchiness or within a relatively restricted nutrient concentration range, outside of which other algal taxa would be the principal consumers of the compound. It is argued that niche differentiation made possible by the simultaneous chemical, temporal, spatial and kinetic partitioning of the dissolved nutrient pool provides a plausible solution to the "paradox" presented by high phytoplankton diversity in seemingly isotropic limnetic environments (see Hutchinson 1961).

That many of the phytoplankton taxa present in Snowbank Lake possess unusual capabilities for nitrogen procurement suggests that resource partitioning may be an important determinant of algal diversity in this ecosystem. Regarding chemical partitioning, several species of mixotrophic flagellates present in the lake are capable of exploiting the

detrital nitrogen pool to meet, in whole or in part, their nitrogen nutritional requirements (Table 26). Similarly, the N_2 -fixation capability of the heterocystous cyanophyte, *Rivularia* sp., may be crucial to its survival in this nitrogen-deficient ecosystem (cf., Smith 1983). Whereas some species of *Peridinium* and *Chroococcus* are known to depend nearly exclusively on NO_3^- -N under certain environmental conditions (Berman et al. 1984; Pollinger et al. 1988; cf., Proctor 1957; Stross 1963), some species of *Chlorella*, *Chlamydomonas* and *Chrysochromulina* are essentially incapable of NO_3^- uptake in the presence of even low concentrations of NH_4^+ (Losada et al. 1970; Herrera et al. 1972; Wehr et al. 1987). Recent studies suggest that nano- and pico-sized (mostly procaryotic) plankton routinely discriminate against NO_3^- in favor of NH_4^+ , whereas larger marine phytoplankton ($>20 \mu m$) tend to depend on NO_3^- as their principal nitrogenous resource (Malone 1980; Nalewajko and Garside 1983; Probyn 1985; Probyn and Painting 1985; Koike et al. 1986; Wheeler and Kirchman 1986; Harrison and Wood 1988). The applicability of this generalization to freshwater ecosystems has not been demonstrated conclusively; however, it is noteworthy that, in the present study, NH_4^+ uptake by small, procaryotic plankton appeared disproportionately large in relation to procaryotic biomass (cf., figures 10 and 18).

That some phytoplankton can take up inorganic nutrients at rates exceeding those required for steady-state growth and store them internally for later use (Goldman and Glibert 1982; Prisco 1987) suggests that at least some degree of temporal nutrient partitioning is possible in lacustrine and oceanic ecosystems (i.e., periods of active nutrient uptake theoretically may vary among phytoplankton populations). The

extent to which nitrogen is partitioned in this manner has received little scientific attention. However, diel periodicities in NH_4^+ and NO_3^- uptake observed in many lakes (see *Coupling of NH_4^+ Assimilative and Regenerative Fluxes*, this chapter) suggest that the ability to sequester inorganic nitrogen at maximal rates during periods of darkness may be comparatively rare among algae (see Raven 1980, pgs 69, 141) and may be an important determinant of phytoplankton community composition under conditions of nitrogen limitation. Seasonal or daily variations in water-column stability, temperature, turbidity or chemistry (e.g., pH; see *Influence of pH on Nitrogen Uptake: A Preliminary Biochemical Model*, this chapter) may impose constraints on inorganic nitrogen utilization by some algae and may further partition the nitrogen resource. Although these abiotic factors contribute markedly to periodicities in N_2 fixation by heterocystous cyanophytes (Carpenter and Price 1976; Howarth *et al.* 1988; Paerl 1988), their effect on inorganic nitrogen uptake, generally, is poorly understood.

Many motile algal species engage in diel vertical migrations of several meters, positioning themselves at depths of optimal nutrient concentration and/or light intensity. Maximum migrational amplitudes reported for freshwater algae include 18 m for *Volvox* sp., Cahora Bassa Reservoir (Sommer and Gliwicz 1986), 10 m for *Peridinium cinctum*, Lake Kinneret (Berman and Rodhe 1971), 5-7.5 m for *Cryptomonas ovata*, *Gymnodinium uberrimum* and *Mallomonas* sp., Finstertaler See (Tilzer 1973), 5 m for *Ceratium hirundinella*, Esthwaite Water (Talling 1971; Frempong 1984) and 5 m for *Peridinium penardii*, Lake Beryessa (Sibley *et al.* 1974). Such migrational capabilities suggest that the nutrients

occurring in deeper (6-11 m) regions in Snowbank lake are readily accessible to the ecosystem's flagellated algal community. Periodic excursions by *Peridinium cinctum* into these waters (which occasionally exhibit somewhat higher nutrient concentrations than do shallower waters; see Figure 6) may explain the seasonal dominance of this dinoflagellate (Figure 10) despite the low specific uptake rates for both NH_4^+ and NO_3^- previously ascribed to the species (see Sherr et al. 1982; Berman et al. 1984). Similarly, the ability of *Volvox tertius* to access regions of elevated nutrient concentrations within the water column may account for the presence of this purportedly poor nitrogen competitor (Smith 1950) in Snowbank Lake. The migrational capabilities of many of the smaller flagellated algae in Snowbank Lake (e.g., *Rhodomonas*, *Cryptomonas*, *Mallomonas*, *Chromulina*) likewise may contribute to the survival of these organisms by permitting them periodic access to the nutrients contained in deeper waters and surficial sediments (cf., Tilzer 1973; Sommer 1982; Dokulil 1988).

In the past decade, several researchers have argued that the small-scale spatial and temporal distribution (i.e., patchiness) of regenerated nutrients potentially influences the outcome of competitive interactions among algae. Turpin and Harrison (1980) were among the first to report that species succession in nitrogen-limited algal cultures could be manipulated via the frequency of NH_4^+ addition (continuous versus periodic). Moreover, these researchers demonstrated that the frequency of nutrient addition also could affect the size distribution of algal communities, with less frequent pulses of high concentration selecting for larger cells (cf., Scavia et al. 1984; Suttle et al. 1988).

Turpin *et al.* (1981) developed a theoretical model for exploring the effects of limiting nutrient patchiness on phytoplankton growth and demonstrated, via computer simulation, that "the degree of patchiness in the environment can affect individual growth rates and thus alter community structure even though there is no change in the average ambient nutrient concentration." Sommer (1985) conducted a comprehensive laboratory investigation of nutrient competition among Lake Constance phytoplankton and concluded that algae could be grouped into three categories based on their utilization of nutrient resources: (1) "affinity specialists" were deemed capable of effectively utilizing low background concentrations of limiting nutrients; (2) "velocity specialists" rapidly exploited nutrient pulses and immediately utilized the acquired nutrients for growth; (3) "storage specialists" also capitalized on nutrient pulses but stored nutrients intercellularly for later use (*i.e.*, luxury consumption). By subjecting phytoplankton assemblages to fluctuating nutrient environments, Sommer found that affinity specialists (which had dominated in chemostat cultures) gradually were eliminated, that storage specialist populations increased asymptotically, and that velocity specialist populations initially increased, then oscillated about a stable mean. Although Sommer and the other researchers were concerned primarily with the influence of nutrient patchiness on phytoplankton competitive interactions, *per se*, their research has direct relevance to the present discussion. Specifically, the marked interspecific differences in nutrient uptake and storage capabilities alluded to by these workers, coupled with the general availability of regenerated nutrient patches of varying concentration, size and longevity, could

permit an intricate partitioning of the regenerated nutrient pool in many lacustrine and oceanic ecosystems. The kinetic partitioning of regenerated NH_4^+ among affinity, velocity and storage specialists, for example, could contribute significantly to phytoplankton diversity and to the complexity of nutrient flow in Snowbank Lake and other nitrogen-deficient surface waters.

In theory, the partitioning of a limiting nutrient resource in the manner described above would permit the attainment of competitive equilibrium among multiple phytoplankton species. Although "resource partitioning" has been invoked by previous researchers as an equilibrium-based solution to Hutchinson's (1961) paradox, the term generally has referred to simultaneous, species-specific restrictions on growth imposed by the availability of an array of different nutrients; i.e., situations in which "several nutrients (are) in relatively short supply and . . . the growth of each species (is) restricted by a single nutrient or a unique combination of several nutrients" (Petersen 1975). This concept is compatible with the extended Volterra-Gause principle advocated by Levin (1970): "No stable equilibrium can be attained in an ecological community in which some r components are limited by less than r limiting factors." However, Stewart and Levin (1973) demonstrated that it is analytically possible to obtain stable states of coexistence for two species competing for a single limiting nutrient, provided one species grows faster at higher concentrations and the other at lower concentrations of the nutrient. They also reported (but did not demonstrate) that stable equilibria are possible for three or more species competing for the same limiting nutrient, provided the growth of each species is

avored within a unique range of nutrient concentrations. Stewart and Levin cautioned that

"... as one adds species, the range of parameter values which allow for stable equilibria appears to become narrower and narrower. Consequently, we do not believe that such a mechanism by itself could explain the coexistence of large numbers of species on few different limiting resources."

Subsequent to the work of Stewart and Levin, it has been demonstrated that nutrient remineralization by heterotrophic plankton imparts a microscale heterogeneity to the dissolved nutrient pool in open water environments (discussed previously). At any moment in time, microscale nutrient concentrations range from extremely high levels in newly formed nutrient patches to near-background levels in older patches subjected to turbulent dispersal or biological depletion. Phytoplankton with high V_{max} values for a limiting nutrient presumably encounter nutrient patches of appropriately high concentration at comparatively infrequent intervals, whereas those possessing high affinity for the nutrient consume it more or less continuously from the background pool. Depending upon several factors, including the frequency and magnitude of nutrient excretion events, the population density and metabolic activity of the various algal species, and the prevailing level of turbulence, the kinetic partitioning of the limiting nutrient among velocity, storage and affinity specialists could preclude or greatly retard the competitive exclusion process. That is, the range of nutrient concentrations encountered by a given phytoplankter could include as a subset the narrow "range of parameter values" physiologically best suited to the organism's survival (cf., Stewart and Levin 1973). The concomitant temporal, spatial and chemical partitioning of the dissolved nutrient pool could

contribute further to niche differentiation and to the alleviation of resource competition among phytoplankton.

The extent to which competitive equilibrium is approached in aquatic ecosystems has received much attention from both theoretical and empirical ecologists. Whereas some contemporary researchers continue to describe algal competitive interactions in purely equilibrium-dependent terms (e.g., Kilham and Hecky 1988), others emphasize the dynamic nature of these interactions and contend that fluctuating environmental conditions greatly "perturb the approach to competitive exclusion and equilibrium" (Harris 1983). The debate concerning the prevalence of equilibrium versus nonequilibrium conditions in natural phytoplankton communities has been carried on by many researchers over a period of many years (see Hutchinson 1941, 1953, 1961, 1965; Levin 1970; Richerson *et al.* 1970; Stewart and Levin 1973; Gilpin 1975; Margalef 1978; Reynolds 1980; Wall and Briand 1980; Harris 1983, 1986; Kilham and Hecky 1988; *cf.*, Connell 1978; Huston 1979) and its definitive resolution is beyond the scope of this report. Rather, this subsection has attempted to demonstrate that the partitioning of the limiting nutrient resource may be an undervalued mechanism whereby phytoplankton diversity is maintained in some oligotrophic freshwaters. In particular, resource partitioning may be largely responsible for the perennial reemergence of a diverse, nitrogen-limited phytoplankton assemblage in Snowbank Lake.

Implications of Regenerated NH_4^+
Uptake by Bacterioplankton

Bacterial secondary production in natural waters often approaches or even exceeds algal primary production due to organic carbon cycling

within the heterotrophic food web (Scavia *et al.* 1986b; Scavia and Laird 1987; Scavia 1988; Strayer 1988), suggesting that bacterioplankton at times impose heavy demands on available nitrogen resources. Bacteria have long been known to possess efficient membrane transport systems for both inorganic and organic nitrogen substrates (reviewed by Payne 1980); however, only organic compounds traditionally have been thought to contribute significantly to bacterioplankton nitrogen requirements (e.g., Dugdale and Goering 1967). Recent studies of bacterial nutrition in coastal and open oceanic waters have demonstrated that the production of assimilatable organic compounds frequently cannot keep pace with bacterial demand for nitrogen (Wheeler and Kirchman 1986) and that NH_4^+ may supply the bulk of the bacterial nitrogen requirement under such circumstances (see Eppley *et al.* 1977; Billen 1984; Hagstrom *et al.* 1984; Laws *et al.* 1985; Probyn and Painting 1985; Kirchman *et al.* 1986; interestingly, neither NO_3^- nor urea appear to be utilized appreciably by most marine bacteria; see Wheeler and Kirchman 1986). Although few similar studies have been conducted in freshwater ecosystems, results from the metabolic inhibitor experiments performed in the present investigation (Figure 18) suggest that NH_4^+ is utilized heavily by bacteria in at least some nitrogen-deficient lakes.

The physiology of NH_4^+ uptake by heterotrophic bacterioplankton has received comparatively little scientific attention. In contrast, much is known about the physiology of bacterial DOM uptake, and some of this information may be of general application to NH_4^+ and other inorganic nutrients. Azam and Ammerman (1984) have theorized that bacteria take up organic substrates in close physical proximity to the sources of the

substrates (e.g., algal cells, detritus). They also contend that a close temporal and metabolic coupling exists between DOM production and consumption processes and that this coupling makes possible the high rates of bacterial secondary production frequently noted in oligotrophic marine environments. Although their argument is analogous to that used by McCarthy and Goldman (1979) to account for high phytoplankton productivity in nitrogen-depleted oceanic waters (see *Coupling of NH_4^+ Regenerative and Assimilative Fluxes*, this chapter), it appears that bacteria are more flexible than algae in their metabolic responses to fluctuating nutrient concentrations. For example, the uptake of amino acids and simple sugars by marine bacterial assemblages is often multiphasic, in that each substrate exhibits multiple values for K_s when uptake is measured over a very wide range of substrate concentrations (see Azam and Hodson 1981; Hagstrom et al. 1984). Similarly, Nissen et al. (1984) have reported that the glucose transport system in the marine bacterial isolate LNB-155 changes its K_m from 10^{-8} to 10^{-3} M at a critical glucose concentration. They suggest that such a response is advantageous to any bacterium living in a heterogeneous nutrient environment, wherein microscale DOM concentrations might easily vary by several orders of magnitude. Whether bacterioplankton possess similar multiphasic transport systems for NH_4^+ and other inorganic nutrients is not known; however, there is little a priori reason to assume they do not. Such metabolic flexibility would allow bacteria living in nitrogen-depleted surface waters to capitalize on microzones of regenerated NH_4^+ during periods of intense eucaryotic remineralization activity and to successfully exploit the background NH_4^+ pool at other times.

In addition to the kinetic plasticity of their nutrient transport systems, bacteria are also capable of chemoreception and directed movement. That is, they are capable of actively seeking microenvironments of optimal nutritive content. Their extremely small size and correspondingly high surface-to-volume ratios also place them in intimate metabolic contact with the surrounding medium and allow them to respond rapidly to changing environmental conditions. This point is emphasized by Azam and Ammerman (1984):

"A 0.2 μm diameter bacterium has a cell volume of merely 0.0033 μm^3 or 3.33×10^{-18} l (It) follows that uptake of a very few effector molecules per cell could profoundly affect the metabolic regulation. The intracellular concentration of cAMP (cyclic adenosine monophosphate, an algal exudate believed to be a key regulator of enzyme synthesis in bacteria and a unifying signal for the nutrient status of the microenvironment) is on the order of 1 μM . . . , which for the very small . . . bacteria corresponds to only 1 or 2 molecules per cell."

By virtue of their ability to actively seek nutrient microzones and to rapidly respond to changing nutrient conditions, bacterioplankton are potentially important competitors for NH_4^+ and other regenerated substrates. Water-column concentrations of amino acids and other low molecular weight organic compounds probably are important in determining whether bacteria act as NH_4^+ sources or sinks in nature. In waters lacking appreciable (nanomolar) quantities of these compounds (e.g., certain oligotrophic to ultraoligotrophic lakes), bacteria would be expected to depend heavily on NH_4^+ and to compete strongly with phytoplankton for this regenerated resource. Such competition conceivably could exacerbate conditions of nutrient deficiency and lead to a significant depression in photoautotrophic production. The extent to which bacterial utilization of NH_4^+ contributes to nitrogen-deficient

conditions in Snowbank lake is currently unknown; however, its determination would appear to be experimentally feasible and would offer much insight into the dynamics of nitrogen flow in this ecosystem.

Water-Column Nitrogen Dynamics in Snowbank Lake: A Revised Conceptual Model

The intricacy of the heterotrophic food web, the partitioning of nitrogenous resources among phytoplankton, and the intensive utilization of both inorganic and organic nitrogen by heterotrophic bacteria each imparts an element of complexity to the limnetic nitrogen cycle in Snowbank Lake. In an attempt to incorporate these elements into the previously described model of nitrogen supply and demand (see Figure 33 and related text), the following additional assumptions have been made: (1) a close spatial and temporal coupling exists between the eucaryotic production and the bacterial utilization of NH_4^+ ; (2) some use is made by bacteria of the background NH_4^+ pool; (3) heterotrophic bacteria consume far more NH_4^+ than they produce; (4) NH_4^+ meets a significant fraction of the bacterial nitrogen requirement; (5) NH_4^+ uptake by bacteria rivals in magnitude that by eucaryotic algae; (6) NO_3^- assimilation by water-column bacteria is negligible; (7) inorganic nitrogen resources are intricately partitioned among phytoplankton; (8) metazoan zooplankton and micro- and nanoflagellates are the principal agents of NH_4^+ regeneration; (9) predation by metazooplankton imposes strong constraints on flagellate regeneration activity; and (10) predation imposes constraints on bacterioplankton secondary production and, therefore, on the consumption and regeneration of NH_4^+ by bacteria. Assumptions 1-9 are derived from the preceding discussion whereas (10), above, is consistent with the

emerging microbial loop paradigm considered briefly at the beginning of this section.

Figure 34 depicts major pathways of nitrogen flow and transformation in Snowbank Lake, based on model modifications 1-10, above. (The temporal/spatial partitioning of nitrogenous resources is not illustrated in the figure but is implicit in the revised model.) Note that the bulk of the photoautotrophic demand for nitrogen is met by advective inputs of NO_3^- and by internally recycled NH_4^+ , that the efficiency of the remineralization/uptake cycle is itself dependent on competition- and predation-based interactions within the plankton community, and that higher level trophic dynamics may exert strong, though indirect, influences on algal primary production (and community structure). For example, a decline in the abundance of crustacean zooplankton conceivably could lead to the rapid proliferation of bacterivorous flagellates; subsequent reductions in bacterioplankton density and nutrient consumption presumably would enhance algal (velocity/storage specialist) access to eucaryotically regenerated NH_4^+ and lessen nutrient constraints on photolithotrophic growth. Recall that similar "trophic cascades" have been documented in whole-lake fishery experiments and in mesocosm zooplankton manipulations and probably contribute to the dynamics of most freshwater ecosystems (see Chapter 1 and *Metazoan Agents of NH_4^+ Regeneration*, this chapter; cf., Carpenter *et al.* 1985, 1987; Carpenter and Kitchell 1988).

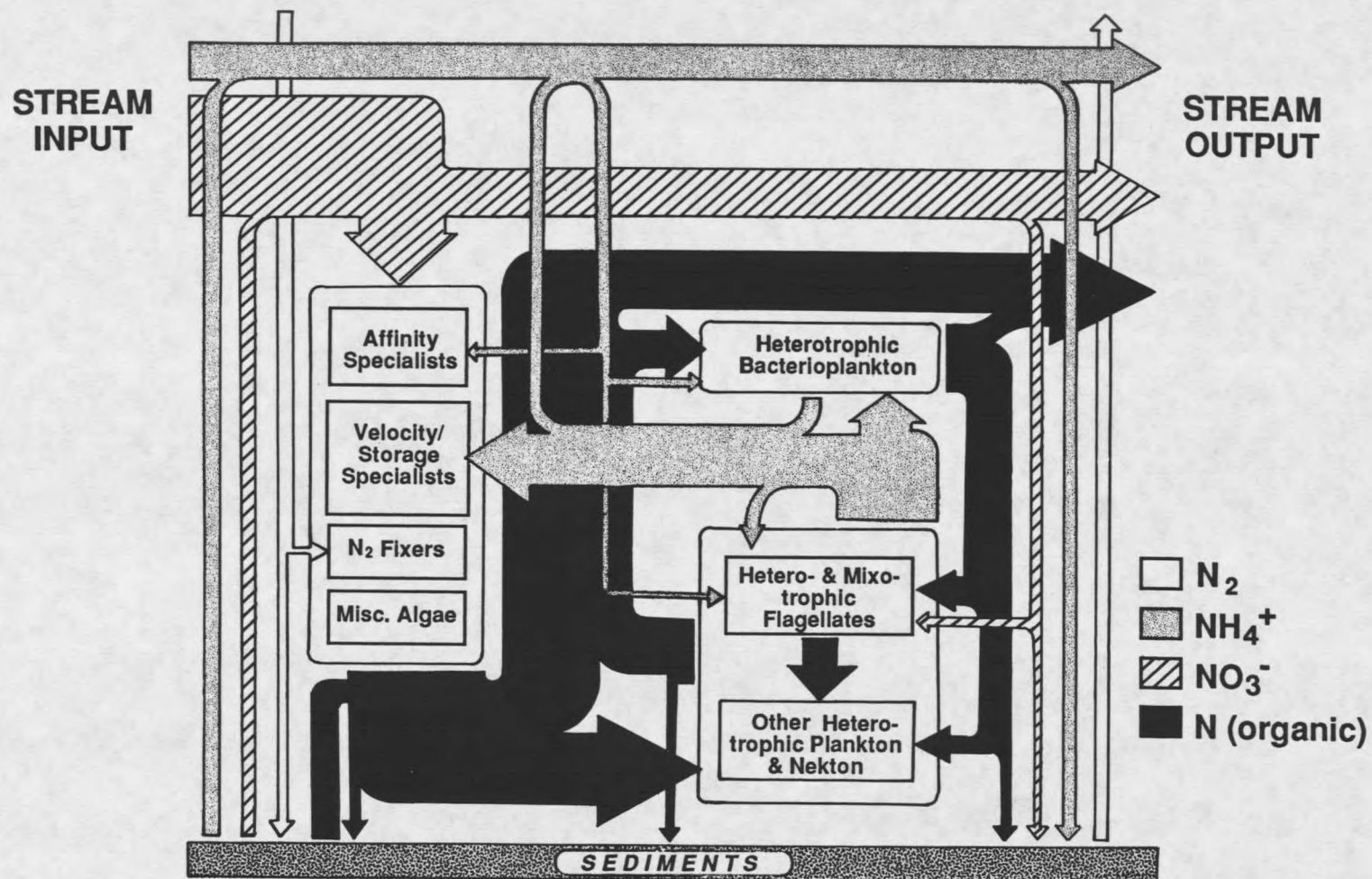


Figure 34. Revised schematic representation of nitrogen supply and demand in Snowbank Lake. See Figure 33 and text for details.

Potential Effects of Lake Acidification on Planktonic Nitrogen Cycling

Results from the present study suggest that nitrogen cycling processes in Snowbank Lake are highly sensitive to changes in ambient pH. Recall from Chapter 4 that regenerative and assimilative fluxes of NH_4^+ in lakewater samples declined rapidly in response to acidification (figures 22 and 23), that the fraction of the biological nitrogen demand supplied by NH_4^+ was much reduced under acidic conditions (Figure 26), and that algal utilization of NO_3^- generally increased following reductions in sample pH, especially when HNO_3 was employed as the acidifying agent (Figure 26). The following discussion considers the physiological and ecological implications of these findings. Particular attention is given to (1) the biochemical basis for the observed effects of acidification on NH_4^+ and NO_3^- uptake, (2) the influence of extracellular $[\text{H}^+]$ on protein synthesis in algae, (3) the biochemical basis for the observed effects of acidification of NH_4^+ regeneration, and (4) the relationship between the biological cycling of inorganic nitrogen and the biogeochemical regulation of aqueous pH. Finally, with insight gained through our discussion of these topics, we consider the potential long-term effects of acidification on patterns of nitrogen supply and demand in Snowbank Lake.

Influence of pH on Inorganic Nitrogen Uptake: A Preliminary Biochemical Model

The uptake of inorganic nitrogen by algae involves both mediated and nonmediated nutrient transport processes. Nonmediated or diffusive fluxes of nitrogenous solutes through the plasmalemma are largely

dependent on the polarity of the solute species and on the intra- and extracellular solute concentrations. For the electroneutral species, NH_3 , diffusive flux is described by the equation

$$J_{\text{NH}_3} = P_{\text{NH}_3}(C_{\text{out}} - C_{\text{in}}) \quad (23)$$

where J_{NH_3} is the transmembrane NH_3 flux ($\text{mol cm}^{-2} \text{ s}^{-1}$), P_{NH_3} is the NH_3 -specific membrane permeability coefficient (cm s^{-1}), and C_{out} and C_{in} are the NH_3 concentrations (mol cm^{-3}) on the two sides of the plasmalemma. The transmembrane free energy difference constitutes the driving force for this flux and is described by

$$\Delta G_{\text{NH}_3} = RT \ln \frac{C_{\text{out}}}{C_{\text{in}}} \quad (24)$$

where ΔG_{NH_3} is the NH_3 -specific, transmembrane free energy difference (kJ mol^{-1}), R is the gas constant ($\text{kJ mol}^{-1} \text{ }^\circ\text{C}^{-1}$) and T is the absolute temperature. For the charged species, NH_4^+ and NO_3^- , the driving force for uptake is given by the equation

$$\Delta G_e = RT \ln \frac{C_{\text{out}}}{C_{\text{in}}} + zF\Delta\psi \quad (25)$$

where ΔG_e is the electrolyte-specific, transmembrane free energy difference (kJ mol^{-1}), z is the charge of the electrolyte (+1 or -1), F is Faraday's equivalent (coulombs mol^{-1}) and $\Delta\psi$ is the transmembrane electrical potential (volts). The relationship between the transplasma-lemma free energy difference and the electrolyte flux (J_e) may be expressed in terms of a permeability coefficient (P_e ; see Raven 1980):

$$J_e = -P_e \frac{zF\Delta\psi/RT}{1 - \exp(zF\Delta\psi/RT)} (C_{out} - C_{in} \exp(zF\Delta\psi/RT)) \quad (26)$$

where the units associated with solute concentration, solute flux, and membrane permeability are as given in equation (23). From equations (23) and (26), the equilibrium distribution of the protonation pair, $\text{NH}_3/\text{NH}_4^+$, across the plasmalemma is described by:

$$P_{\text{NH}_3}(C_{\text{NH}_3, \text{out}} - C_{\text{NH}_3, \text{in}}) = -P_{\text{NH}_4^+} \frac{zF\Delta\psi/RT}{1 - \exp(zF\Delta\psi/RT)} (C_{out} - C_{in} \exp(zF\Delta\psi/RT)) \quad (27)$$

The $[\text{NH}_4^+]/[\text{NH}_3]$ ratio occurring on either side of the plasmalemma presumably conforms to the Henderson-Hasselbalch distribution ($\text{pK}_{a, \text{NH}_4^+} \approx 9.4$, subject to correction for temperature and ionic strength influences; see Emerson *et al.* 1975; Thurston *et al.* 1979; Messer *et al.* 1984).

The permeability of the plasmalemma lipid bilayer to hydrophilic species such as NH_4^+ and NO_3^- is very low, and diffusion alone cannot account for the measured fluxes of these solutes. Although the presence of uniporter proteins may increase transport rates by several orders of magnitude, they do not by themselves alter the solute equilibrium distribution across the cell membrane. Given equation (26) and a transmembrane electrical potential of, say, 58 mV (Raven 1980), the facilitated transport of NH_4^+ or NO_3^- via uniporter proteins would require that the solute concentration on the sink side of the membrane be less than one-tenth the concentration on the source side. Data from Dortch (1982), however, suggest that the intracellular concentration of these solutes typically exceeds the extracellular concentration by a factor of

10^3 or more. Clearly, some additional source of free energy must be coupled to the transport process (cf., Smith and Walker 1978; Wright and Syrett 1983; Balch 1986).

In theory, either an exergonic biochemical reaction (primary active transport) or an exergonic transmembrane solute flux (secondary active transport) could power nutrient uptake (specifically, nutrient transport) in the presence of an unfavorable electrochemical gradient. Although primary active transport generally involves ATP-powered H^+ ion extrusion rather than nutrient uptake, the electrochemical gradients generated by this process make possible a large variety of secondary transport systems (see Mitchell 1976, 1979; Raven and Smith 1976; Raven 1980). The transplasmalemma electrochemical potential resulting from the active extrusion of protons comprises an electrical potential component and a $[H^+]$ gradient component, as described in the following equation (Mitchell 1979):

$$\Delta\bar{\mu}_{H^+} = \Delta\psi - \left(\frac{2.3RT}{F}\right) \Delta pH \quad (28)$$

where $\Delta\bar{\mu}_{H^+}$ is the total electrochemical or "chemiosmotic" potential (in volts), ΔpH equals the difference between extracellular and cytoplasmic pH, and $\Delta\psi$, R and T are defined as in equations 24 and 25. Note that secondary transport processes dependent on $\Delta\bar{\mu}_{H^+}$ are also dependent on external pH, as the latter parameter influences both ΔpH and $\Delta\psi$. Whereas $\Delta\bar{\mu}_{H^+}$ and internal $[H^+]$ are nearly invariant over a wide range of external pH values, ΔpH and $\Delta\psi$ fluctuate markedly (Smith and Raven 1979; cf., Padan et al. 1981; Padan 1983).

The nutrient transport model depicted in Figure 35 attempts to explain the findings of the present study in a chemiosmotic context. It assumes that inorganic nitrogen uptake is predominantly active and coupled to $\Delta\bar{\mu}_{\text{H}^+}$. The uptake of NH_4^+ is associated with a uniport and is driven by $\Delta\psi$; NO_3^- transport is associated with an electroneutral symport and is driven by ΔpH . The diffusion or "leakage" of NH_3 through the lipid bilayer represents an inherent inefficiency in the nitrogen acquisition/retention process (i.e., $C_{\text{in}} \gg C_{\text{out}}$; see equation 23). As extracellular pH decreases relative to cytoplasmic pH, the following outcomes are assumed: (1) ΔpH increases at the expense of $\Delta\psi$ (i.e., $\Delta\bar{\mu}_{\text{H}^+}$ is constant; see equation 28); (2) uniport function is impeded whereas symport function is enhanced; and (3) $V_{\text{NO}_3^-}$ becomes larger whereas $V_{\text{NH}_4^+}$ becomes smaller. It is assumed also that pH-related changes in $V_{\text{NO}_3^-}$ compensate for corresponding changes in $V_{\text{NH}_4^+}$ over a wide $[\text{H}^+]$ range. Acidification beyond this range reduces cytoplasmic pH, in addition to ΔpH , $\Delta\bar{\mu}_{\text{H}^+}$, and total nitrogen ($\text{NH}_4^+ + \text{NO}_3^-$) uptake (cf., figures 25 and 26).

Interestingly, Merezhko *et al.* (1986) have reported that submergent macrophytes also respond to acidification by increasing $V_{\text{NO}_3^-}$ and decreasing $V_{\text{NH}_4^+}$; moreover, macrophytic uptake of total inorganic nitrogen is essentially invariant over the pH range 8.5 to 5.5. The similarities between these responses and those demonstrated by phytoplankton in the present study suggest that compensatory nutritional shifts represent a real and widespread photolithotrophic adaptation to fluctuating ambient pH. The ecological ramifications of this phenomenon are considered shortly.

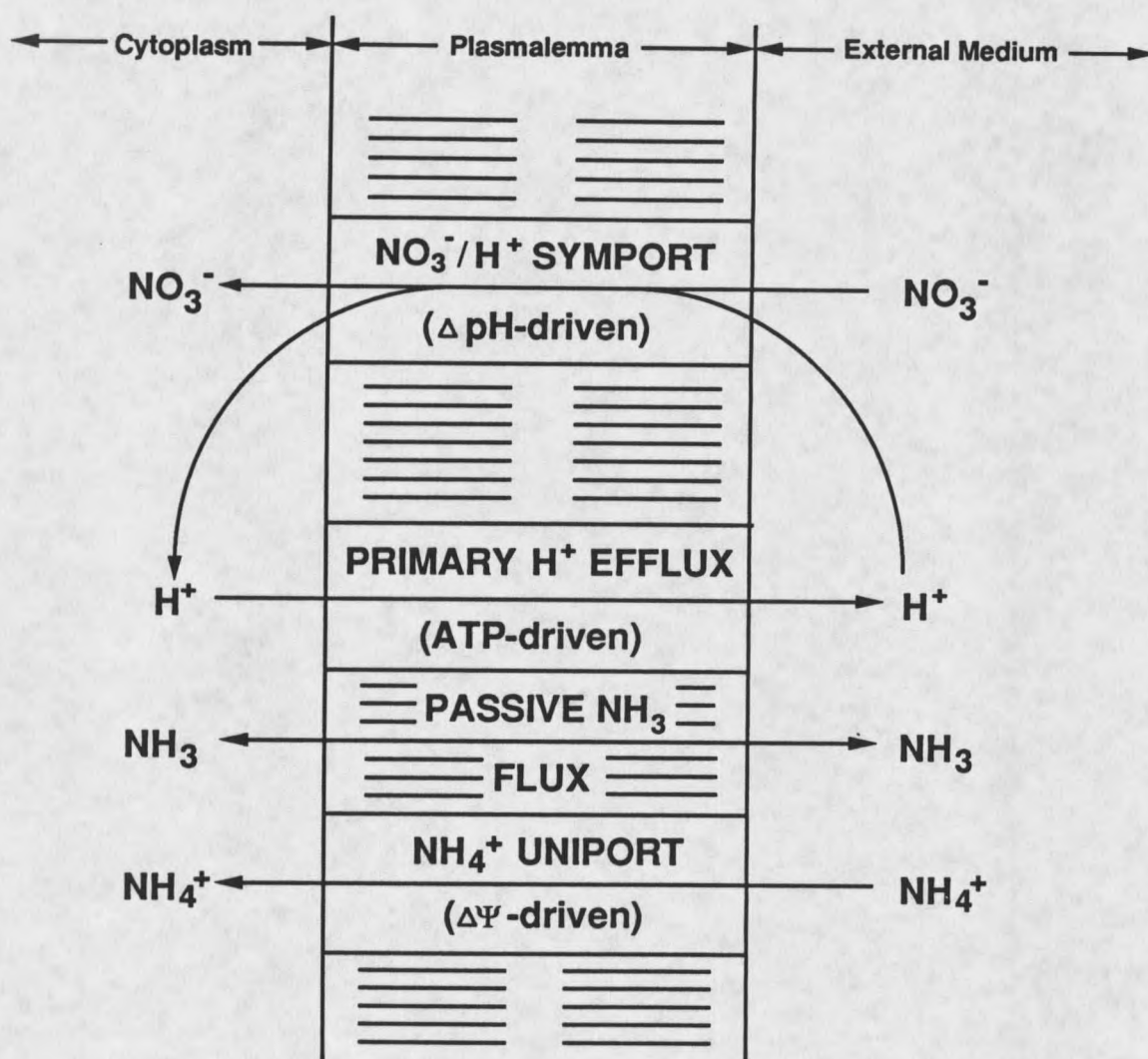
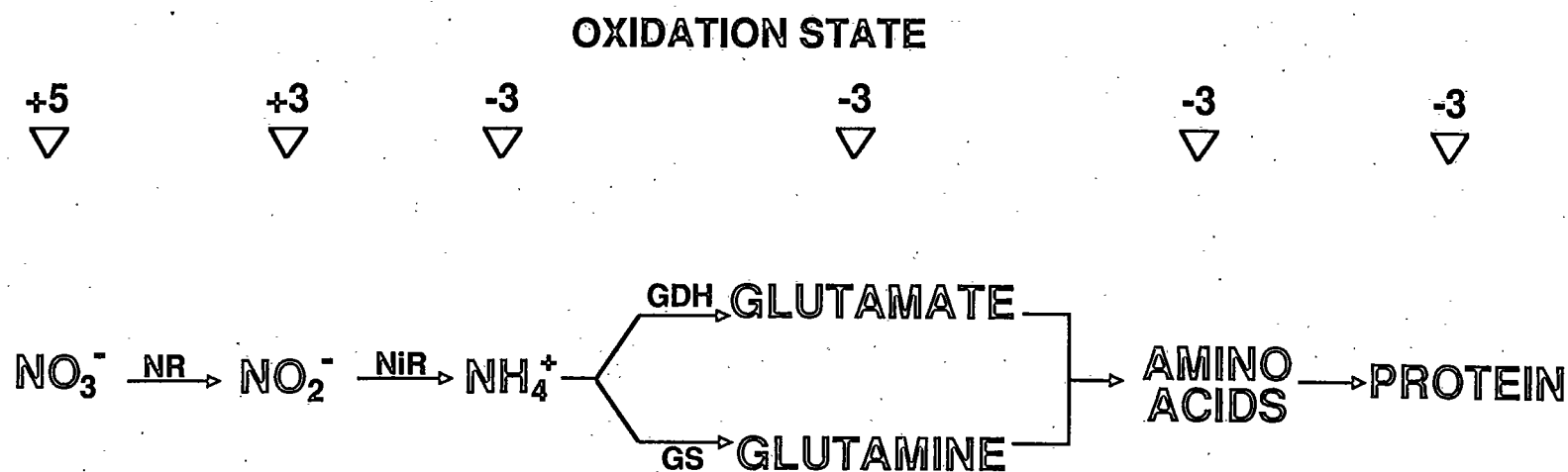


Figure 35. Schematic representation of mediated and nonmediated nitrogen transport systems in phytoplankton. Only those systems relevant to the discussion are illustrated; see text for details.

Effects of Acidification on Algal Protein Synthesis

Phytoplankton regulate internal pH through the active uptake and extrusion of protons. Although essential to cell function, this homeostatic process imposes a high energetic cost during extended periods of acidification or alkalization (see Smith and Raven 1979). Similarly, an added investment of assimilatory reductant (NAD(P)H) is required if protein synthesis is to continue unabatedly following acidification-induced, compensatory nutritional shifts from NH_4^+ to NO_3^- . Because the diversion of metabolic energy and reducing power from biosynthetic to homeostatic functions conceivably could detract from cellular growth, environmental H^+ ion concentration must be regarded as yet another physicochemical factor capable of influencing photolithotrophic production.

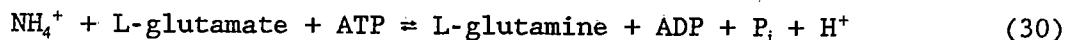
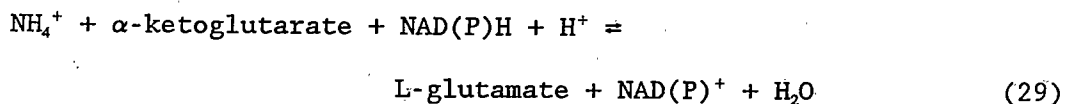
The aforementioned study by Merezhko *et al.* (1986) represents one of the few attempts to examine the influence of pH on nitrogen metabolism in aquatic plants. Interestingly, these researchers found that protein synthesis in submergent macrophytes proceeded less rapidly under mildly acidic conditions than under weakly alkaline conditions (pH 5.5 versus pH 8.5). This response could not be attributed to a decrease in total nitrogen uptake at low pH (combined NH_4^+ and NO_3^- influx was unaffected by pH; see preceding subsection), nor did it appear to be caused by a reduction in NO_3^- reductase activity (which should have resulted in the feedback inhibition, rather than the observed, compensatory enhancement, of NO_3^- uptake; *cf.*, Pistorius *et al.* 1978). Instead, it appeared that one or more of the post-reductive steps in the nitrogen assimilatory sequence (Figure 36) was impeded somehow under acidic conditions. These



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Figure 36. Generalized pathway for inorganic nitrogen assimilation in phytoplankton. Note that NO_3^- -N and NO_2^- -N must be reduced prior to incorporation into cellular protein. Abbreviations: NR, NO_3^- reductase; NiR, NO_2^- reductase; GDH, glutamate dehydrogenase; GS, glutamine synthetase.

steps presumably began with one of the following alternative reactions (Stryer 1981):



where reactions (29) and (30) were mediated by glutamate dehydrogenase and glutamine synthetase, respectively. Note that homeostatic demands on reductant and ATP (or a general shortage of suitable carbon skeletons) could have impeded glutamate/glutamine formation and accounted for the effects of reduced pH on protein synthesis. Subtle interactions between external pH and the activity of the mediating enzymes were also possible. For example, glutamate dehydrogenase activity may have been sensitive to the cationic composition of the intracellular medium (cf., Schoffeniels and Gilles 1963; Schoffeniels 1964) and thus responsive to $\Delta\psi$ -related fluctuations in electrolyte transport (see *Effects of Acidification on NH_4^+ Regeneration: Physiological Implications*, this chapter). Also, the activity of glutamine synthetase, a key control element in intermediary metabolism, may have been affected by the intracellular availability of cAMP and various other allosteric effectors potentially involved in homeostatic regulation (see Stadtman and Ginsburg 1974; Tyler 1978). Transient decreases in intracellular (mitochondrial) pH shortly after experimental acidification could have affected the activity of both enzymes (i.e., the binding of substrate to an active site of an enzyme generally involves electrostatic interaction; thus, the formation of an

enzyme-substrate complex is highly pH dependent; cf., Ahmed *et al.* 1977). Even minor increases in cytoplasmic $[H^+]$ could have altered the activity of certain glycolytic enzymes important in the production of ATP and NADH and in the generation of carbon skeletons necessary for protein synthesis (e.g., phosphofructokinase activity decreases 10-20 fold, *in vitro*, when the pH of the incubating medium decreases by as little as 0.1 pH unit; see Trivedi and Danforth 1966).

Recall that the rate at which total inorganic nitrogen uptake was assimilated into protein by Snowbank Lake phytoplankton was not influenced significantly by a 100-fold increase in ambient $[H^+]$ (Figure 27). Changes in NH_4^+ - and NO_3^- -based protein production resulting from acidification were of the same sign and approximately the same magnitude as corresponding changes in NH_4^+ and NO_3^- uptake (cf., figures 26 and 27), suggesting that protein synthesis was limited by the influx of inorganic nitrogen rather than by enzymatic constraints or by the availability of carbon skeletons, reductant, or ATP. The apportionment of $^{14}CO_2$ -C among protein, lipid and polysaccharide fractions was approximately the same at pH 7.4 as at pH 5.4 (Figure 29), indicating that cellular metabolism was not "distorted" seriously within this $[H^+]$ range, at least over the short term. The proportion of ^{14}C inoculant recovered in the LMWM fraction was significantly less at pH 5.4, however, perhaps owing to homeostatic demands on simple sugars and other readily mobilizable energy reserves.

Data from this study and from that of Merezko *et al.* (*ibid.*) suggest that the influence of environmental pH on protein synthesis and other anabolic processes may vary markedly among photolithotrophic taxa.

In submergent macrophytes and other aquatic plants which tend to be self-shading and light-limited (reviewed by Wetzel 1983), the homeostatic mechanisms invoked by acidification probably increase cellular respiration at the expense of cellular growth. Contrastingly, nutrient-limited phytoplankton inhabiting relatively transparent water bodies such as Snowbank Lake likely have access to more radiant energy than can be utilized effectively in growth and replication. Such energy surpluses (and the internal store of sugars, starches and oils arising therefrom) presumably mitigate the effect of fluctuating ambient pH on intracellular energy supply and metabolite synthesis. Only persistent or unusually severe decreases in pH are likely to affect the anabolic patterns in these algae, either through the eventual failure of cellular homeostatic mechanisms or through the excessive and continued diversion of metabolic energy from biosynthetic to homeostatic functions.

Influence of pH on NH_4^+ Regeneration:
Physiological Implications

From a biochemical perspective, environmental pH probably influences NH_4^+ regeneration in at least three ways. First, the electrostatic interactions that occur between NH_4^+ and the integral membrane proteins responsible for NH_4^+ transport presumably are influenced by the immediate pH environment. Acidification from pH 7 to pH 5, for example, would tend to ionize histidine residues ($\text{pK} \approx 6.0$) located on the exterior surfaces of NH_4^+ porters; further reductions in ambient pH and the concurrent protonation of other amino acid residues (e.g., glutamic and aspartic acids; $\text{pK} \approx 4.3$ and 3.9 , respectively) could have major effects on porter conformation, activity and substrate specificity (see Stryer 1981).

Second, pH-related changes in the electrolytic composition of the internal medium (discussed below) could reduce NAD⁺-linked glutamate dehydrogenase activity and impede the catabolic formation of NH₄⁺ (see equation 29). This is suggested by the rather exacting [Na⁺] and [Cl⁻] requirements demonstrated by glutamate dehydrogenase *in vitro* (Schoffeniels and Gilles 1963; Schoffeniels 1964; Chaplin *et al.* 1965; Gilles 1969, 1974). Third, reductions in external pH could influence the energetics of NH₄⁺ extrusion by substantially altering transmembrane electrochemical potentials. This possibility is somewhat more abstruse than those mentioned previously and receives further elaboration in the following discussion.

The transport of an electrolyte through a biological membrane frequently involves some form of ion exchange. This arrangement preserves the circumelectroneutrality of the internal and external fluid environments and minimizes the expenditures of energy required for ion transport (Krough 1939; Shaw 1960; Maetz 1963; Maetz and Garcia-Romeu 1964; Stobbart 1971; Payan and Maetz 1973; Dietz and Alvarado 1974; Evans 1975). Ammonium extrusion ($r_{\text{NH}_4^+}$) generally is coupled to Na⁺ uptake (P_{Na^+} ; see Shaw 1960; Evans 1975; Silverthorn and Krall 1975) and, although water quality influences on $r_{\text{NH}_4^+}$ have received comparatively little scientific attention, the effects of environmental pH on P_{Na^+} have been studied intensively during the past decade. It is now apparent that lake acidification decreases P_{Na^+} in many eucaryotic fauna (e.g., Potts and Fryer 1979; Havas *et al.* 1984) and in some neutrophilic bacteria (reviewed by Padan *et al.* 1981); furthermore, reductions in internal [Na⁺] under acidic conditions have been documented in several species of

crustacean zooplankton (Potts and Fryer 1979; Havas 1981; Havas and Hutchinson 1982, 1983; Havas *et al.* 1984) as well as in fish (Leivestad and Muniz 1976; McWilliams and Potts 1978; McWilliams *et al.* 1980; Muniz and Leivestad 1980) and appear to be most severe in waters depleted of Ca^{2+} and other divalent cations (see Evans 1975; Oduleye 1973; McWilliams and Potts 1978; McDonald *et al.* 1980; Brown 1981; Havas *et al.* 1984; *cf.*, Shaw 1959; Potts and Fleming 1970, 1971). These pH-related effects on Na^+ uptake and retention suggest that Na^+ -linked transport processes such as $r_{\text{NH}_4^+}$ also may be impeded under acidic conditions.

Results from the present study imply that NH_4^+ regeneration rates in softwater Snowbank Lake are strongly dependent on ambient pH. This dependence does not differ dramatically among heterotrophic size classes (Figure 22), suggesting that the transport processes affected by external $[\text{H}^+]$ are similar in a wide variety of metazoan and protozoan forms. The experimental findings may be interpreted in a chemiosmotic context given the following assumptions: (1) transplasmalemma (transepithelial) electrical potentials are influenced by external pH and range from strongly negative in circumneutral environments to zero or weakly positive under moderately acidic conditions (see McWilliams and Potts 1978); (2) reductions in $r_{\text{NH}_4^+}$ resulting from acidification are accompanied by declines in P_{Na^+} and by the partial dissipation of the Na^+ concentration gradient across the plasmalemma ($\Delta[\text{Na}^+]$; *cf.*, Hutchinson and Collins 1978; Havas *et al.* 1984); and (3) P_{Na^+} is balanced electrolytically by the sum of NH_4^+ and H^+ efflux rather than by $r_{\text{NH}_4^+}$ alone (Maetz 1973). Figure 37 presents one possible interpretation of the experimental results which is consistent with these assumptions.

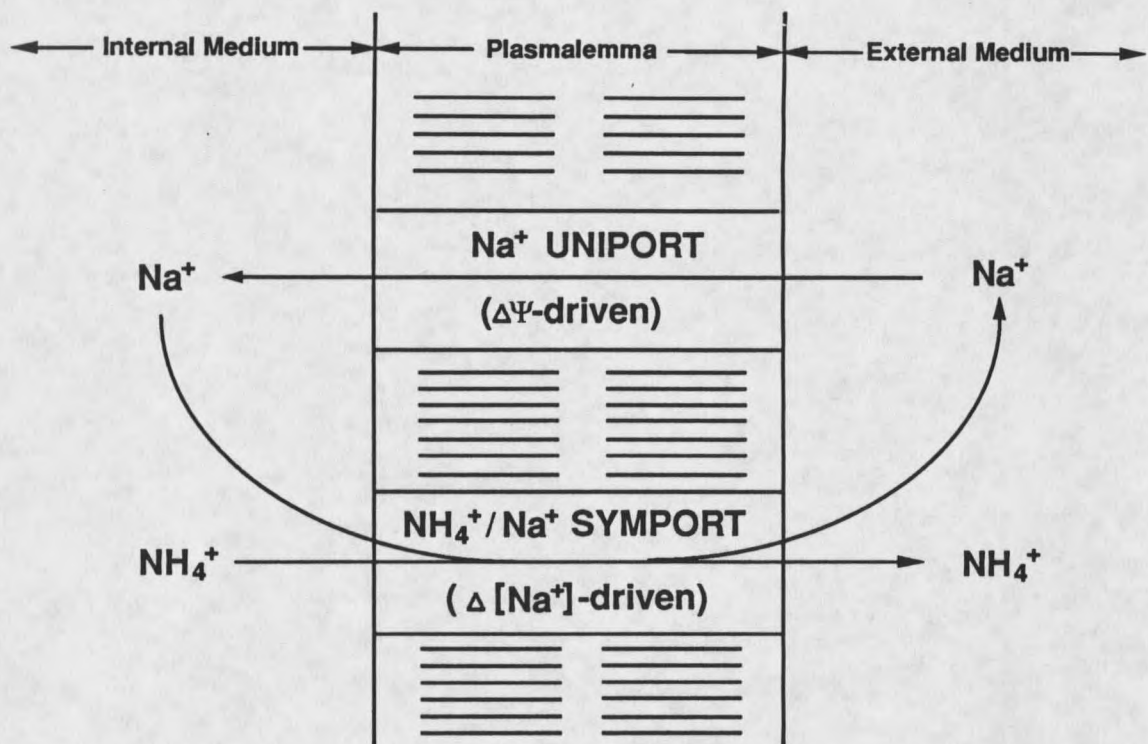


Figure 37. Biochemical model of $\text{NH}_4^+/\text{Na}^+$ ion exchange across zooplankton external membranes. See text for details.

According to this model, P_{Na^+} is mediated by an inward-directed, $\Delta\psi$ -driven uniport, whereas $r_{NH_4^+}$ is mediated by a $\Delta[Na^+]$ -driven NH_4^+/Na^+ symport (note: $\Delta[Na^+]$ -driven symports occur in a wide variety of procaryotic and eucaryotic organisms; see Raven 1980; Padan *et al.* 1981; Stryer 1981). Acidification of the external medium presumably decreases $|\Delta\psi|$, thereby reducing P_{Na^+} and contributing to the disruption of the internal Na^+ balance (*i.e.*, total Na^+ efflux exceeds total Na^+ influx), to the dissipation of $\Delta[Na^+]$ and, ultimately, to a decline in $r_{NH_4^+}$. The increased Na^+ efflux at low pH noted by some workers (*e.g.*, McWilliams and Potts 1978; Havas *et al.* 1984) may be interpreted as an increase in the diffusional permeability or "leakage" of Na^+ or, alternatively, as an enhancement of some facilitated or active Na^+ extrusion process not depicted in Figure 37.

The actual physiological mechanism through which pH exerts its influence on NH_4^+ efflux is admittedly uncertain, and it is possible that the enzymatic, electrostatic and chemiosmotic explanations given above are not applicable under natural circumstances. These explanations provide a hypothetical framework for future studies, however, and their eventual substantiation or repudiation will do much to improve our understanding of nitrogen metabolism in freshwater heterotrophs.

Biological Contributions to Lake Acid Neutralizing Capacity: Importance of Nitrogen Assimilative Processes

The acid neutralizing capacity of a water body is a relatively complex biogeochemical property, dependent not only on the availability of dissolved alkaline species and on the exchangeable base content of

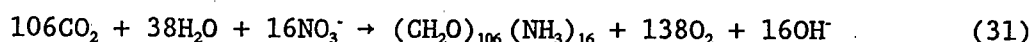
surficial sediments, but also on the ability of the aquatic microbial community to serve as a net biochemical sink for free H^+ (Table 26). This latter factor has received much attention in recent whole-lake acidification studies, and it is now apparent that the dissimilatory reduction of protolytic ions represents an especially important mechanism of H^+ neutralization in many freshwater ecosystems (e.g., Schindler and Turner 1982; Kelly and Rudd 1984; Schindler *et al.* 1985). Cook *et al.* (1986) have estimated that nearly 85% of the *in situ* alkalinity production in Lake 223 (Experimental Lakes Area or ELA, northwestern Ontario) is associated with bacterial SO_4^{2-} and Fe^{3+} reduction and FeS formation, processes largely confined to the lake's anoxic sediments and hypolimnetic waters. Bacterial NO_3^- reduction in anaerobic waters and sediments also contributes seasonally to the acid neutralizing capacity of other ELA lakes (e.g., Kelly *et al.* 1982). Although these biological processes are not "100% efficient" at neutralizing incoming acids, they may delay the onset or reduce the severity of acidification in some lakes receiving substantial anthropogenic loadings of H_2SO_4 and HNO_3 (Schindler 1988a).

The contribution made by microbial organisms to the acid neutralizing capacity of softwater alpine lakes has received comparatively little scientific attention (Stoddard 1987, 1988). In rapidly flushing, freely mixing water bodies such as Snowbank Lake, the uniformly oxygenated water column (Figure 5) and the general paucity of organic sediments (Chapter 2) would appear to preclude the high rates of dissimilatory SO_4^{2-} and NO_3^- reduction noted in the ELA studies. Microbial components may contribute more to the total alkalinity of such ecosystems through the

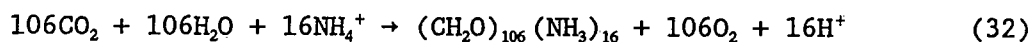
Table 26. Major biologically mediated processes affecting pH in surface water ecosystems (from Weber and Stumm 1963).

Process	Reaction	Effect on pH
Photosynthesis	$6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$	Increase
Respiration	$\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$	Decrease
Methane fermentation	$\text{C}_6\text{H}_{12}\text{O}_6 + 3\text{CO}_2 \rightarrow 3\text{CH}_4 + 6\text{CO}_2$	Decrease
Nitrification	$\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{H}^+$	Decrease
Denitrification	$5\text{C}_6\text{H}_{12}\text{O}_6 + 24\text{NO}_3^- + 24\text{H}^+ \rightarrow$ $30\text{CO}_2 + 12\text{N}_2 + 42\text{H}_2\text{O}$	Increase
Sulfide oxidation	$\text{HS}^- + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + \text{H}^+$	Decrease
Sulfate reduction	$\text{C}_6\text{H}_{12}\text{O}_6 + 3\text{SO}_4^{2-} + 3\text{H}^+ \rightarrow$ $6\text{CO}_2 + 3\text{HS}^- + 6\text{H}_2\text{O}$	Increase

assimilatory reduction of protolytic chemical species. The uptake of NO_3^- by phytoplankton may be especially important in this regard, as indicated by the following equation (expressed in Redfield elemental proportions):



Contrastingly, the uptake of NH_4^+ by phytoplankton detracts from lakewater total alkalinity:



In a series of laboratory experiments involving three species of marine phytoplankton, Brewer and Goldman (1976) found that NO_3^- -supported algal growth was associated with an increase in the acid neutralizing

capacity of the culture medium, whereas NH_4^+ -based algal growth was associated with a marked decrease in this capacity (see also Pratt and Fong 1940). In a whole-lake eutrophication experiment, Schindler *et al.* (1985) demonstrated that additions of NH_4Cl (together with H_3PO_4) caused significant reductions in lakewater alkalinity and pH; this trend was reversed by the subsequent application of NaNO_3 (together with H_3PO_4). There exists little doubt, therefore, that the form of inorganic nitrogen utilized by phytoplankton may exert a significant influence on the pH and alkalinity of the aqueous environment.

Saturating additions of inorganic nitrogen had no measurable effect on sample pH in the present study, perhaps owing to the relatively short time frame of the field and laboratory experiments and/or to the low algal biomass and correspondingly low rates of nitrogen uptake. Even when substrate enrichment dramatically increased the rate of nitrogen uptake, attendant changes in H^+ or OH^- production apparently were offset by chemical buffering reactions. For example, sample $[\text{H}^+]$ presumably would have decreased from pH 7.2 to pH 8.0 in the NO_3^- uptake kinetics experiment conducted on 24 August 1986 were it not for the conversion of OH^- and H_2CO_3 to HCO_3^- and H_2O ; similarly, $[\text{H}^+]$ would have increased from pH 7.0 to pH 5.7 in the NH_4^+ uptake kinetics experiment conducted in August 1985 were it not for the conversion of H^+ and HCO_3^- to H_2CO_3 (Table 11; equations 31 and 32; see also Stumm and Morgan 1981). Interestingly, sample pH also was stable in acidification treatments amended with 3.6-7.1 μM additions of NH_4^+ or NO_3^- , even though the aqueous HCO_3^- reserve was much reduced in these treatments.

The absence of any observable change in background sample pH in field and laboratory experiments did not preclude the possibility of microscale fluctuations in environmental $[H^+]$. In particular, the pH of the quiescent boundary layer extending only a few μm (at most) from the surface of algal cells undoubtedly was influenced more rapidly by changes in OH^- or H^+ production than was the background pH. This raises an important question: Could the compensatory nutritional shift observed at moderately low pH have represented an external $[H^+]$ buffering mechanism, capable of restoring the pH of the immediate extracellular environment to a physiologically acceptable level? Although no direct evidence for such a mechanism was gathered in this study, the results of the laboratory time course experiment conducted on 26 September 1987 support such a hypothesis. For example, the acidification-induced increase in $V_{NO_3^-}$ (i.e., the discrepancy between experimental and control $V_{NO_3^-}$ values) ceased after approximately 19 hr, perhaps signifying the restoration of boundary layer $[H^+]$ to preacidification levels (Figure 24). Significant differences in $V_{NH_4^+}$ between control and acidification treatments likewise were eliminated during the course of the incubation (Figure 23). Because every mole of NO_3^- that was assimilated in lieu of an equal quantity of NH_4^+ would have generated, in effect, 2 moles of OH^- , the presumed stoichiometric substitution of NO_3^- for NH_4^+ would have provided a rather efficient means of H^+ neutralization. The initial concentration of much of this biologically generated alkalinity in the immediate vicinity of algal cells would have minimized the magnitude and duration of the effect of acidification on $[H^+]$ -dependent assimilative processes (Table 27).

Table 27. Theoretical changes in inorganic nitrogen uptake and alkalinity generation by phytoplankton following experimental acidification.

Post-acidification response	
Moderate (≤ 2 unit) pH reduction	Severe (> 2 unit) pH reduction
Increased rates of NO_3^- uptake and OH^- generation; decreased rates of NH_4^+ uptake and H^+ generation	Rates of NO_3^- and NH_4^+ uptake decrease precipitously; little net increase in OH^- generation
Acid neutralizing capacity and pH of immediate extracellular environment increase	No significant change in buffering capacity or pH of immediate extracellular environment
Rapid return to preacidification rates of NH_4^+ and NO_3^- uptake	Continued depression of NH_4^+ and NO_3^- uptake rates

The suggestion that phytoplankton exert some degree of control over their aqueous chemical environment is not new. For some 50 years, the close relationship between the carbon:nitrogen:phosphorus composition of seawater and that of marine algae has been thought to reflect a long-term biogeochemical feedback mechanism mediated primarily by photosynthetic and oxidative decomposition processes (Redfield 1934; see also Harvey 1926). More recently, Morel and Hudson (1985) have proposed that "many major and trace elements, essential and toxic, may be simultaneously controlling biological production in the oceans . . . , in turn, the (cycling and concentration) of these elements (are) controlled by the biota." Schindler (1985) has remarked on the resiliency of biogeochemical cycles in freshwater ecosystems and has compared the microbial mechanisms that "exist for maintaining these nutrient balances over wide ranges" to those that occur in the sea. The processes contributing to

the acid-base balance of lakes (Table 26) also tend to respond to perturbation (acidification or alkalization) in a manner which eventually restores the original H^+ concentration and turnover rate (see Schindler 1985; cf., Schindler and Turner 1982; Kelly and Rudd 1984; Kelly et al. 1984).

In contrast to the long-term biogeochemical feedback mechanisms which stabilize proton flux and concentration on an ecosystem scale, this study suggests that algal assimilative responses to acidification may rapidly restore the pH of the immediate extracellular environment and minimize the influence of environmental $[H^+]$ on nutrient procurement and related aspects of cellular metabolism. Such responses may have considerable adaptive value in poorly buffered aquatic ecosystems undergoing periodic (i.e., diel, seasonal) fluctuations in aqueous pH (cf., Stoddard 1987, 1988).

Long-Term Effects of Lake Acidification on Water-Column Nitrogen Cycling and Biological Production

Among the earliest ecological concerns associated with lake acidification was its potential for affecting organic decompositional and nutrient cycling processes in sediments and limnetic waters. This concern was founded on a relatively large body of direct observational and experimental evidence. For example, early researchers noted thick accumulations of coarse organic detritus in many anthropogenically acidified lakes and attributed these accumulations to a general, pH-related decrease in microbial decompositional activity (e.g., Grahn et al. 1974). In whole-lake neutralization experiments, the adjustment of epilimnetic pH to preacidification levels led to the rapid decay of

detrital accumulations (Andersson *et al.* 1978) and to the proliferation of water-column bacteria (Sheider *et al.* 1975). In various field and laboratory investigations, decreases in aqueous pH were associated with reduced microbial consumption of O_2 (Traaen 1974), with hindered decomposition of leaf litter (e.g., Traaen 1976) and with decreased uptake of radioactive phosphorus in lake sediment cores (Laake 1976). These early findings were corroborated in numerous subsequent investigations (e.g., Traaen 1980; Burton *et al.* 1982; Hultberg and Anderson 1982; McKinley and Vestal 1982; Rao and Dutka 1983; Francis *et al.* 1984; Allard and Moreau 1986), although exceptions to the above generalizations were documented (Kelly *et al.* 1984).

Recently, Rudd *et al.* (1988) reported that under-ice concentrations of inorganic nitrogen in two ELA lakes varied significantly in response to experimental declines in pH. Acidification from pH 6.2 to pH 5.4-5.1 via H_2SO_4 addition over a period of several years was accompanied by an increase in $[NH_4^+]$ and a decrease in $[NO_3^-]$; the discontinuation of H_2SO_4 inputs in one of the lakes led to the gradual alkalization of the water column (from pH 5.1 to pH 5.5) and to the eventual restoration of the preacidification NO_3^- level. Rudd *et al.* attributed these results to the disruption of bacterial nitrification activities at low pH (even though nitrification rates were not monitored during the study) and concluded that, of all the major elemental cycles, the nitrogen cycle was by far the most sensitive to environmental pH. Although these researchers did not consider possible algal assimilative responses to acidification (see *Influence of pH on Inorganic Nitrogen Uptake: A Preliminary Biochemical Model*, this chapter) nor other biological manifestations which may have

contributed to the observed changes in inorganic nitrogen concentration, their study demonstrated unequivocally that lake acidification could engender long-term changes in the availability and speciation of assimilatable nitrogen.

In addition to the documented effects of acidification on aquatic nutrient cycling, inputs of mineral acid anions to surface waters may exert a strong stimulatory influence on algal growth. Paerl (1985) documented that rainfall and terrigenous runoff containing high concentrations of NO_3^- substantially increased phytoplankton production in near-surface coastal waters depleted of inorganic nitrogen. Observed increases in chlorophyll a concentration persisted for as long as several weeks following major storms, suggesting that ombrogenic nitrogen contributed significantly to the overall productivity of these waters. Although similar phenomena apparently have not been reported for freshwater ecosystems, a series of nutrient/precipitation enrichment bioassays performed by Manny *et al.* (1987) suggested that the presence of PO_4^{3-} in rainfall could stimulate phytoplankton growth in perennially phosphorus-deficient Lake Michigan. Havens and DeCosta (1986) conducted a two-month mesocosm experiment in Lake O'Woods, West Virginia, comparing algal photosynthetic responses to nutrient additions under circumneutral versus acidic (HCl ; pH 4.5) conditions. Phytoplankton biomass increased most dramatically in acidified treatments containing added NO_3^- and PO_4^{3-} ; unfortunately, the fraction of this increase attributable to nutrient enrichment was obscured owing to the extirpation of acid sensitive zooplankton and to the concomitant relaxation of grazing pressures on algal growth.

Experimental data gathered in the present study and discussed earlier in this chapter suggested that nitrogen cycling processes in Snowbank Lake were highly sensitive to acidification, at least over the short term. Of equal interest was that the mineral acid anion associated with pH reduction appeared to exert a strong influence on algal nitrogen uptake. The addition of HNO_3 (alone or in combination with H_2SO_4) to lakewater samples invariably resulted in a greater enhancement (or lesser suppression) of NO_3^- uptake than did the addition of HCl or H_2SO_4 alone (Figure 26). Moreover, the uptake of *total* inorganic nitrogen sometimes was enhanced by the addition of HNO_3 (see 2 and 26 September entries, Figure 26), implying that this mineral acid could ameliorate nitrogen-limited conditions and increase algal growth despite a substantial (2-unit) decrease in environmental pH. High rates of algal $^{14}\text{CO}_2$ incorporation observed in HNO_3 and $\text{HNO}_3/\text{H}_2\text{SO}_4$ treatments (relative to those observed in HCl or H_2SO_4 treatments; see 26 September entry, Figure 28) likewise suggested that any potential negative effect of acidification on the growth of nitrogen-limited phytoplankton could be offset, at least in part, by drastically elevated concentrations of NO_3^- and, presumably, by an enhanced algal capacity for NO_3^- utilization at low pH (figures 24-26).

Because HNO_3 represents the principal H^+ donor in rainfall throughout much of the western United States (including heavily industrialized or populated locations receiving unusually acidic precipitation; see Liljestrand and Morgan 1978; McColl and Bush 1978), the potential for widespread acidification of lakes within this region relates, first and foremost, to the deposition of this compound. Nitrate derived from the dissociation of HNO_3 would be expected to comprise an increasingly greater

fraction of the assimilatable nitrogen supply in lakes affected by acidification but incapable of a significant denitrification response (see Stoddard 1987, 1988; cf., Kelly 1988; Schindler 1988b; Psenner 1989); moreover, the importance of NO_3^- to algal growth would tend to increase disproportionately to its rate of supply if, as suggested by this study, the physiological capacity for NH_4^+ uptake gradually diminished under acidic conditions. The nitrogenous nutrition of algae within a lake undergoing acidification conceivably could become progressively less dependent on the efficient utilization of heterotrophically regenerated NH_4^+ and progressively more dependent on the deposition of anthropogenically derived NO_3^- . Under persistently nitrogen-limited, increasingly acidic conditions, photolithotrophic production could cease to benefit from the purportedly stabilizing influence of nutrient cycling (i.e., from the nutritional "positive feedback" interactions occurring among trophic components; see Perry et al. 1989) and become increasingly vulnerable to the vagaries of air quality and climatological influences on HNO_3 supply. In addition to the marked changes in biological community structure which invariably accompany surface water acidification, accelerated inputs of HNO_3 could detract from both the efficiency and the constancy of the water-column nitrogen cycle and substantially alter the long-term productivity of chronically nitrogen-deficient lakes.

CHAPTER 6

CONCLUSIONS

Relative to the role played by nitrogen in the regulation of algal primary production in Snowbank Lake, the following major points should be drawn from the preceding discussion:

1) Low concentrations (low supply rates) of assimilatable nitrogen in Snowbank Lake impose strong constraints on phytoplankton growth during the open-water season.

2) The phytoplankton community as a whole exhibits no discernable preference for NH_4^+ over oxidized forms of assimilatable nitrogen; rather, substrate utilization is commensurate with substrate availability.

3) Advectively supplied NO_3^- comprises the bulk of the phytoplankton nitrogen ration in Snowbank Lake; however, *in situ* ammonification supplies at least 30-40% of the algal nitrogen demand.

4) Nitrogen is efficiently recycled between heterotrophic and autotrophic components of the lake plankton community; specifically, a close spatial and temporal coupling exists between NH_4^+ production and consumption processes, and regenerative and assimilative fluxes of NH_4^+ are approximately in balance on an ecosystem scale.

Regarding the food web components contributing most to water-column nitrogen supply and demand, the following findings are worthy of reiteration:

1) Bacterioplankton contribute little to nitrogen regeneration in Snowbank Lake; instead, they are net consumers of NH_4^+ and probably compete strongly with algae for this regenerated resource.

2) Mixo- and heterotrophic flagellates are relatively abundant in Snowbank lake and account for most of the microbial regeneration activity.

3) Reductions in rotifer and crustacean zooplankton populations markedly enhance rates of NH_4^+ regeneration, presumably by reducing predation pressures on metabolically active nano- and microflagellates; hence, grazing interactions between meta- and protozooplankton may exert strong influences on water-column nitrogen supply and algal primary production.

4) An apparent intricate partitioning (chemical, spatial, temporal, kinetic) of nitrogenous resources among phytoplankton presumably reduces interspecific competition and facilitates the perennial reemergence of a diverse, predominantly nitrogen-limited phytoplankton assemblage.

Finally, with respect to the influences (or potential influences) exerted by ambient pH on the limnetic nitrogen cycle in Snowbank Lake, the following conclusions are inferred from the present study:

1) Water-column regenerative and assimilative fluxes of NH_4^+ and bacterioplankton secondary production are reduced under mildly acidic conditions (pH 5 versus pH 7); contrastingly, the algal capacity for NO_3^- utilization is greatest at moderately low pH (pH $\approx$ 5).

2) Acidification-induced algal nutritional shifts from NH_4^+ to NO_3^- are interpretable in a chemiosmotic context and have important physiological (homeostatic) and ecological (lake buffering) implications.

3) Hypothetically, the acidification of the water column via HNO_3 addition would stimulate increases in total nitrogen uptake and permit higher rates of algal photosynthesis than would corresponding additions of "nonnutritive" mineral acids; more importantly, greatly increased NO_3^- concentrations (coupled with an enhanced algal capacity for NO_3^- utilization at low pH) would tend to ameliorate nutrient constraints on water-column primary production following HNO_3 enrichment.

4) Acidification of Snowbank Lake through inputs of HNO_3 -enriched precipitation and runoff would detract from the efficiency of the NH_4^+ regenerative/assimilative cycle and increase algal dependence on allochthonously supplied NO_3^- .

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APPENDIX

Table 28. Aquatic macroinvertebrate taxa collected from the Snowbank Lake watershed during 24-26 August 1985 by D. L. Gustafson, Department of Biology, Montana State University.

Phylum Arthropoda
 Class Crustacea
 Order Anostraca
 Family Branchinectidae
 Branchinecta paludosa (O. F. Muller)
 Class Insecta
 Order Ephemeroptera
 Family Baetidae
 Baetis bicaudatus Dodds
 Family Heptageniidae
 Cinygmula par (Eaton)
 Order Plecoptera
 Family Perlodidae
 Arcynopteryx compacta (McLachlan)
 Megarcys watertoni (Ricker)
 Order Trichoptera
 Family Brachycentridae
 Brachycentrus americanus (Banks)
 Family Limnephilidae
 Asynarchus aldinus (Ross)
 Chyranda centralis (Banks)
 Dicosmoecus atripes (Hagen)
 Ecclisiomyia maculosa Banks
 Hesperophylax incisus Banks
 Psychoglypha subborealis (Banks)
 Family Rhyacophilidae
 Rhyacophila angelita Banks
 R. tucula Ross

Collector's comments:

1. "All species listed are common and well known in this area except for *Arcynopteryx (compacta)*, which is poorly known south of the Canadian tundra. It is known from northwestern Montana, (and) also in alpine areas of Wyoming. The collection is a new (Carbon County) record . . . but not unexpected."
 2. "All species are typical of alpine or subalpine conditions except for *Brachycentrus (americanus)* and *Hesperophylax (incisus)*, both of which indicate the presence of strong groundwater connections for the streams."
-

Table 29. Metazooplankton taxa collected from Snowbank Lake during the 1985-87 ice-free seasons.

Phylum Arthropoda

Class Crustacea

Order Calanoida

Family Diaptomidae

Diaptomus (Hesperodiaptomus) shoshone Forbes

Order Dipotraca

Family Daphnidae

Daphnia pulex Leydig

D. rosea Sars

D. schodleri Sars

Phylum Rotatoria

Class Monogononta

Order Flosculariacea

Family Testudinellidae

Filinia sp.

Order Ploima

Family Synchaetidae

Polyarthra sp.

Family Brachionidae

Brachionus sp.

Kellicottia sp.

Keratella sp.

Notholca sp.

Table 30. Phytoplankton (and flagellated protistan) taxa collected from Snowbank Lake during the 1985-87 ice-free seasons.

Division Chlorophyta

Class Chlorophyceae

Order Volvocales

Family Chlamydomonadaceae

Pandorina morum (Muell.) Bory

Volvox tertius Meyer

Order Tetrasporales

Family Tetrasporaceae

Asterococcus limneticus G. M. Smith

Tetraspora sp.

Order Chlorococcales

Family Chlorococcaceae

Planktosphaeria gelatinosa G. M. Smith

Schroederia (Ankyra) judayi G. M. Smith

S. setigera Lemmermann

Tetraedron sp.

Family Palmellaceae

Sphaerocystis schroeteri Chodat

Family Oocystaceae

Ankistrodesmus braunii (Naeg.) Collins

A. falcatus (Corda) Ralfs

A. sp. #3

Oocystis borgei Snow

Qudrigula chodatii (Tanner-Fullman) G. M. Smith

Family Radiococcaceae

Mycanthococcus sp.

Family Micractiniaceae

Micractinium pusillum var. *longisetum* Tiffany et Ahlstrom

Family Dictyosphaeriaceae

Botryococcus sp.

Dictyosphaerium pulchellum Wood

Westella botryoides (West) de Wildemann

Family Scenedesmaceae

Coelastrum microporum Nageli

Crucigenia tetrapedia (Kirchn.) West et West

Scenedesmus bijuga (Turp.) Lagerheim

Tetrastrum sp.

Family Hydrodictyaceae

Pediastrum boryanum (Turp.) Meneghini

Family Coccomyxaceae

Elakatothrix viridis (Snow) Printz

Family Ulotrichaceae

Ulothrix sp.

Order Microsporales

Family Microsporaceae

Microspora sp.

Table 30. Continued.

Order Oedogoniales

Family Oedogoniaceae

Oedogonium sp.

Order Zygnematales

Family Gonatozygaceae (Mesotaeniaceae)

Roya obtusa var. *montana* West et West

Family Desmidiaceae

Closterium sp. #1*C.* sp. #2*Cosmarium tinctum* var. *subretusum* Messik*C. margaritatum* (Lund.) Roy et Bissett*Euastrum* sp.*Hyalotheca dissiliens* forma *tridentula* Nordstedt*Micrasterias* sp.*Spondylosium planum* (Wolle) West et West*Staurostrum furcigerum* Brebisson*S. hexacerum* (Ehr.) Wittrock*S. manfeldtii* var. *fluminense* Schumacher*S. paradoxum* Meyen*S. paradoxum* var. *cingulum* West et West*S. punctulatum* Brebisson*S. tetracerum* Ralfs

Family Zygnemataceae

Mougeotia sp.

Division Chrysophyta

Class Chrysophyceae

Order Chrysomonadales

Family Ochromonadaceae

Ochromonas (*Chlorochromonas*) sp.

Family Synuraceae (Mallomonadaceae)

Mallomonas sp. #1*M.* sp. #2*M.* sp. #3*Synura uvella* Ehrenberg*S.* sp. #2

Family Dinobryaceae

Dinobryon sertularia Ehrenberg

Order Rhizochrysidales

Family Rhizochrysidaceae

Rhizochrysis limnetica G. M. Smith

Order Chromulinales

Family Chromulinaceae

Chromulina pascheri Hoffeneeder

Family Chrysococcaceae

Chrysococcus sp.

Table 30. Continued.

Class Bacillariophyceae

Order Centrales

Family Coscinodiscaceae

Cyclotella meneghiniana Kuetzing*Melosira italica* var. *subarctica* Mueller

Order Pennales

Family Tabellariaceae

Diatomella sp.*Tabellaria fenestrata* (Lyngb.) Kuetzing*T. flocculosa* (Roth) Kuetzing*T.* sp. #3

Family Meridionaceae

Meridion circulare (Grev.) Agardh

Family Diatomaceae

Diatoma sp.

Family Fragilariaceae

Asterionella formosa Hassall*Fragilaria construens* (Ehr.) Grunow*F. crotonensis* Kitton*F.* sp. #3*Synedra* sp. #1*S.* sp. #2

Family Eunotiaceae

Eunotia (*Ceratoneis*) sp. #1*E.* sp. #2*Hannaea* (*Ceratoneis*) *arcus* (Ehr.) Patrick

Family Achnanthaceae

Achnanthes sp.*Cocconeis* sp.*Rhoicosphenia curvata* (Kutz.) Grunow ex. Rabenhorst

Family Naviculaceae

Diploneis sp.*Frustulia* sp.*Gyrosigma* sp.*Navicula* sp. #1*N.* sp. #2*N.* sp. #3*N.* sp. #4*Neidium* sp.*Pinnularia* sp.*Stauroneis* sp.

Family Gomphonemataceae

Gomphonema sp.

Family Cymbellaceae

Amphora sp.*Cymbella* sp. #1*C.* sp. #2

Family Epithemiaceae

Epithemia sp.

Table 30. Continued.

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- Family Nitzschiaceae
 - Hantzschia virgata* (Roper) Grunow
 - H. virgata* var. *capitellata* Hustedt.
 - Nitzschia* sp. #1
 - N.* sp. #2
 - Family Surirellaceae
 - Surirella* sp.
 - Class Xanthophyceae (Heterokontae)
 - Order Mischococcales (Heterococcales)
 - Family Pleurochloridaceae
 - Botrydiopsis arhiza* Borzi
 - Family Sciadaceae
 - Centritractus belanophorus* Lemmermann
 - Order Heterotrichales
 - Family Tribonemataceae
 - Tribonema bombycinum* (Ag.) Derbes et Solier
 - Division Cryptophyta
 - Class Cryptophyceae
 - Order Cryptomonadales
 - Family Cryptomonadaceae
 - Cryptomonas* sp.
 - Rhodomonas lacustris* Pascher et Ruttner
 - Division Cyanophyta
 - Class Myxophyceae
 - Order Chroococcales
 - Family Chroococcaceae
 - Chroococcus dispersus* (Keissl.) Lemmermann
 - Gomphosphaeria aponina* Kuetzing
 - Order Hormogonales
 - Family Rivulariaceae
 - Rivularia* sp.
 - Division Pyrrhophyta
 - Class Dinophyceae
 - Order Peridiniales
 - Family Peridiniaceae
 - Peridinium cinctum* (Muell.) Ehrenberg
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