



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

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences  
Montana State University  
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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 ( $^{15}\text{N}$ ,  $^{14}\text{C}$ ,  $^3\text{H}$ ) methodologies, plankton size-fractionation and metabolic inhibitor techniques, and short-term bioassay procedures; supporting data were gathered on lake physicochemical and biological properties. Measured aqueous nutrient concentrations, the results of  $^{14}\text{C}$ -based snowmelt and nutrient enrichment bioassays, and physiological indicators of algal nutrient status collectively demonstrated that phytoplankton nitrogen demand greatly exceeded nitrogen supply. Both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were quantitatively important forms of assimilable nitrogen under ambient conditions. Mass balance considerations indicated that within-lake biogeochemical processes constituted a net sink for  $\text{NO}_3^-$ , whereas  $\text{NH}_4^+$  production and consumption rates were approximately in balance on an ecosystem scale. Water-column regenerative and assimilative fluxes of  $\text{NH}_4^+$  were strongly correlated. Meta- and protozooplankton were the principal sources of regenerated  $\text{NH}_4^+$ ; heterotrophic bacterioplankton were net consumers of  $\text{NH}_4^+$ . Experimental reductions in metazooplankton populations markedly enhanced rates of  $\text{NH}_4^+$  regeneration, apparently by reducing predation pressures on metabolically active nano- and microflagellates. Ammonium regeneration,  $\text{NH}_4^+$  uptake and bacterial secondary production declined under acidic conditions (pH 5 versus pH 7; HCl), whereas algal  $\text{NO}_3^-$  utilization increased and compensated for reductions in  $\text{NH}_4^+$  uptake. Acidification via  $\text{HNO}_3$  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  $\text{H}_2\text{SO}_4$ . These findings collectively suggested that the efficiency of the  $\text{NH}_4^+$  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  $\text{NO}_3^-$ .

BIOLOGICAL CYCLING OF NITROGEN IN A ROCKY MOUNTAIN  
ALPINE LAKE, WITH EMPHASIS ON THE PHYSIOLOGICAL  
AND ECOLOGICAL EFFECTS OF ACIDIFICATION

by

Robert Thomas Angelo

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

Doctor of Philosophy

in

Biological Sciences

MONTANA STATE UNIVERSITY  
Bozeman, Montana

September 1989

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16 October 1989

Date

John C. Pruce  
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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21 September 1989

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

## ACKNOWLEDGEMENTS

Many individuals and organizations merit recognition for their role in this research effort. Dr. John Priscu, who served as my academic advisor and graduate committee chairperson, introduced me to several of the field and analytical procedures used in this study and made many suggestions which improved the overall quality of the research. Other committee members included Drs. Daniel Goodman, Calvin Kaya, Samuel Rogers and Vance Thurston, each of whom read the dissertation manuscript and provided valuable and constructive criticisms. The late Professor Gordon Pagenkopf served on the graduate committee during the initial, formulative phase of the project; his advice and encouragement at that critical time were much appreciated. John Beehler, Lisa Campbell, Daniel Gustafson, Samuel Lohr, Dr. Robert Murray, and Linda and Dr. John Priscu provided capable assistance in the field. Computer and statistical advice and thoughtful dialogue were provided by Milo Atkinson, Peter Boveng, Dr. Walter Dodds, Daniel Gustafson, Kirk Johnson, Dr. Randall Ryti, Paul Wade and Lizhu Wang. The dissertation manuscript was typed and proofread by Wanda Myers; final illustrative materials were prepared by Martha Lonner. This project was funded through grants from the Five Valleys Audubon Society, the Montana State University College of Graduate Studies, and the Gary Lynch Memorial Awards Program.

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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 ( $^{15}\text{N}$ ,  $^{14}\text{C}$ ,  $^3\text{H}$ ) methodologies, plankton size-fractionation and metabolic inhibitor techniques, and short-term bioassay procedures; supporting data were gathered on lake physicochemical and biological properties. Measured aqueous nutrient concentrations, the results of  $^{14}\text{CO}_2$ -based snowmelt and nutrient enrichment bioassays, and physiological indicators of algal nutrient status collectively demonstrated that phytoplankton nitrogen demand greatly exceeded nitrogen supply. Both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were quantitatively important forms of assimilatable nitrogen under ambient conditions. Mass balance considerations indicated that within-lake biogeochemical processes constituted a net sink for  $\text{NO}_3^-$ , whereas  $\text{NH}_4^+$  production and consumption rates were approximately in balance on an ecosystem scale. Water-column regenerative and assimilative fluxes of  $\text{NH}_4^+$  were strongly correlated. Meta- and protozooplankton were the principal sources of regenerated  $\text{NH}_4^+$ ; heterotrophic bacterioplankton were net consumers of  $\text{NH}_4^+$ . Experimental reductions in metazooplankton populations markedly enhanced rates of  $\text{NH}_4^+$  regeneration, apparently by reducing predation pressures on metabolically active nano- and microflagellates. Ammonium regeneration,  $\text{NH}_4^+$  uptake and bacterial secondary production declined under acidic conditions (pH 5 versus pH 7; HCl), whereas algal  $\text{NO}_3^-$  utilization increased and compensated for reductions in  $\text{NH}_4^+$  uptake. Acidification via  $\text{HNO}_3$  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  $\text{H}_2\text{SO}_4$ . These findings collectively suggested that the efficiency of the  $\text{NH}_4^+$  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  $\text{NO}_3^-$ .

## 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  $\text{NH}_4^+$  (*i.e.*,  $\text{NH}_4^+$  derived from macrozooplankton excretion and microbial ammonification activities) rather than by newly available (*i.e.*, externally derived) forms of assimilatable nitrogen such as  $\text{N}_2$  or  $\text{NO}_3^-$ . 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 assimilable 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 assimilable 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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  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  $\text{NO}_3^-$  (as  $\text{HNO}_3$ ) 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  $\text{NO}_3^-$  (or a decreased reliance on regenerated  $\text{NH}_4^+$ ) 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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  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  $\text{NH}_4^+$  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  $\text{CO}_2$  fixation, giving particular attention to any stimulatory effects resulting from  $\text{HNO}_3$  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,

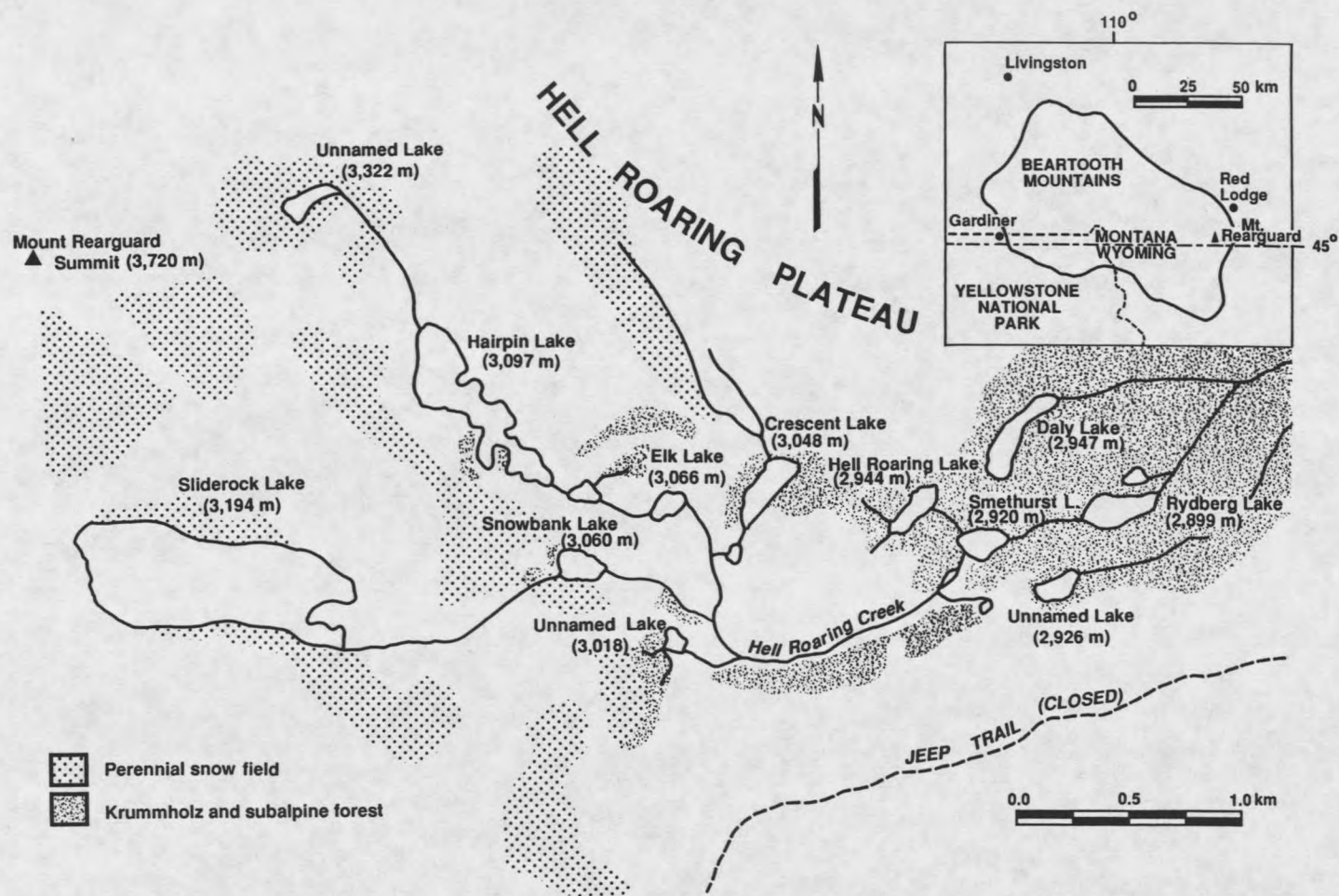


Figure 1. Location and physiographic features of the upper Hell Roaring Creek watershed. Parenthetical values refer to elevation above mean sea level (as determined by Marcuson 1980d).

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



































































































































































































































































































































































































































































































































































