

Lake-specific responses to elevated atmospheric nitrogen deposition in the Colorado Rocky Mountains, U.S.A.

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Abstract

We explored variability among subalpine lakes sharing very similar climate and atmospheric conditions, but differing in watershed characteristics, hydrology, and food web structure. Special attention was given to nitrogen (N) dynamics because the study area receives some of the highest levels of atmospheric N deposition in the Rocky Mountains. We asked if the effect of regional N deposition would be manifested uniformly among neighboring lakes both in terms of ambient conditions and responses to greater nutrient inputs. Catchment vegetation appeared to be the main determinant of ambient nitrate (NO₃), phosphate (PO₄), and dissolved organic carbon (DOC) concentrations, although in-lake differences in recycling produced variable and contrasting NH₄ levels. Phytoplankton chlorophyll *a* temporarily responded to early season NO₃ peaks in the lakes with rocky watersheds, but chlorophyll means over the ice-free season were remarkably similar among lakes despite differences in both nutrient supply and zooplankton grazing. In most cases, phosphorus was limiting to phytoplankton growth, although the importance of N deficiencies was greater in lakes with forested watersheds and fringing wetlands.

Introduction

Mountain lakes are especially sensitive to atmospheric deposition; alpine watersheds have limited ability to immobilize nutrients due to steep slopes, sparse vegetation, shallow soils, a short growing season, and snowmelt dominated hydrology (Williams et al., 1996; Campbell et al., 2000). In the eastern United States and Europe, high levels of nitrogen (N) and sulfur deposition to mountain ecosystems have resulted in increased nitrate (NO₃) concentrations and acidification of surface waters (Baker et al., 1991; Fott et al., 1994; Camarero et al., 1995; Kopáček et al., 1995; Mosello et al., 1995). In the western United States, more moderate levels of N deposition are associated with changes in mountain lake community

and ecosystem properties (Jassby et al., 1994; Interlandi & Kilham, 1998; Baron et al., 2000). In the Colorado Front Range, lakes experience elevated NO₃ concentrations and altered algal composition, but even episodic acidification of headwaters is rare (Williams et al., 1996; Williams & Tonnessen, 2000; Wolfe et al., 2001). Front Range lakes thus represent an opportunity to study the effects of elevated N deposition without the confounding influence of chronic acidification, which can eliminate zooplankton grazers and reduce both total phosphorus (TP) and chlorophyll *a* concentrations (Kopáček et al., 1995).

The eastern flank of the Colorado Front Range receives the highest N deposition in the Rocky Mountains (Baron et al., 2000), but lake NO₃ concentrations range from less than 7 to 280 µg N l⁻¹ (Lafrancois et

al., 2003) indicating a large amount of variability in the response to a regional perturbation. In other lake districts, variability among lakes often is predictable and dependent on factors such as exposure to climatic conditions and hydrologic or landscape position (Magnuson et al., 1990; Kratz et al., 1997; Riera et al., 2000). In the Colorado Front Range, however, individual lake characteristics may overwhelm regional responses, despite similarities in altitude and exposure to atmospheric pollutants (Baron & Caine, 2000).

In this study, we asked if the effect of regional N deposition would be manifested uniformly among neighboring lakes, and if lakes would respond similarly to further increases in nutrient inputs. Although P limitation of freshwater productivity often is assumed (Schindler, 1977; Kopáček et al., 1995), N limitation of phytoplankton has been documented in a number of mountain lakes in the western U.S. (Axler et al., 1982; Goldman, 1988; Morris and Lewis, 1988; Wurtsbaugh et al., 1997) and is fairly common in the U.S. in general (Elser et al., 1990). Furthermore, nutrient limitation can vary seasonally within individual lakes as well as among lakes within a region (Morris & Lewis, 1988; Axler et al., 1994).

Physical, chemical, and biological trends were monitored throughout the ice-free season in three oligotrophic subalpine lakes situated in close proximity to one another on the east side of Rocky Mountain National Park, Colorado. *In situ* bioassays were used to assess phytoplankton biomass and community responses to further nutrient enrichment. We hypothesized that ambient lake conditions and phytoplankton nutrient limitation would be influenced by lake-specific responses to elevated N deposition in the Colorado Front Range.

Study sites

There are hundreds of lakes in the Colorado Front Range. Most are small and shallow with low ionic strength, although NO_3 concentrations vary greatly. Autumn NO_3 concentrations in a survey of lakes east of the Continental Divide averaged $117 \pm 68 \mu\text{g N l}^{-1}$ (SD), and mean alkalinity was $72 \pm 40 \mu\text{eq l}^{-1}$ (Lafrancois et al., 2003).

The study lakes are located in Loch Vale Watershed (3000–4000 m), a long-term ecological research and monitoring site on the east side of the Continental Divide in Rocky Mountain National Park, Colorado (Fig. 1). Geology is predominantly Precambrian gran-

Table 1. Characteristics of the study lakes

	The Loch	Embryo Pond	Mystery Pond
Lake Type	Drainage	Semi-drainage	Seepage
Inlet/Outlet	Permanent streams	Marsh/Stream	Marsh/ Marsh
Lake Area (ha)	5.0	0.2	1.0
Lake Elevation (m)	3102	3164	3090
Watershed Area (ha)	660	21	18
Mean / Max. Depth (m)	1.5 / 4.7	0.8 / 2	1.0 / 3
% watershed rock & talus	82	89	<1
Fish	Rainbow & greenback cutthroat trout	none	none
Other		Talus drains directly into lake; wetlands	Wetlands and macrophytes

ite, schist, and gneiss. Precipitation to these high elevations is primarily as snow in winter and snow-melt occurs from April to July. Subalpine vegetation is Englemann spruce-subalpine fir; tundra occurs at elevations above approximately 3300 m. Loch Vale is located within 60 km of the Denver to Fort Collins corridor, a region with many urban and agricultural sources of N emissions. Mean annual wet deposition to Loch Vale was $3.5 \text{ kg inorganic N ha}^{-1} \text{ y}^{-1}$ from 1984 to 1999. More remote locations in Colorado report half as much wet N deposition (NADP, 1999).

The Loch, Mystery Pond, and Embryo Pond are shallow, oligotrophic subalpine lakes within 2 km of each other at an elevation of about 3100 m (Fig. 1). They do not stratify during summer, and ice cover lasts from November to June. All are at least 3 km from the nearest road. Although the lakes are similar in certain respects, they vary in hydrologic and catchment characteristics and food web structure (Table 1). The Loch is a drainage lake (Pennak, 1969) with a permanently flowing inlet and outlet. It is the largest lake (5 ha) in this study and also has the largest watershed (660 ha). The Loch's drainage basin is comprised of bare rock and talus (82%), tundra and meadow (12%), and forest (6%) (Baron & Campbell, 1997). Although originally fishless, The Loch was stocked with trout in the early to mid 1900s (Roselund & Stevens, 1990). Currently, The Loch is dominated by rainbow \times cutthroat hybrids. Embryo Pond is a small, shallow, fishless semi-drainage pond with a temporary inlet stream. It has a 21 ha watershed consisting of bare rock and talus

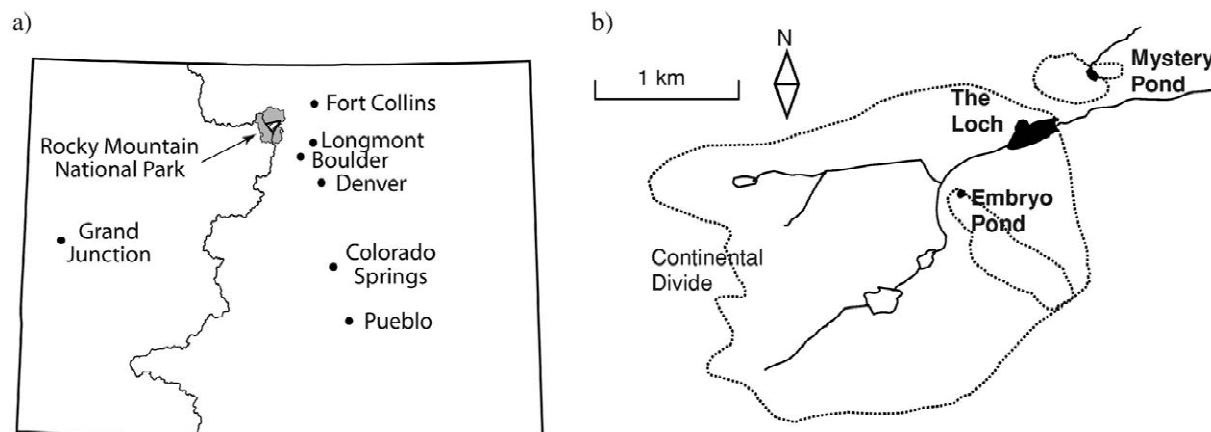


Figure 1. Location of Rocky Mountain National Park in Colorado. Loch Vale Watershed is indicated by a hatched triangle (a). The approximate extent of each study lake's drainage basin is shown with dashed lines (b).

(89%), tundra and meadow (10%), and <1% forest. Its inlet and outlet areas are small wetlands. Snowmelt and storm runoff also drain directly into the lake from a steep talus slope. Mystery Pond is 1 ha in area and has an 18 ha watershed dominated by meadow (66%) and forest (32%). The inlet and outlet areas of this fishless, seepage pond are small wetlands without distinct channels. Sedges and aquatic macrophytes fringe a portion of the perimeter.

Methods

Ambient lake conditions

We sampled each lake weekly for temperature, pH, specific conductance, NO_3 , NH_4 , PO_4 , dissolved organic carbon (DOC), and chlorophyll *a* during the summer of 1999. SiO_2 was measured only in The Loch. Total phosphorus (TP) was not measured in this study, but values from 2001 give an indication of summer concentrations. Samples were collected and processed using standard Loch Vale Watershed project methods (Allstott et al., 1999). Briefly, water was collected at one meter depth above the deepest part of each lake using a hand pump connected to rinsed acid washed tubing. Samples were stored in acid washed HDPE bottles previously rinsed three times with lake water (glass was used for DOC samples). Water for inorganic and organic analyses was passed through Nucleopore membrane (0.45 μm) and pre-combusted Whatman GF/F (1.0 μm) filters, respectively, and stored at 4°C until analysis. Temperature, specific conductance, and pH were measured with meters (Or-

ion Model 128 and VWR Model 8000). NO_3 , NH_4 , and PO_4 were determined colorimetrically with a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Detection limits were 5, 5, and 1 $\mu\text{g l}^{-1}$ for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$, respectively. Standard deviation on five runs of low PO_4 standards (2, 3, and 5 $\mu\text{g P l}^{-1}$) was zero. SiO_2 was analyzed colorimetrically with the molybdate method modified by Hach, Inc. DOC was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey, Boulder, CO). TP was calculated as the sum of dissolved P and particulate P, which were determined with persulfate oxidations (Ameel et al., 1993; Lampman et al., 2001) followed by the ascorbic acid method. Lake water samples for chlorophyll *a* analyses were filtered immediately through Whatman GF/C filters. Filters were frozen in dark containers and chlorophyll *a* was extracted with methanol (Riemann, 1980). Chlorophyll *a*, corrected for phaeophytin, was determined with a Sequoia-Turner Model 450 Digital Fluorometer.

Phytoplankton samples were collected twice monthly from one meter depth at the deepest part of each lake and preserved with Lugol's solution. Phytoplankton were identified to species when possible by Dufford Consulting, Fort Collins, CO. Biovolume was not calculated due to concerns about species-specific distortion of cell sizes and shapes induced by the fixative (Jerome et al., 1993; Zinabu and Bott, 2000; Menden-Deuer et al., 2001) and growth condition-related variability in cell volumes (Sicko-Goad et al., 1977; Hillebrand et al., 1999).

Zooplankton were sampled approximately weekly. Depth-integrated samples were collected by bilge pump at three sites within each lake until mid-summer. Samples for each lake were pooled, collected on 80 μm mesh, and preserved with sugared buffered 4% formalin. Beginning on July 29, we collected zooplankton with an 80 μm mesh Wisconsin net in three to five locations for each lake. Both methods were used on July 29. The Wisconsin net allowed us to sample a greater lake volume, but had lower capture efficiency compared to the bilge pump method and a correction factor determined from the ratio of Wisconsin net: bilge pump dry mass per l was applied to post-July 29 samples. Zooplankton were rinsed of preservative and observed under a dissecting scope to remove as much detritus as possible. Samples were then filtered onto Whatman GF/C filters and dried at 60°C for 24 h to obtain dry mass. Chaoborids were present in late summer Mystery Pond samples; we removed them and measured chaoborid dry mass separately. Fairy shrimp, *Branchinecta coloradensis* (Anostraca) were observed in Embryo Pond, but were not captured in zooplankton samples. Although qualitative observations and dry mass measurements were made on all samples, zooplankton were enumerated for the June 29 sample date only. Rotifers were identified to genus, copepods to order, and cladocera as *Daphnia* sp. or Chydorinae. Immature copepods were identified as nauplii.

Phytoplankton nutrient bioassays

Phytoplankton nutrient bioassays were performed three times during the 1999 ice-free season in Embryo and Mystery Ponds and twice for The Loch. The early bioassay occurred from June 29 to July 5 during the end of the snowmelt period. The mid-summer bioassay ran from August 4 to 10, and we conducted the late-summer bioassay from September 11 to 18. For each experiment, a depth-integrated water sample was collected from the deepest area of each lake. Zooplankton grazers were removed by filtering through 80 μm mesh prior to distributing lake water into clear, plastic 2 l bottles containing nutrient treatments. The bottles were previously acid-washed and rinsed three times with the filtered water sample. Treatments, in triplicate, were control, +2.26 mg l⁻¹ NO₃-N, +0.19 mg l⁻¹ PO₄-P, +NO₃ and PO₄ combined, +5 mg l⁻¹ SiO₂, and +0.8 mg l⁻¹ dissolved organic N (DON). High concentrations ensured that nutrient uptake rates would be maximal and that enrichments would not

be depleted during the incubation. NO₃ was added as KNO₃, PO₄ as K₂HPO₄, and DON as urea (CH₄N₂O). NO₃ and DON, rather than NH₄, were used because these are the dominant forms of dissolved N input from the watershed (Baron et al., 1992). Silica limitation was explored during the first two bioassays because diatoms are known to be an important component of The Loch flora (McKnight et al., 1990). Bottles containing a small headspace were shaken gently and incubated *in situ* at 0.5–1 m depth for 6 days.

We measured chlorophyll *a* and collected phytoplankton and water samples at the end of each experiment. Samples for phytoplankton taxonomic analysis were pooled among the three replicates and preserved with Lugol's solution. Cell counts and enumeration were conducted using Utermöhl settling chambers and a Leitz-Wetzlar Diavert inverted microscope with phase contrast.

Statistical analyses

We initially compared chlorophyll *a* responses to nutrient enrichment in three ways: (1) across all lakes for the mid- and late- summer bioassays (excluding the silica treatment), (2) across all bioassays for Embryo and Mystery Ponds (excluding the silica treatment), and (3) across all lakes and bioassays for the silica treatment only. We used lake, time (early, mid, or late summer), and treatment as factors in a full ANOVA with all interactions (SAS Institute, Inc., Version 8.2). The interaction of lake*time*treatment was important in the two analyses without silica ($p < 0.0001$ and $p = 0.05$, respectively). Because of this significant interaction, we combined all bioassays and compared responses for each experiment separately using lake*time and treatment as the two ANOVA factors. When the treatment main effect was significant at the $\alpha = 0.05$ level, we used least squared means to compare treatments against the control (and among treatments if more than one was different from the control) for each individual bioassay. One outlier was removed because its residual was more than four standard deviations from the predicted value. Chlorophyll *a* responses were log-transformed to meet the assumptions of normality and homogeneity of variance for all analyses. For each bioassay experiment, the mean percent of the control was used to indicate the magnitude of each treatment's chlorophyll *a* response.

Correlations were calculated with Pearson product moments (r ; SAS Institute, Inc., Version 8.2). Correlations with p -values ≤ 0.02 were considered significant at the $\alpha = 0.05$ level corrected for multiple comparisons.

Results

Ambient lake conditions

Mean daytime water temperatures over the summer were 8.0, 9.0, and 10.7°C for The Loch, Embryo Pond, and Mystery Pond, respectively (Table 2, Fig. 2). Temperatures were similar at the beginning and end of sampling, but Mystery and Embryo Ponds attained higher mid-summer temperatures than The Loch. pH generally increased slightly over the ice-free season, and seasonal means were ~ 6.0 . All three lakes were dilute and had low specific conductance. Average DOC concentrations were 1.2, 4.0, and 9.2 mg l⁻¹ for The Loch, Embryo, and Mystery Ponds, respectively.

The mean NO₃-N concentration was highest for The Loch (224 $\mu\text{g l}^{-1}$), followed by Embryo Pond (111 $\mu\text{g l}^{-1}$), and lowest in Mystery Pond (5 $\mu\text{g l}^{-1}$). Peaks of 350 and 300 $\mu\text{g l}^{-1}$ N occurred in mid to late June during snowmelt in The Loch and Embryo Pond, respectively (Fig. 2, Table 2). In The Loch, NO₃ remained elevated ($>150 \mu\text{g l}^{-1}$ N) until the end of sampling, but Embryo Pond had lower NO₃ concentrations during mid-summer. Mystery Pond did not experience an early-summer NO₃ pulse. The mean NH₄ concentrations in The Loch and Embryo and Mystery Ponds were 12, 25, and 17 $\mu\text{g l}^{-1}$ N, respectively. The highest NH₄ concentrations occurred during or after chlorophyll *a* peaks. Mean summer PO₄ concentrations were 3, 6, and 8 $\mu\text{g l}^{-1}$ P for The Loch, Embryo, and Mystery Pond. TP in The Loch averaged 9 $\mu\text{g l}^{-1}$ in mid-summer 2001 when PO₄ concentrations were 1–2 $\mu\text{g P l}^{-1}$. SiO₂ averaged 1.70 mg l⁻¹ for The Loch, and the lowest concentration (1.38 mg l⁻¹) was recorded during mid-summer. SiO₂ was not measured in Mystery and Embryo Ponds.

Phytoplankton cell counts were correlated with chlorophyll *a* concentrations over time for The Loch and Mystery Pond. Cell density per unit chlorophyll *a* varied greatly among lakes indicating differences in cell size or chlorophyll *a* allocation (Table 2). Mean summer chlorophyll *a* concentrations were similar (1.32–1.63 $\mu\text{g l}^{-1}$), but temporal dynamics and community composition varied among lakes (Fig. 3).

The Loch had a mixed assemblage of mostly chrysophytes and non-heterocystous blue-green algae during the early to mid-summer. The diatom *Asterionella formosa* Hassall was also present in early summer, with highest density measured on June 22 (1.35 $\mu\text{g l}^{-1}$ chlorophyll *a*). A late summer increase in chrysophytes, mainly *Chrysochromulina parva* Lackey, occurred from mid-August until the end of sampling (max. 4.43 $\mu\text{g l}^{-1}$ chlorophyll *a*). The green alga *Chlorella* sp. also increased at the end of the summer in The Loch.

Embryo Pond was dominated by non-heterocystous blue-green algae, especially *Aphanothece smithii* Komarkova-Legnerova et Cronberg, in early June. Chrysophytes, mainly *Kephyrion boreale* Skuja and *Chromulina mikrop plankton* Pascher, increased in June, and peak cell counts and chlorophyll *a* (2.74 $\mu\text{g l}^{-1}$) were detected on July 14, one week after the minimum summer NO₃ concentration. Cell numbers decreased rapidly, but chlorophyll *a* remained approximately 1.5 $\mu\text{g l}^{-1}$ for the remainder of sampling. Late season phytoplankton were composed mainly of chrysophytes (*Chromulina* sp.) and a green alga, *Chlorella* sp.

The phytoplankton assemblage of Mystery Pond during June and July was comprised mainly of chrysophytes (*Chromulina* sp.) and non-heterocystous blue-greens, *Synechococcus sigmoides* (Moore et Carter) Komarek and *A. smithii*, with fewer greens and cryptophytes. A dramatic increase of the non-heterocystous blue-green *Merismopedia glauca* Ehrenberg began in August and continued into September (max. 3.44 $\mu\text{g l}^{-1}$ chlorophyll *a*).

Both qualitative and quantitative observations indicated differences among lakes in zooplankton community composition and density. Average dry mass was similar for Embryo and Mystery Ponds, but was lower for The Loch (Table 2). Zooplankton generally increased over the summer in all lakes, although abundant early summer filamentous algae obscured this trend in terms of dry mass for The Loch since the algae could not be separated completely from the zooplankton on the filters (Fig. 2). The Loch had a sparse zooplankton community of rotifers and cyclopoid copepods, with few *Daphnia* sp. appearing later in the summer. Zooplankton in Embryo Pond were mostly cyclopoid copepods in early summer with *Daphnia* sp. and fairy shrimp, *B. coloradensis*, gaining dominance later in the season. Mystery Pond had a community of mostly rotifers (*Keratella* sp.) with fewer cyclopoid copepods and nauplii, and *Daphnia*

Table 2. Summer 1999 measurements for The Loch, Embryo Pond, and Mystery Pond. Values are the mean \pm standard deviation, with n in parentheses. *TP is for July, 2001

	The Loch	Embryo Pond	Mystery Pond
Temperature ($^{\circ}\text{C}$)	8.0 ± 2.7 (11)	9.0 ± 4.9 (13)	10.7 ± 5.0 (12)
pH	6.2 ± 0.3 (14)	6.1 ± 0.3 (16)	5.9 ± 0.3 (16)
Conductivity ($\mu\text{S cm}^{-1}$)	11.0 ± 2.9 (14)	13.9 ± 2.9 (14)	8.9 ± 0.4 (14)
$\text{NO}_3\text{-N}$ ($\mu\text{g l}^{-1}$)	224 ± 60 (15)	111 ± 97 (16)	5 ± 1 (16)
$\text{NH}_4\text{-N}$ ($\mu\text{g l}^{-1}$)	13 ± 15 (15)	25 ± 15 (16)	17 ± 11 (16)
$\text{PO}_4\text{-P}$ ($\mu\text{g l}^{-1}$)	3 ± 1 (15)	6 ± 1 (16)	8 ± 3 (16)
TP ($\mu\text{g l}^{-1}$)	9 ± 1 (3)*	—	—
SiO_2 (mg l^{-1})	1.70 ± 0.25 (15)	—	—
DOC (mg l^{-1})	1.2 ± 0.5 (13)	4.0 ± 0.2 (14)	9.2 ± 1.1 (13)
Chlorophyll a ($\mu\text{g l}^{-1}$)	1.41 ± 1.24 (14)	1.37 ± 0.63 (15)	1.66 ± 0.82 (14)
Cells per ml ($\times 1000$)	3.3 ± 2.7 (6)	11 ± 15 (6)	44 ± 43 (6)
Zooplankton dry mass (mg m^{-3})	51 ± 40 (12)	228 ± 157 (11)	230 ± 145 (11)
Chaoborid dry mass (mg m^{-3})	0	0	60 ± 60 (11)

sp. *Chaoborus* sp. were first observed on July 21 in Mystery Pond and appeared to grow larger over the course of the season. *Daphnia* sp. were less common during late summer, while copepods remained abundant. During this time we observed many copepod individuals associated with plant material, suggesting that many were captured from the macrophyte portion of Mystery Pond. Zooplankton density on July 29 (excluding *Chaoborus* and fairy shrimp) was 1, 10, and 189 l^{-1} for The Loch, Embryo, and Mystery Ponds, respectively. Zooplankton dry mass and chl a were not correlated in The Loch or Embryo Pond, but were positively related in Mystery Pond ($r = 0.86$, $p = 0.007$).

Phytoplankton nutrient bioassays

Significant positive responses to nutrients were observed for 13 of 37 amendments (Table 3). These occurred 5, 6, and 2 times for the P, N+P, and DON treatments, respectively. No significant SiO_2 treatment effects were detected. Nutrients were not depleted and pH remained fairly stable (5.5–7.3) even for bioassays with substantial chlorophyll a gains.

During the mid-summer bioassay in The Loch, phytoplankton chl a increased in response to PO_4 enrichment (Table 3). Chlorophyll a in the N+P treatments was similar to controls while NO_3 caused a negative response, indicating phytoplankton limitation by P alone. Ambient lake NO_3 concentrations had decreased after the snowmelt pulse, but were still above $200 \mu\text{g N l}^{-1}$. The late summer bioassay occurred

under similar ambient lake NO_3 concentrations but higher chlorophyll a levels than the mid-summer trial. Responses to both P and N+P treatments increased similarly above the control, also indicating that just P was limiting to phytoplankton growth.

Phytoplankton in the Embryo Pond bioassay during early summer increased above the control in response to P. Enrichment with both N+P caused a greater response, however, signifying that phytoplankton were primarily limited by P but secondarily limited by N (Table 3). Ambient lake NO_3 levels decreased to $76 \mu\text{g N l}^{-1}$ and chlorophyll a was increasing at the start of this bioassay. Similar responses were observed in Embryo Pond's mid-summer experiment; P elicited an increase above control chlorophyll a values, while N+P caused a greater increase. The DON addition led to a slight negative chlorophyll a response. During the late summer trial, the P and N+P treatments caused similar phytoplankton chlorophyll gains, indicating deficiency in P only.

In the early and mid-summer bioassays in Mystery Pond, fertilization with N+P caused a dramatic increase in chlorophyll a . The DON additions led to smaller, yet significant, increases (Table 3). In the late summer experiment during the increase in *M. glauca*, the N+P treatment was 126% of the control, but this response was only marginally significant. Enrichment with P alone gave a negative response.

The magnitude of phytoplankton chlorophyll a response (i.e. mean percent of the control) and cell density were positively correlated ($r = 0.65$; $p < 0.0001$)

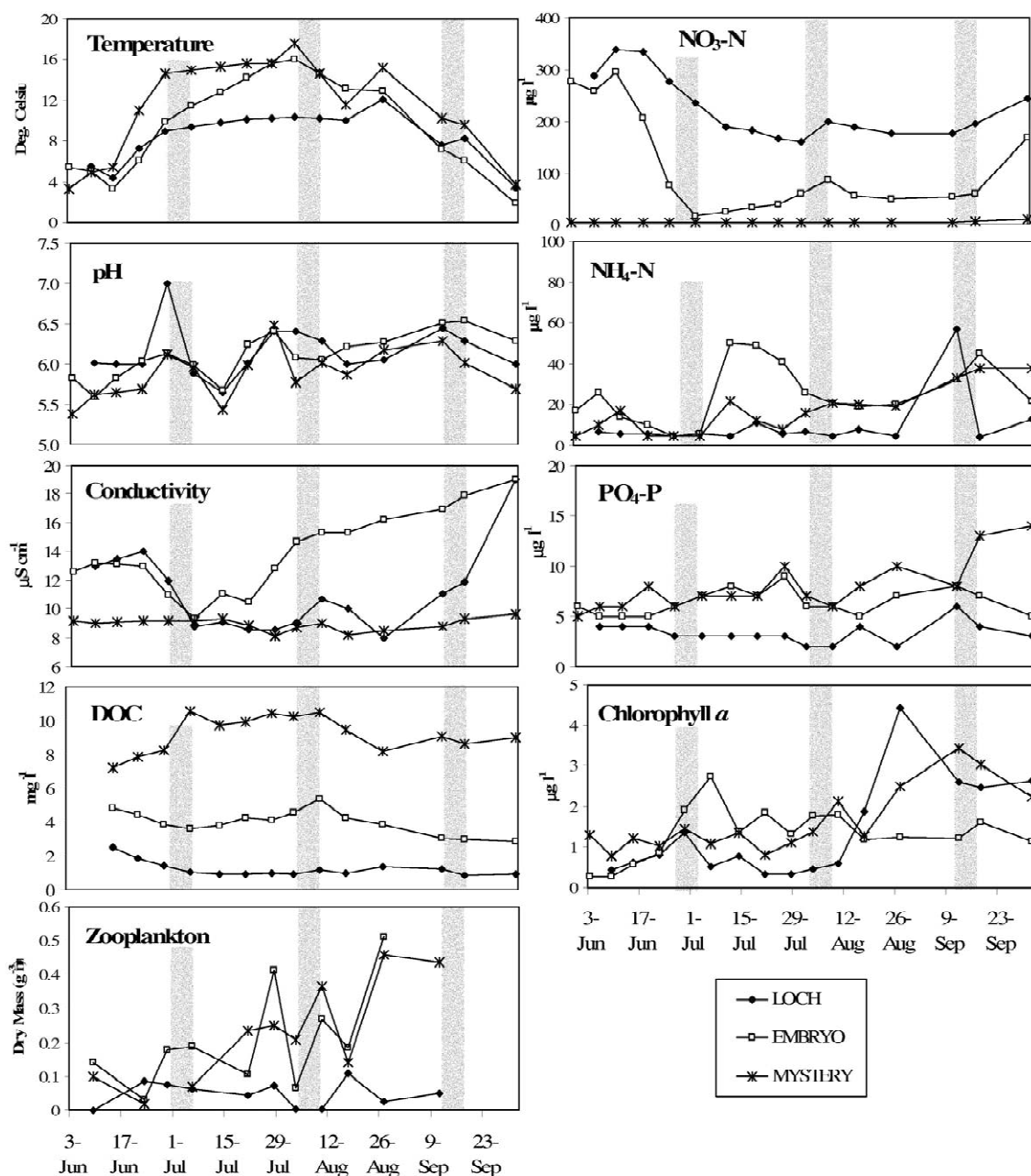


Figure 2. Summer trends in ambient lake conditions for The Loch (LH), Embryo Pond (EM), and Mystery Pond (MP). The timing of the early, mid, and late bioassays is indicated with shaded bars. The dashed line on the zooplankton plot represents Chaoborid dry mass in Mystery Pond.

over all bioassays. Qualitative observations of cell size revealed little change or small increases in cell size accompanying significant chlorophyll *a* gains. Large changes in cell density (< 55% or > 175% of control) corresponded to significant chlorophyll *a* responses roughly half the time. In general, when chlorophyll *a* and cell density responses were inconsistent, a ma-

ajor taxonomic shift occurred. Taxonomic shifts were common, but not consistent within particular nutrient treatments or lakes.

Table 3. Nutrient limitation status of phytoplankton determined from bioassay experiments conducted during early, mid, and late-summer 1999. "P lim" is P limitation, "2nd N lim" refers to secondary N limitation, and "N+P co-lim" means closely coupled N and P limitation. *p*-values (*n* = 3) are for the treatment compared to control, except where indicated as a comparison between treatments (i.e. "P-NP"). Also shown are the magnitudes of significant chl *a* responses compared to the control. Treatments are: N = NO₃, P = PO₄, NP = NO₃ + PO₄, DON = urea, and Si = SiO₂

	Early			Mid			Late		
	Trts	%	<i>p</i> -value	Trts	%	<i>p</i> -value	Trts	%	<i>p</i> -value
The Loch					P lim			P lim	
				N	29	<0.0001	N		0.39
				P	203	<0.0001	P	245	<0.0001
				NP		0.24	NP	245	<0.0001
				DON		0.33	DON		0.56
				Si		0.19			
Embryo		P lim, 2nd N lim			P lim, 2nd N lim			P lim	
	N		0.68	N		0.99	N		0.50
	P	136	0.04	P	146	0.02	P	172	0.001
	NP	194	<0.0001	NP	206	<0.0001	NP	160	0.003
	DON		0.13	DON	79	0.02	DON	131	0.09
	Si		0.40	Si		0.97			
	P-NP		0.03	P-NP		<0.0001	P-NP		0.77
Mystery		N lim, N+P "co-lim"			N lim, N+P "co-lim"			N and P sufficiency	
	N		0.14	N		0.25	N	71	0.06
	P		0.80	P		0.52	P	57	0.001
	NP	369	<0.0001	NP	227	<0.0001	NP	126	0.09
	DON	156	0.004	DON	168	0.001	DON		0.51
	Si		0.48	Si		0.59			
	NP- DON		<0.0001	NP- DON		0.06			

Discussion

Variability in ambient conditions

Seasonal nutrient trends varied among the study lakes despite regionally elevated N deposition and appeared to be influenced mainly by watershed characteristics and hydrology. High seasonal means and snowmelt pulses of NO₃ were associated with rocky watersheds and talus slopes, while consistently low lake NO₃ concentrations were observed for a heavily forested catchment. Unvegetated terrain similarly was linked to high autumn NO₃ concentrations in lakes in the High Tatra Mountains, central Europe (Kopáček et al., 1995, 2000) and to high mean annual NO₃ levels in Colorado Rocky Mountain streams (Clow & Sueker, 2000). Lack of vegetation is often associated with steep, rocky slopes which limit NO₃ removal. In addition, talus environments are sites of N mineralization and nitrification, processes that contribute to high NO₃

concentrations in drainage waters (Clow & Sueker, 2000).

Watershed hydrology also influenced lake NO₃ concentrations by controlling NO₃ influx. Permanent flow to The Loch provided continuous input throughout the summer, resulting in sustained high NO₃ concentrations. In contrast, lack of inflow to Embryo Pond after snowmelt was reflected by reduced lake NO₃ concentrations during mid-summer. These differences in mid-summer NO₃ concentrations are thought to be due to the amount of NO₃ input rather than lack of NO₃ uptake in The Loch. When enclosures in The Loch interrupted inflow, NO₃ concentrations fell below detection limits within a week, demonstrating substantial capacity for NO₃ uptake (K. Nydick, pers. obs.).

In contrast to NO₃, higher lake DOC and slightly elevated PO₄ concentrations were associated with fringing wetlands and/or forest soils. Wetlands retain NO₃ (Baron et al., 1983; Saunders et al., 2000), but also release DOC and P (Meili, 1992). Thus, dis-

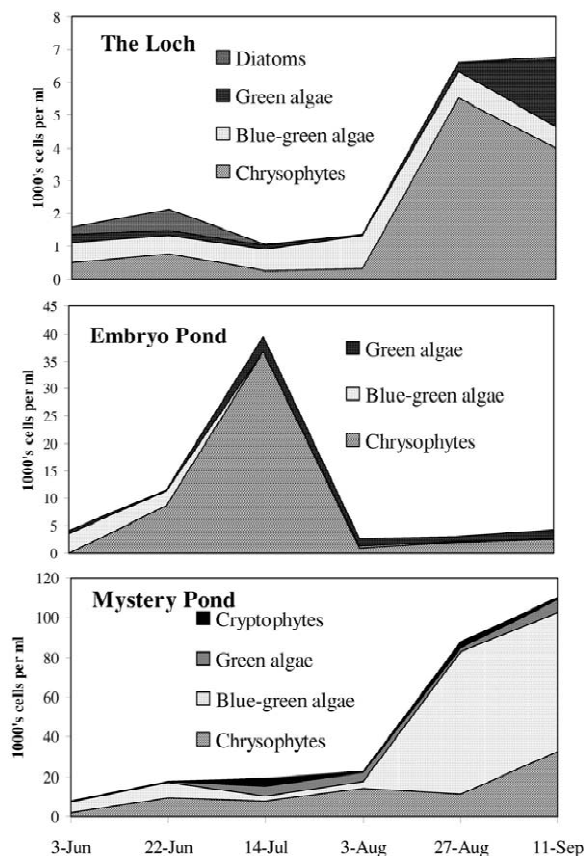


Figure 3. Abundance of major phytoplankton divisions for The Loch (a), Embryo Pond (b), and Mystery Pond (c).

solved and particulate P often are correlated with DOC (Nurnberg & Shaw, 1998; Saunders et al., 2000), and a similar relationship existed between PO_4 and DOC in our study lakes ($r = 0.69$, $p < 0.0001$). Forest soils can also have a similar effect on DOC and P concentrations. For example, lakes with forested catchments had the highest concentrations of DOC and TP in the High Tatra Mountains (Kopáček et al., 1995, 2000).

Phytoplankton biomass is the result of multiple influences, including not only nutrient inputs, and zooplankton grazing, but also factors such as nutrient recycling, temperature, and hydraulic flushing (Carpenter et al., 1996, 1998; Elser & Urabe, 1999). In the present study, phytoplankton biomass increased following early season pulses of NO_3 . Such snow-melt driven phytoplankton peaks are common in high-elevation drainage lakes (McKnight et al., 1990). Average summer chlorophyll *a* concentrations were remarkably similar among lakes, however, despite differing nutrient concentrations, zooplankton grazing

pressure, and water temperatures. Zooplankton grazing did not seem to control phytoplankton biomass in any of the study lakes. Warmer maximum water temperatures corresponded with higher DOC concentrations among the lakes, but effects of temperature on phytoplankton and zooplankton biomass were not apparent. N recycling, evident in the study lakes from the increases in NH_4 following chlorophyll *a* peaks, can maintain productivity even in lakes with very low mid-summer NO_3 inputs (Axler et al., 1982; Dodds & Priscu, 1990). Nutrient recycling can be particularly important in lakes with aquatic macrophytes, which obtain nutrients from the sediment and release them to the water column during senescence (Landers, 1982). This was evident during late summer in Mystery Pond where elevated NH_4 and PO_4 concentrations accompanied a dramatic increase in chlorophyll *a*. Thus, late summer phytoplankton biomass was probably influenced by differential nutrient recycling in the study lakes.

Response to additional nutrients

The study lakes also varied in terms of their response to nutrient enrichment experiments. P was the nutrient most limiting to phytoplankton growth, but N was also important in some cases. Phytoplankton P limitation decreased and N deficiencies increased with greater ambient PO_4 and DOC concentrations presumably due to input from forest soils and fringing wetlands. For example, in Mystery Pond, which had both a forested watershed and fringing wetlands, phytoplankton N deficiencies were the strongest among the study lakes. Interestingly, in Mystery Pond's early and mid-season bioassays enrichment with DON or $\text{NO}_3 + \text{PO}_4$ elicited increased phytoplankton biomass, but neither NO_3 nor PO_4 -alone caused additional growth. This situation may have been due to taxa-specific requirements for different N sources, although we could not ascertain the nature of these preferences. The apparent lack of phytoplankton nutrient deficiency in the late summer experiment may have been due to enhanced nutrient availability associated with macrophyte senescence (Landers, 1982), or the predominance of *Merismopedia* sp., which is less capable of using NO_3 as its N source and may also control growth responses of other taxa via allelopathy (Blomqvist, 2001).

Certain chemical and biological conditions may affect phytoplankton response to added nutrients. For example, phytoplankton can be efficient at taking up both NO_3 and NH_4 at very low concentrations (Axler

et al., 1982), but molybdenum (Mo) deficiency and high NH_4 availability can inhibit NO_3 uptake (Axler et al., 1980, 1982; Wheeler & Kokkinakis, 1990). Summer Mo concentrations in The Loch were $0.4\text{--}0.5\ \mu\text{g l}^{-1}$ during 1996–1998 (Shiller, 1999), which are similar to the median value in Sierra Nevada mountain lakes (Bradford et al., 1968), but higher than Castle Lake, CA where Mo deficiencies exist (Bachman & Goldman, 1964). Mo concentration in The Loch increased throughout the summer (Shiller, 1999) suggesting that it was not being depleted by biological processes. Furthermore, $^{15}\text{NO}_3$ isotope tracer studies in The Loch suggest that phytoplankton NO_3 uptake occurs even during mid-summer (K. Nydick, pers. obs.). High DOC concentrations may reduce the availability of micronutrients (Williamson et al., 1999) such as Mo, however, and this may be a factor explaining why DON, but not NO_3 caused phytoplankton increases in Mystery Pond.

Nutrient excretion by zooplankton and fish also may influence which nutrient or nutrients limit algal growth. Top-down control of zooplankton by trout predation was evident in The Loch, which had much lower zooplankton dry mass and smaller taxa, but similar chlorophyll *a* levels compared to the fishless lakes. Trout stocking can increase P availability by regenerating terrestrial and benthic nutrients (Schindler et al., 2001), and by shifting the zooplankton size structure away from large zooplankton with low N:P nutritional requirements (Elser et al., 1988). These relationships favor N limitation in The Loch, where trout were present but zooplankton were small and sparse, and P limitation in Mystery and Embryo Ponds, which did not have fish but had larger and more abundant zooplankton. We found just the opposite, however, and infer that nutrient recycling by fish and invertebrates was not a major influence on phytoplankton nutrient limitation among the study lakes. Hydrology and watershed characteristics, most notably the amount of forested versus unvegetated terrain and the presence of fringing wetlands, appeared to be more important in determining nutrient availability, and hence phytoplankton response to nutrient additions.

Summary and conclusions

Nutrient and DOC concentrations differed among neighboring lakes affected by regionally elevated atmospheric N deposition. The presence of unvegetated terrain in the watershed appeared to control NO_3 in-

put, while forest soils and fringing wetlands retained NO_3 and contributed PO_4 and DOC. These differences influenced phytoplankton biomass during snow-melt, but average summer concentrations were similar despite differing algal community composition, nutrient levels, and zooplankton grazing pressure. P was most limiting to phytoplankton growth, but N deficiencies became more important in lakes with forest soils and/or fringing wetlands that contributed PO_4 and DOC. The presence/absence of fish influenced zooplankton biomass, but was not a strong influence on either ambient phytoplankton biomass or response to additional nutrients. Thus, we found that the effects of regionally elevated N deposition are tempered by individual lake characteristics, most notably watershed vegetation and hydrology in the study lakes.

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