Community and Species-level Phosphorus Limitation Patterns in Alpine Lakes

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ABSTRACT

While the growth of algae in alpine lakes is often co-limited by the nutrients nitrogen (N) and phosphorus (P), nitrogen has historically been the main limiting nutrient. However, over the past century, rates of N deposition to alpine lakes have increased, resulting in more production of algae in these lakes. With the increased N in these lakes, the question arises as to whether the alpine lakes are now becoming more limited by P. The purpose of this study was to assess whether P limitation is occurring in 9 lakes of the Central Rocky Mountains by measuring the total rates of and species-specific patterns in alkaline phosphatase activity produced by algae in these lakes. Out of the 9 lakes sampled, 8 were determined to be severely limited by P, while the remaining lake had no P limitation. The lake found to have no P limitation may have some unknown source of phosphorus coming from the surrounding landscape or from dust deposition.

INTRODUCTION

In recent decades, inputs of nutrients such as phosphorus (P) and nitrogen (N) to aquatic ecosystems have increased. In lakes, rivers, and estuaries, these inputs have lowered water quality by promoting large blooms of phytoplankton, a process known as eutrophication. Eutrophication has many adverse effects on aquatic systems, including depletion of oxygen, increased incidence of fish kills, alteration of food web structure, reduction of biodiversity and loss of aesthetic value (Carpenter et al. 1998). Although point sources of nutrient inputs have been reduced, nonpoint sources, which are more difficult to control, remain high and are the major source of water pollution in the U.S. today (Havens and Steinman 1995; Carpenter et al. 1998; EPA 1996).

Potential nonpoint sources of N to aquatic systems include agricultural runoff, urban runoff, and atmospheric deposition (Howarth et al. 1996). Vitousek et al. (1997) estimate that human activities, such as fossil fuel combustion, the planting of N-fixing crops such as soybeans, and the manufacturing of inorganic fertilizer, have at least doubled the amount of fixed N transferred from the atmosphere to land-based ecosystems. Fixed N includes forms such as nitrate (NH_3) and ammonium (NH_4^+), and can be transferred from the atmosphere to aquatic and terrestrial ecosystems via wet or dry deposition. Clearly, this pool of fixed N can be a potentially large contributor to aquatic systems and can affect systems far removed from the source of N emission. For example, an increase in fixed N is detectable in glacial ice in Greenland (Vitousek et al. 1997).

Alpine lakes are excellent systems for investigating the extent to which N deposition affects aquatic ecosystems. There is typically little development on the watersheds of these lakes, thus agricultural and urban runoff tends to be low. Increased N inputs into these systems would therefore likely be from atmospheric sources. In addition, unlike in many other freshwater lakes, the production of algae in alpine lakes is primarily limited by nitrogen, and secondarily limited by P (McKnight et al. 1990; Interlandi and Kilhan 1998; Saros et al. 2005). Saros et al. (2005) demonstrated that phytoplankton communities in alpine lakes of both the southern and central Rocky Mountains have changed as a result of N additions.

With the continuation of enhanced N deposition to alpine lakes, these lakes may be driven towards P limitation of primary production. A change in nutrient limitation patterns may affect the phytoplankton community structure, which, in turn, can affect organisms higher up in the food chain, such as invertebrates and fish. Many of these lakes are situated in national parks and forests; hence they are valued for their fisheries. It is difficult to assess P limitation using nutrient measurements in these lakes, however, because P cycles rapidly among various pools (e.g., particulate versus dissolved, organic versus inorganic) in these lakes. Alternatively, assessing P limitation can be done by using alkaline phosphatase activity (APA) assays.

Alkaline phosphatase is an enzyme that can cleave phosphate from phosphomonoesters, which are organic compounds that algae can't use directly. The production of this enzyme is only induced if free phosphate is not available in the environment. Therefore, the detection of APA serves as an indicator of P limitation. APA can be measured in bulk (i.e., the whole community) and for each species, as techniques are available to label individual

cells that are expressing APA. The objective of this study was to use community and species-specific measurements of APA from primary producers in alpine lakes to assess whether P limitation is occurring. The study site consisted of 9 remote, alpine lakes within the Beartooth Mountain range of the central Rocky Mountains.

METHODS

Lake stratification was pre-determined using a Hydrolab device to determine the water temperature at 1 meter intervals. Whole water samples were collected from three different depths (epilimnion, thermo cline, and hypolimnion) in each lake using a Van Dorn column sampler. At each of the three depths a 30 mL sample was collected in 200-mL opaque bottles to be brought back to the lab. The methods of Healy and Hendzel (1979) were used to measure the bulk APA using 4-methylumbelliferyl phosphate (MUP). When P is cleaved from MUP by alkaline phosphatase, MU results- this is a fluorescent molecule. A Turner Designs Field Fluorometer was used to quantify fluorescence in these samples as a measure of APA. To run the APA assay, 0.5 mL of 0.1 mM 4-MUP solution was added to 7 one-milliliter cuvettes. A control cuvette with 3.8 mL of deionized water was used for each assay. For each lake the three depths were measures in duplicate with 3.8 mL of sample water added to each cuvette. The fluorescence was recorded initially, then at every 15 minutes thereafter, for no more than 60 minutes. An APA rate was then determined, according to Wetzel (1983), for each depth and lake, and compared to pre-determined chlorophyll *a* levels to determine the degree of P limitation based on the APA: chlorophyll *a* ratio.

For the species-specific APA, enzyme labeled fluorescence (ELF) of alkaline phosphatase was used, in which a fluorescent substrate precipitated at the site of APA on the cell surface. Phytoplankton was concentrated from 1.0 L of lake water by passing through a 10 µm Nitex mesh. Four replicates for each lake depth were collected. Each sample was then centrifuged, and the resulting pellets were incubated in 70% ethanol overnight. An ELF probe + buffer solution was added to three of the samples; buffer alone was added to one of the samples to serve as a control. Samples were then incubated in the dark for 30 minutes, at the end of which they were centrifuged. The ELF/buffer solution was aspirated off and the samples were washed with 0.2 µm filtered 0.1M phosphate buffer saline (PBS) a total of four times. Samples were then examined under brightfield and epifluorescence with a DAPI (4', 6' – diamidino-2-phenyl-indole) filter set. For each species 50 cells were to be counted and examined for fluorescence to assess the percentage of the population exhibiting APA.

RESULTS AND DISCUSSION

Species-specific APA could not be accurately determined with the phytoplankton samples collected. The ELF method sufficiently displayed individual cells with fluorescence (Figure 1). However, there was an insufficient number of individual phytoplankton in the samples to accurately determine a percentage of the population that showed fluorescence.

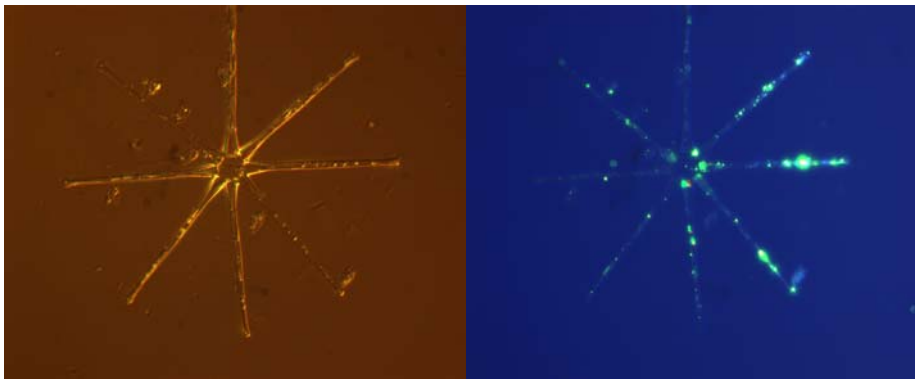

Figure 1. Left- the diatom species *Asterionella* under brightfield microscopy;
Right- *Asterionella* showing APA in green under fluorescent microscopy.

The APA: chlorophyll *a* ratios for Beartooth, Emerald, Island, Beauty, Kersey, Fossil, and Glacier lakes indicated moderate to severe P limitation from at least one depth sampled (Table 1). Heart Lake lacked P limitation at all three depths sampled (Table 1).

Table 1. Alkaline phosphatase activity and average chlorophyll *a* in alpine lakes used to determine the degree of P limitation based on an APA: Chl *a* ratio. Alpine lakes of the central Rocky Mountains, July 2006.

	Layer	APA	Chl *a*	APA:Chl (umol/ug)h^-1	º P Limitation
Beartooth (7/8/06)					
1m	Epilimnion	53	0.817	0.0649	Severe
7m	Metalimnion	48.5	1.283	0.0378	Severe
12m	Hypolimnion	27.5	3.15	0.0087	Severe
Emerald (7/9/06)					
1m	---	34.7	1.4	0.0248	Severe
9m	---	97.5	5.25	0.0186	Severe
18m	---	8	3.267	0.0024	None
Island (7/10/06)					
3m	Epilimnion	56	1.458	0.0384	Severe
6m	Metalimnion	51.5	2.1	0.0245	Severe
9m	Hypolimnion	39.5	3.15	0.0125	Severe
15m	Hypolimnion	1	3.325	0.0003	None
Beauty (7/11/06)					
3m	Epilimnion	49.5	1.167	0.0424	Severe
6m	Metalimnion	66.5	1.12	0.0594	Severe
9m	Hypolimnion	67	1.75	0.0383	Severe
15m	Hypolimnion	39.5	2.38	0.0166	Severe
Kersey (7/12/06)					
3m	Epilimnion / Metalimnion	92	1.575	0.0584	Severe
6m	Metalimnion	23	2.45	0.0094	Severe
9m	Hypolimnion	7.5	1.96	0.0038	Moderate
12m	Hypolimnion	4	1.89	0.0021	None
Heart (7/13/06)					
3m	Epilimnion / Metalimnion	12.5	4.638	0.0027	None
6m	Hypolimnion	20.5	17.15	0.0012	None
9m	Hypolimnion	28	36.75	0.0008	None
12m	Hypolimnion	15.5	16.275	0.0010	None
Fossil (7/15/06)					
5m	Epilimnion / Metalimnion	18	2.888	0.0062	Severe
9m	Hypolimnion	19.5	11.9	0.0016	None
12m	Hypolimnion	14.5	13.183	0.0011	None
25m	Hypolimnion	8.5	3.675	0.0023	None
Beartooth (7/16/06)					
3m	Epilimnion	46.5	0.875	0.0531	Severe
6m	Metalimnion	66	0.98	0.0673	Severe
9m	Hypolimnion	74.5	2.1	0.0355	Severe
12m	Hypolimnion	31.5	4.375	0.0072	Severe
Glacier (7/17/06)					
3m	---	698.5	8.925	0.0783	Severe
6m	---	733.5	10.15	0.0723	Severe
9m	---	701.5	11.725	0.0598	Severe
12m	---	685	10.5	0.0652	Severe

Heart Lake may have some unknown source of phosphorus coming from the surrounding landscape or from a source of dust deposition contributing to its pool of phosphorus. Since there was an insufficient number of phytoplankton to quantify results from the ELF method, future studies may concentrate on a more accurate method for sampling phytoplankton. Phosphorus limitation patterns had not previously been examined on these lakes, therefore, the results from the community APA assay may serve as an initial baseline to determine how P limitation patterns on these alpine lakes change over time.

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