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Orthophosphate concentration and flux within
the epilimnia of two Canadian Shield lakes

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Introduction

The need to re-investigate the forms and movements of phosphorus in lakes is acute. Recent eutrophication studies have confirmed the hypothesis that, in most lakes, phosphorus limits productivity (WINBERG & LYAKHNOVICH 1965; VOLLENWEIDER 1968; SCHINDLER et al. 1973). Concurrently, evidence indicating that the universally used molybdate blue colorimetric method for measuring inorganic phosphate measures more than orthophosphate has accumulated (KUENZLER & KETCHUM 1962; JONES & SPENCER 1963; RIGLER 1966, 1968; STANTON 1975). STANTON has demonstrated that the difference between soluble reactive phosphorus and orthophosphate is due to the hydrolyzation of phosphate from colloidal compounds under the acidic conditions of the molybdate blue reaction. Because the amount of phosphate hydrolyzed is not predictable, the value of most studies of orthophosphate cycling in lakes is questionable.

A sensitive, alternative, analytical technique for orthophosphate determination has not been devised. Because orthophosphate is believed to be the form of phosphorus most readily available to algae, we cannot afford to ignore its presence while new methods are explored.

Two crude means of estimating orthophosphate concentration exist. One, suggested by LEAN (unpubl.), is to measure total dissolved phosphorus analytically, and then determine the fraction of dissolved phosphorus which is orthophosphate by Sephadex fractionating the Millipore filtrate of P^{32} labeled lakewater. The ratio of P^{32} to P^{31} must be equal in all phosphorus compartments at the time of filtration.

Two major drawbacks limit the usefulness of this method. First, the fraction of orthophosphate in the filtrate must be high enough to elute a detectable peak. P^{32} labeled Millipore filtrate from lakes within the Experimental Lakes Area, in the Canadian Shield, generally produces only a colloidal phosphorus peak when fractionated. Second, colloidal phosphorus may continue to acquire radioactive label several days after P^{32} is added, making a determination of radioactive equilibrium difficult (LEVINE 1975). A variation on this method would be to Sephadex fractionate filtered lakewater and measure soluble reactive phosphorus only in those fractions which elute as orthophosphate. For Canadian Shield lakes, however, the orthophosphate fractions would have to be concentrated many times before they would be within the limits of sensitivity of the molybdate blue reaction.

A more sensitive technique for estimating orthophosphate concentration is the radioassay presented by RIGLER (1966) as evidence that the molybdate blue method greatly overestimates orthophosphate concentration. The method assumes that the rate constant for orthophosphate uptake decreases in a regular manner with increasing orthophosphate concentration. By calculating uptake velocity at several added orthophosphate concentrations, one can obtain a maximum estimate of what the original concentration must have been. The method requires a considerable amount of time, maintenance of in situ temperature, and extreme precautions to prevent contamination. It would not be practical for routine analysis.

The RIGLER bioassay has been used since May, 1973 to estimate orthophosphate concentration in Lake 227 and the south basin of Lake 302 in the Experimental Lakes Area, northwestern Ontario (see *J. Fish. Res. Board Can.* 28 (2), 1971).

Methods

During the summers of 1973 and 1974, Lake 227 received .024 g of P as H_3PO_4 and .31 g of N as $NaNO_3$ per square meter per week as part of a fertilization experiment (SCHINDLER et al. 1973). The annual phosphorus load was increased to about 20 times the natural load. The south basin of Lake 302 is unfertilized. Phosphorus, nitrogen, and carbon, however, are being added to the hypolimnion of the north basin during the summer.

Integrated epilimnetic samples were collected approximately biweekly during the summers of 1973 and 1974 and monthly during the winter. Temperature was measured and analyses were conducted for total dissolved phosphorus and suspended phosphorus (STAINTON, CAPEL & ARMSTRONG 1974). Information concerning other parameters was obtained from other ELA projects.

Bioassays were begun within two hours of water collection. During the summer, determination of the rate constants for uptake required about 10 minutes per concentration tested. Very slow uptake rates during the winter, however, made incubations of several hours necessary. The water was maintained at in situ temperature at all times and was stirred with a magnetic stirring rod during the bioassays.

Both P^{32} and orthophosphate solutions were prepared a few hours before each experiment and irradiated with ultraviolet light until needed. Unsterilized solutions were converted to colloidal phosphorus within hours of preparation even when stored at 4 °C.

Because uptake velocity calculations were no more reliable than $\pm .001 \mu g/l/min$, any assumed original concentration which produced a positive slope and a y-intercept of $.001 \mu g/l/min$ on a velocity-concentration graph was accepted as a maximum estimate of orthophosphate concentration. If the slope was negative when the y-intercept was $.001 \mu g/l/min$, the maximum estimate was taken to be the assumed original concentration which produced a slope of 0.

Results

Orthophosphate-phosphorus concentration was found to be very low during summer stagnation (Fig. 2). The maximum estimates of concentration fluctuated around $.015 \mu g/l$ in both lakes. During dense algal blooms in Lake 227, concentration dropped below $.001 \mu g/l$.

With fall turnover, concentration estimates increased severalfold. High concentrations, however, did not develop until late winter, when algal biomass declined to minimum values. Concentration estimates between .2 and $2.3 \mu g/l$ were obtained from this time until iceout.

Total dissolved phosphorus remained fairly constant throughout the year in both lakes, averaging $5 \mu g/l$ in 302 South and $8 \mu g/l$ in 227. Sephadex fractionation indicated that almost all Millipore filtrate phosphorus was colloidal.

Suspended phosphorus varied seasonally, roughly in proportion to changes in algal biomass and chlorophyll *a* (Fig. 1). In Lake 302 South the winter average was $8 \mu g/l$, and the summer, $5 \mu g/l$. In Lake 227, the winter average was $17 \mu g/l$ and the summer $36 \mu g/l$.

The rate constant, *K*, is the reciprocal of turnover time. Summer turnover times were very low, between .4 and 15 minutes in Lake 227, and between 5

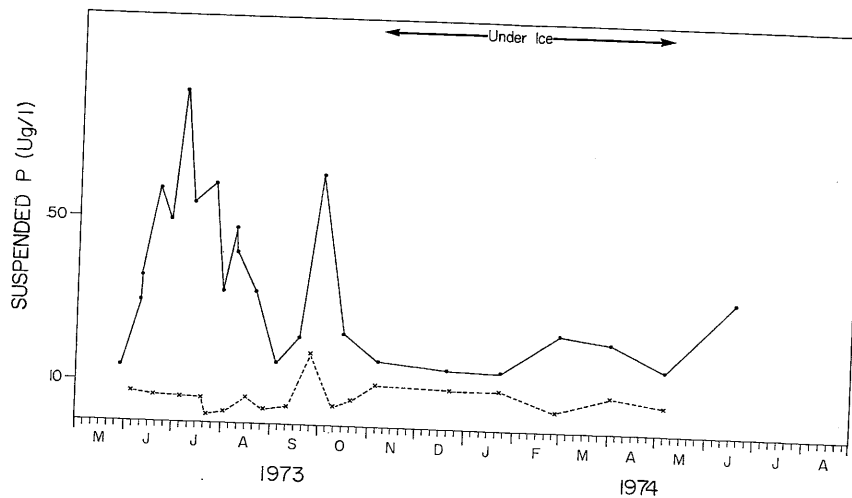


Fig. 1. Seasonal changes in suspended phosphorus.

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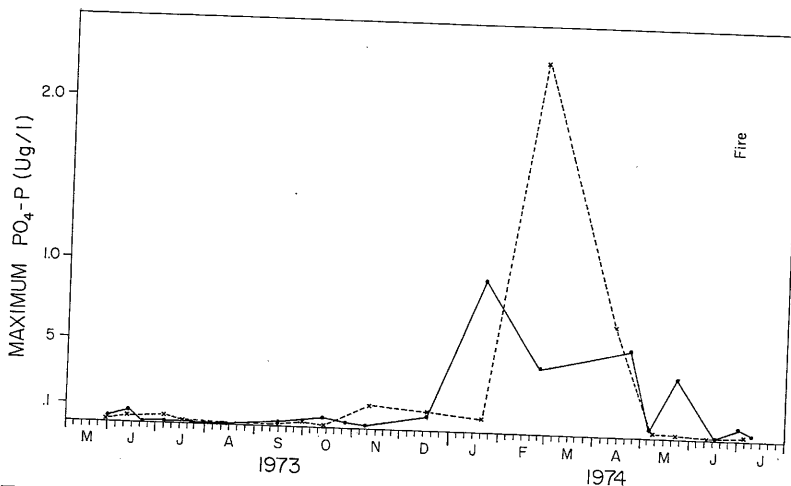


Fig. 2. Seasonal changes in maximum estimated orthophosphate concentration.

and 26 minutes in Lake 302 South (Fig. 3). During the winter, turnover time dragged to from 2 to 195 hours.

The product of the rate constant and the concentration is the flux of orthophosphate to planktonic algae and bacteria. The maximum estimated flux remained between .001 and .013 $\mu\text{g}/\text{l}/\text{min}$ in both lakes throughout the year (Fig. 5). A minor peak occurred in the fall while the phytoplankton crop was high, and the thermocline was eroding. Maximum flux values were obtained when a forest fire introduced ash into the lakes.

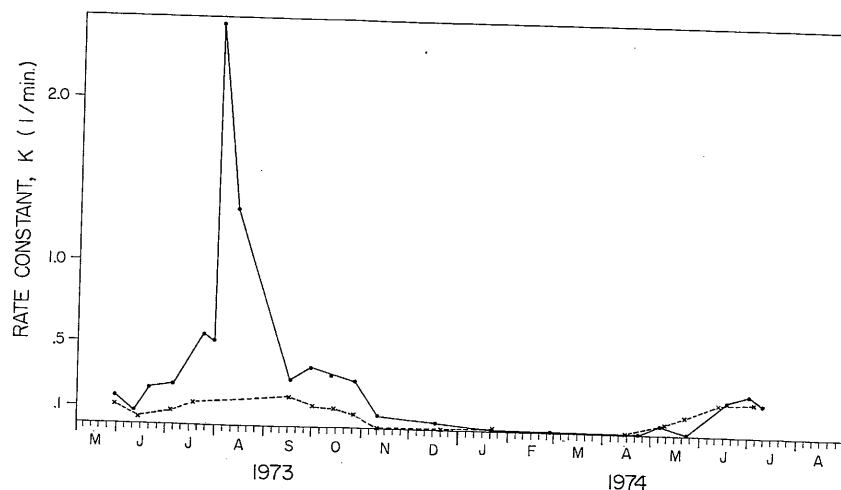


Fig. 8. Seasonal changes in the rate constant for orthophosphate uptake.

Discussion

The RIGLER bioassay does not provide minimum estimates of orthophosphate concentration. Its maximum estimates, however, seem to be reasonable. LEAN (1973) was able to integrate Sephadex fractionation peaks that were 2% of the total filtrate P^{32} activity. The lack of an orthophosphate peak from ELA lake-water during the summer, indicates that concentrations must be less than 2% of the total dissolved phosphorus (less than $1 \mu\text{g/l}$).

As long as orthophosphate additions are small, RIGLER's major assumption, that the rate constant for uptake will decrease as concentration is increased, is true in the lab. A statistically significant negative relationship between K and estimated maximum orthophosphate concentration was obtained in the two lakes as well. This relationship, however, was complicated by an additional significant correlation between K and temperature (see Fig. 4 for seasonal trends). K was not significantly correlated with suspended phosphorus or with chlorophyll a . This is not surprising since these parameters are strongly influenced by dead material and by large algae. Rapid orthophosphate uptake generally is associated with particles less than 10μ across in ELA lakes. The fact that orthophosphate flux is nearly constant throughout the year, despite 1000 fold changes in concentration and in K , argues that the relationship between concentration and K is strong. Some of the constancy, however, may be an artifact of the decision to accept an intercept of $.001 \mu\text{g/l/min}$ as equivalent to zero. If actual concentrations are much lower than their estimated maximum, the addition of $.001 \mu\text{g/l/min}$ to flux values may hide fluctuations.

The high orthophosphate concentration during the winter is probably the result of mineralization occurring in the absence of significant primary production. Production is limited by light during the winter. Mass balance calculations indicate that no phosphorus is added to the lakes from sediments during the winter (SCHINDLER 1975).

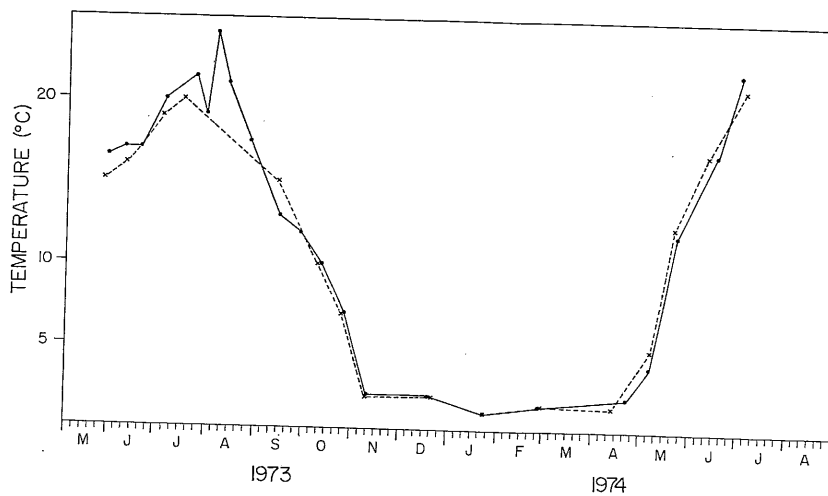


Fig. 4. Seasonal changes in temperature.

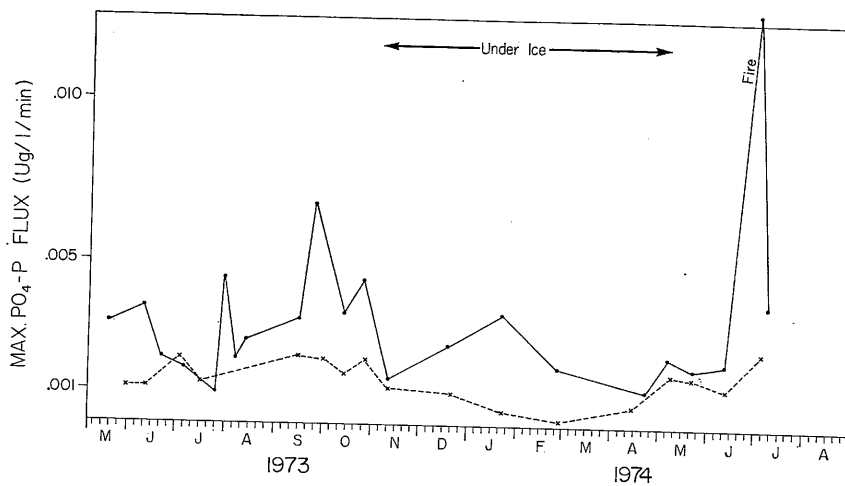


Fig. 5. Seasonal changes in maximum estimated orthophosphate flux.

During the summer, when algal biomass is great, phosphorus loading seems to have little influence on orthophosphate concentration. Phosphorus added to Lake 227 as phosphate is incorporated quickly into suspended phosphorus and into colloidal phosphorus. The fallacy of using orthophosphate concentration as a measure of eutrophication is obvious.

This paper has been concerned only with epilimnetic orthophosphate concentrations. During 1974 and 1975 concentration estimates will be calculated for water from the hypolimnia of Lakes 227 and 302S as well. The possibility of orthophosphate movement between the two strata then may be considered.

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Discussion

GOLTERMAN: Do you and the preceding speaker consider colloidal phosphate to be unavoidable for algae?

LEVINE: Only sparingly available. When ^{32}P labeled colloid obtained from Sephadex fractionation, is added to lake water, the rate constant for uptake is two orders of magnitude less than that for orthophosphate uptake.

EBERLY: Could you give more information on the uptake of phosphate by particles "less than $10\ \mu$ in size"?

LEVINE: E. L. A. lakewater which has been filtered through a $10\ \mu$ mesh takes up P^{32}O_4 as rapidly as whole lakewater. No uptake occurs in $.45\ \mu$ filtrate and very little in $3\ \mu$ filtrate. Thus particles between 3 and $10\ \mu$ seem to be primarily responsible for rapid P^{32}O_4 uptake. 50% of the chlorophyll *a* in these lakes is in this fraction. Nannoplankton, as well as bacteria, may be important.

RICHEY: Does your ^{32}P method require achieving an equilibrium between dissolved and particulate forms?

LEVINE: No. I use only the initial slope of a plot of $\log\ ^{32}\text{P}$ in filtrate vs. time. This is gross P^{32}O_4 uptake.