THE UNIVERSITY OF MANITOBA

A PRELIMINARY INVESTIGATION OF ORTHOPHOSPHATE CONCENTRATION AND THE UPTAKE OF ORTHOPHOSPHATE BY SESTON IN TWO CANADIAN SHIELD LAKES

by

SUZANNE NANETTE LEVINE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

NOVEMBER, 1975

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ABSTRACT

Past investigators have proved that conventional chemical techniques do not give accurate estimates of the biologically-active phosphorus in lake water. Soluble reactive phosphorus, long believed to be orthophosphate-phosphorus, the form of phosphorus most readily available to plankton, includes large amounts of phosphorus which are hydrolyzed from colloidal compounds during the molybdate-blue analysis. This colloidally-bound phosphorus is not removed by phytoplankton at rates comparable to PO_4-P uptake.

During the current study several previously untested methods for estimating PO_4-P concentration were tried. None of these methods proved to be very satisfactory. The chemical methods which were investigated either lacked the sensitivity necessary for PO_4-P measurement or suffered from interference by colloidal phosphorus or other compounds in lake water. Many of the radiochemical methods which were considered required isotopic equilibrium. Colloidal phosphorus acquires label so slowly that meeting this condition was impractical.

Based on the above results, it was decided that Rigler's bioassay (Rigler, 1966) was the most reliable method available for estimating PO_4-P concentration. This method was used during seasonal studies of an oligotrophic and a fertilized lake in the Experimental Lakes Area, Northwestern Ontario. Rigler's bioassay gives only maximum concentration estimates. During summer stagnation, these estimates were very low (from less than 1 to 180 ng/l) for both epilimnion and hypolimnion samples. PO_4-P concentration was only slightly higher in the hypolimnion than in

the epilimnion during the summer, although the hypolimnions of both lakes were anoxic. The flux of PO_4-P to seston was substantial (about 3 µg/l/day, on average) and was nearly equal in the epilimnion and hypolimnion. This suggests that organisms, rather than inorganic reactions, control PO_4-P concentration at all depths in a lake. The highest concentration estimates were obtained for epilimnion samples during the winter.

Although the concentrations of particulate phosphorus, ATP, total dissolved phosphorus, and chlorophyll-a were much greater in the fertilized lake, the PO_4-P concentrations in the fertilized and unfertilized lakes were similar. Apparently, the added PO_4-P was removed by seston before samples were taken. It then either was sedimented or was converted to colloidal phosphorus.

ACKNOWLEDGMENTS

I am indebted to my supervisor, Dr. D.W. Schindler, for advice and encouragement during the course of this project. R.A. Reid, D. Findlay, M. Buckley, D.W. Schindler, R. Flett, E. Matheson and A. Turner all helped with sampling on one or more occasions. J. Prokopowich and the staff of the E.L.A. analytical laboratory performed analyses for particulate phosphorus and total dissolved phosphorus.

Drs. D.W. Schindler and F.P. Healey offered constructive criticism of the manuscript in its early stages.

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GENERAL INTRODUCTION

In most lakes phosphorus limits primary production (Winberg and Lyaknovich, 1965; Vollenweider, 1968; Schindler et al., 1973). By exercising this control, it also curtails production at higher trophic levels and regulates the abundance and distribution of the combined forms of other nutrients.

An awareness of the forms and movements of phosphorus in lakes should serve as a skeleton for many limnological studies. Nevertheless, the phosphorus cycle is one of the least understood nutrient cycles. Part of the confusion about phosphorus cycling has resulted from the common practice of measuring nutrient concentrations, without considering fluxes or loading rates. Even more important has been the use of separation and analytical techniques which often measure meaningless phosphorus compartments.

The Molybdate Blue Method

Until recently molybdate blue colorimetric methods have been used almost exclusively to estimate phosphorus concentrations in lake water. These techniques involve the reaction of orthophosphate with molybdate, under acidic conditions, to form yellow heteropoly acids. These acids then are reduced to blue compounds of unknown composition. The intensity of the blue color is proportional to orthophosphate concentration.

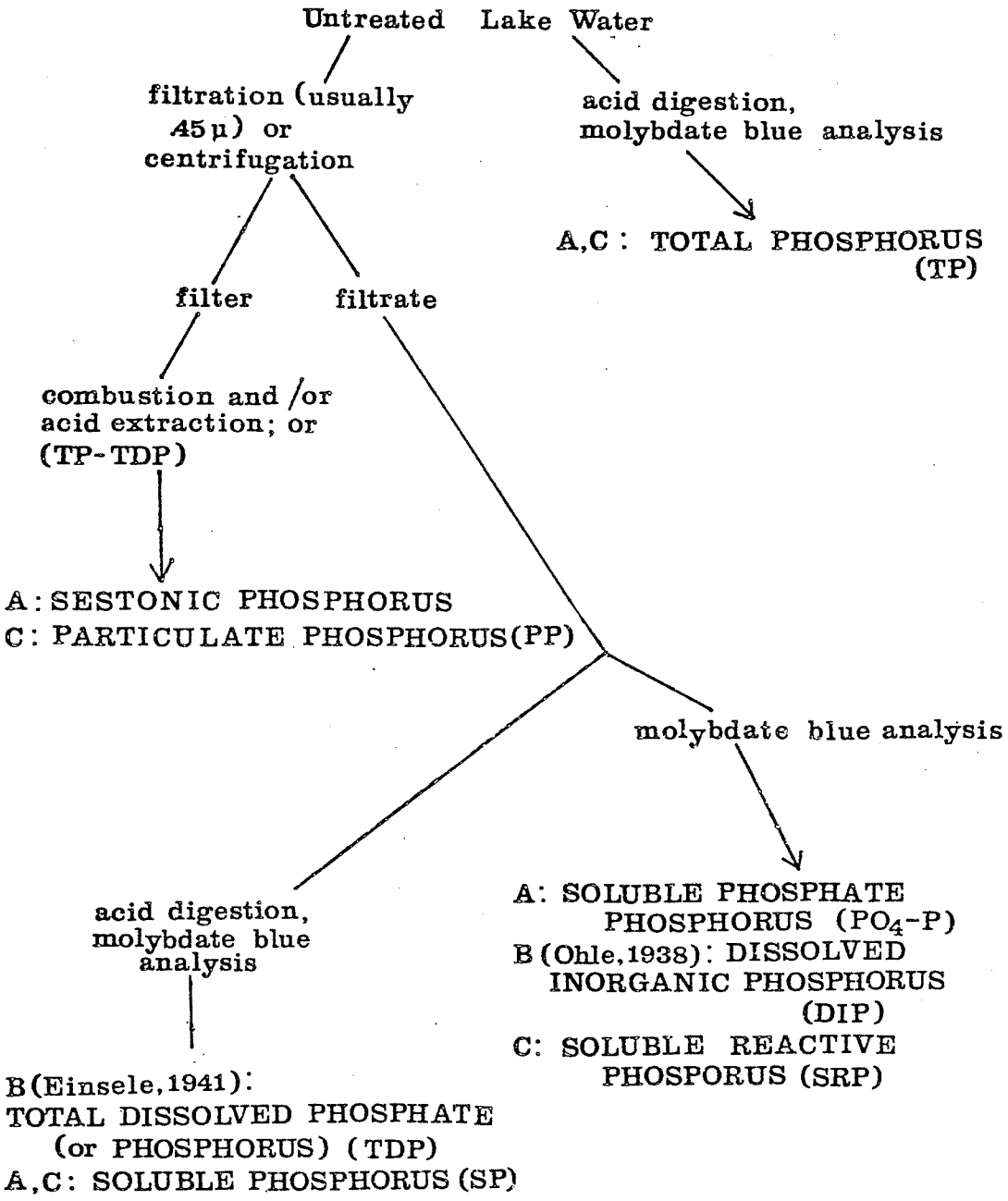
The basic procedure was developed by Osmond (1887) to measure orthophosphate concentration in laboratory solutions. During the

1920's, Deniges (1921) and Atkins (1923) modified the procedure so that it was sensitive enough to produce color in lake water samples. Over the years limnologists began to apply the technique, and concepts about the forms of phosphorus in lake water evolved. Juday, Birge, Kemmerer and Robinson (1928) measured"orthophosphate"in both undigested and digested whole lake water. They called the former "soluble phosphorus" and the latter "total phosphorus". The difference between the two became known as "organic phosphorus".

Ohle (1938) further differentiated the phosphorus fractions by using 0.75μ filters to separate suspended from "dissolved" phosphorus. He recognized that "dissolved organic phosphorus" might be in colloidal form. The four phosphorus fractions which Ohle defined, "dissolved inorganic phosphorus", "dissolved organic phosphorus", "suspended inorganic phosphorus", and "suspended organic phosphorus", along with combinations of these fractions, have been measured ever since. A key to the most commonly measured fractions is presented in Figure 1. Few workers have measured all the phosphorus fractions. Often only soluble reactive phosphorus is determined. This fraction generally is interpreted as free orthophosphate, the form of phosphorus believed to be most readily available to phytoplankton (see Hooper, 1973).

The details of the molybdate blue method have been modified many times since the 1930's. A large variety of filter pore sizes and centrifuge techniques have been used to separate suspended from "dissolved" phosphorus. In 1964, Rigler demonstrated that most of the range in the relative proportions of the phosphorus fractions

Figure 1. A key to the most commonly measured phosphorus fractions. Terms preceded with an "A" are those popularized by Hutchinson (1957). If another term has been as or more widely used, it is listed as well (B). The name of the investigator first to use the term is included. Those terms preceded with a "C" were recommended by Strickland and Parsons (1965) as being less presumptuous than previously used terms. In the text of this thesis the abbreviation "PO_4-P" refers to orthophosphate-phosphorus, not to soluble phosphate phosphorus.


Untreated Lake Water
filtration (usually 45 µ) or centrifugation
acid digestion, molybdate blue analysis
A,C : TOTAL PHOSPHORUS (TP)
filter
filtrate
combustion and /or acid extraction; or (TP-TDP)
A: SESTONIC PHOSPHORUS
C: PARTICULATE PHOSPHORUS(PP)
molybdate blue analysis
acid digestion, molybdate blue analysis
A: SOLUBLE PHOSPHATE PHOSPHORUS (PO_4-P)
B (Ohle, 1938): DISSOLVED INORGANIC PHOSPHORUS (DIP)
C: SOLUBLE REACTIVE PHOSPORUS (SRP)
B (Einsele, 1941): TOTAL DISSOLVED PHOSPHATE (or PHOSPHORUS) (TDP)
A,C: SOLUBLE PHOSPHORUS (SP)

reported in the literature could be due to differences in separation techniques.

A diversity of reducing agents, additives, and solvent extraction methods also have been used. Olsen (1966), surveying the orthophosphate literature between 1960 and 1966, counted about 100 variations in the analytical technique for soluble reactive phosphorus. The authors also reported their results in a great variety of units.

Lack of standardization, however, has not been the only problem with the molybdate blue method. The method is so simple and the results so replicable that few limnologists have questioned its validity.

Errors Involved in the Molybdate Blue Method

Two potentially major sources of error in the molybdate blue method exist. First, anions of Group IV (Si and Ge) and other Group V (As) elements of the periodic table react with molybdate under acidic conditions. Fortunately, the optimum pH for the reaction of molybdate with silicate and germanate is higher than that for the reaction with orthophosphate and arsenate. Interference of Group IV elements with orthophosphate measurement, therefore, usually can be avoided. Occasionally, however, the silicate concentration in samples (i.e. in some sediment fusions) is extremely high and silicate interference is significant.

Until recently, no one was greatly concerned about arsenate interference with orthophosphate measurement because arsenic seemed to be rare. Recently, however, Chamberlain and Shapiro (1969) reported that the arsenate concentration in Minnesota lakes is great enough to account for the discrepancies in orthophosphate estimates

which have been obtained by different molybdate blue techniques in that state. Orthophosphate and arsenate react optimally with molybdate under similar conditions, but molybdoarsenic acid is formed and reduced at a slower rate than molybdophosphoric acid. Arsenate interference with orthophosphate measurement, therefore, can be minimized by extracting the molybdophosphate into isobutyl alcohol before acid reduction is complete (Chamberlain and Shapiro, 1969). For total phosphorus and total dissolved phosphorus analyses, arsenic interference may be eliminated by reducing the samples with sodium metabisulfite and sodium thiosulfate before analysis (van Schauwenburg and Walinga, 1967). This mixture converts arsenate to arsenite, but is too weak to reduce orthophosphate. Because organic phosphorus compounds may hydrolyze under reducing conditions, this method can not be used to avoid arsenate interference with SRP measurement.

The second potential source of error in the molybdate blue method is the hydrolysis of organic phosphorus compounds. This possibility was recognized almost from the beginning of inorganic phosphorus measurement, but it never was believed to be serious. Some workers attempted to avoid hydrolysis by working fast (Fiske and Subbarow, 1925; Crouch and Malmstadt, 1967; Chamberlain and Shapiro, 1969; and Javier, et al., 1969), by lowering the reaction pH (Lowry and Lopez, 1946) (which actually augmented the problem), or by separating the phosphomolybdate from solution with an organic solvent (Berenblum and Chain, 1938).

In the last decade, evidence that the molybdate blue method may, in fact, greatly overestimate orthophosphate concentration in

natural waters has accumulated. The first major indication arose during a study by Kuenzler and Ketchum in 1962. They were working with Phaeodactylum tricornutum cultures, attempting to assess the influence of algal populations on phosphorus recycling rates in the ocean. To obtain flux values, they measured phosphorus partitioning between the algae and the 0.8μ filtrate at what they believed to be equilibrium conditions, using both analytical and radiotracer methods. Surprisingly, the orthophosphate concentration predicted from the equation PO_4-P=TP x ^{32}P filtrate/^{32}P total was only 4% of the chemically determined value. Rigler (1968) obtained similar results using lake water.

More evidence appeared when Jones and Spencer (1963) attempted to remove orthophosphate from sea water by running the water through a column of ion exchange resin. The column removed 98% of the SRP from an 0.55 M NaCl solution containing 31 μg PO_4-P/l, but only 15% of the molybdate reactive material was removed from sea water containing 15.5 μg "PO_4-P"/l. Rigler (1968) found that the same resin would remove 96% of the ^{32}P added as orthophosphate to a sample of lake water, but only 74% of the SRP. In studies with hydrous zirconium oxide, a material with a high affinity for orthophosphate, Rigler (1968) observed a similar discrepancy between the uptake of $^{32}PO_4$ and SRP.

Rigler presented yet another observation condemning the molybdate blue method in 1966. He reasoned that the rate constant for orthophosphate uptake would decrease in a regular manner as orthophosphate concentration was increased. Adding $^{32}PO_4$ and various amounts of unlabeled orthophosphate to samples of lake water, he

calculated uptake velocities by multiplying each orthophosphate concentration (the amount he had added plus the SRP) by its corresponding uptake rate constant. If the molybdate blue estimate of orthophosphate concentration had been correct, a plot of uptake velocity versus concentration would have intercepted the x and y axes at (0,0). The actual curve, however, swung upward as it approached the y-axis, intercepting it at a very high value. By assuming various original orthophosphate concentrations, and testing each with his plot, Rigler discovered that the molybdate blue method was overestimating orthophosphate concentration by at least 10 to 100 times.

Proof that the molybdate blue method measures forms of phosphorus other than orthophosphate has been obtained this year. Chamberlain (1968) and Lean (1973) demonstrated that most "dissolved" phosphorus actually is in colloidal form. Using Sephadex gel fractionation, Stainton (1975) showed that these colloidal phosphorus compounds hydrolyze when they are subjected to the acidic conditions of the molybdate blue reaction. He then demonstrated that the cleavage products are measured as soluble reactive phosphorus. Five popular molybdate blue procedures were tested. Hydrolysis was significant in every case.

The Present State of Phosphorus Measurement

With the demonstration that the molybdate blue method does not separate inorganic from organic phosphorus, one of the two dichotomics used to define the phosphorus compartments in lake water

was shown to be meaningless.

The validity of the remaining step, the separation of suspended from "dissolved" phosphorus, also has been questioned. First, the term "dissolved phosphorus" is not appropriate for the largely colloidal filtrate.[1] Furthermore, colloidal phosphorus is only partially passed through an 0.45μ filter (Rigler, 1964, Chamberlain, 1968). The proportion of colloidal phosphorus passed may not be constant over a season or between lakes.

Thus, after 50 years of estimating phosphorus concentrations with the molybdate blue method, the only trustworthy data available are total phosphorus measurements. Even this information may be faulty for lakes where arsenic concentrations are high.

Objectives of the Present Study

The work described in this thesis is an attempt to separate orthophosphate from other forms of phosphorus, especially from those forms which are included in the SRP fraction, and to make a preliminary study of the seasonal dynamics of orthophosphate concentration and its flux to seston.

Orthophosphate was chosen for study largely because it has the reputation of being the biologically most active form of

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1. During the remainder of this thesis these two phosphorus compartments will be referred to as "filter retainable" and "filtrate" phosphorus when theories are presented or when radioisotope experiments are discussed. To conform with existing terminology, the terms TDP and PP will be used when the results of chemical analyses are discussed.

phosphorus in lakes and because the radioisotopes ^{32}P and ^{33}P are conveniently available as orthophosphate salts. The latter property facilitates the use of radiochemical methods for estimating concentration and flux. These methods generally are far more sensitive than chemical ones.

To treat the many aspects of this study with the least amount of confusion for the reader, the body of this thesis has been divided into two sections. The first is concerned with the methods for estimating orthophosphate concentration which were investigated, and the second with seasonal changes in orthophosphate cycling.

SECTION I

METHODS FOR ESTIMATING ORTHOPHOSPHATE CONCENTRATION

Introduction

Finding a new chemical method to measure orthophosphate concentration is a difficult, and thus far, unfulfilled task. Any technique devised either must be gentle enough not to hydrolyze colloidal phosphorus (the pH and the ionic content of the sample must not be altered) or the orthophosphate must be separated from other forms of phosphorus prior to the analysis. Furthermore, the method must be very sensitive. The concentration of SRP, of which orthophosphate-phosphorus may be but a minor component, often is reported to fall below the limit of detection, 1 µg/l. The most sensitive tools now available for quantitative anion analysis are flame photometry combined with gas chromotography (Matthews et al., 1971) and fluorometry (Guyton and Shults, 1969). Flame photometry can detect a few nannograms of phosphorus, but only about 5 µl of sample can be injected at a time. The limit of detection, therefore, is about 20 µg/l. Fluorometry, using the aluminum-morin system, is about as sensitive as the molybdate blue method. Methods for determining orthophosphate concentration without interference from other anions have not been devised for either of these approaches. Flame photometry, furthermore, requires that the phosphorus be silylated (converted to a gaseous form, tris-(trimethylsilyl) phosphate) before analysis.

During the course of this study, several untested means of

estimating orthophosphate concentration were investigated. To maintain a sense of continuity, these methods are presented here in separate sub-sections before they are compared in the discussion. Experimental procedures which were common to all of the methods and which could alter either the rate constant for orthophosphate uptake, K, or orthophosphate concentration are assessed in Appendix I.

Sephadex Fractionation

The first method considered was one suggested by Schindler, Lean and Fee (1975). The principle was to measure the TDP concentration of a sample, and then determine the fraction of this phosphorus which is PO_4-P by Sephadex fractionating the 0.45μ filtrate of ^{32}P-labeled lake water. This method requires that the ratio of ^{32}P to ^{31}P be equal in all phosphorus compartments at the time of filtration. A variation on this method would have been to Sephadex fractionate filtered lake water and measure SRP only in those fractions which eluted as orthophosphate.

Methods

Although both Chamberlain (1968) and Lean (1973) had published orthophosphate uptake curves showing that an initial rapid uptake is followed by several hours of what seems to be equilibrium conditions, it was decided to label <u>in situ</u> columns of water with $^{32}PO_4$ and follow uptake in them to be certain that long-term changes in ^{32}P distribution were not occurring.

The columns were made of polyethylene, were 3 m long, and 27 cm in diameter, and were suspended from wooden floats at the lake surface. During the summer of 1973, 3 columns were set up in Lake 227 and 2 in the south basin of Lake 302, in the Experimental Lakes Area, Northwestern Ontario (see J. Fish. Res. Board Can. Vol. 28, No. 2, Feb. 1971). About 2 mCi of carrier-free $H_3{}^{32}PO_4$ (obtained from

Atomic Energy of Canada) were added to each column. The procedure was to mix the $^{32}PO_4$ with an amount of lake water equal to the volume of a 2 m long PVC tube. The tube was held vertically in the column while the mixture was added to its mouth at the surface. After addition, the tube was raised slowly, with a swirling motion, so that the $^{32}PO_4$ would be distributed throughout the column. The columns were incubated from 4 to 7 days. An epilimnion water sample was returned to the laboratory on the day of each "spike", and $^{32}PO_4$ uptake was followed in this sample until the first in situ aliquot was obtained. Samples were collected with another 2 m long PVC tube, which was stoppered with a rubber bung.

Sephadex fractionation of the 0.45μ filtrate of column samples was performed at least once during each incubation. TDP and PP analyses (see Figure 1) also were made. G-25 (fine) Sephadex columns with bed dimensions of 2.5 cm by 45 to 55 cm were prepared according to the Pharmacia technical literature. The eluant contained 0.3% NaCl and 0.01% NaN_3. Although the eluant was boiled during preparation, and although NaN_3 is a strong bacteriacide, the Sephadex columns occasionally became contaminated with bacteria. In 1974, the concentration of NaN_3 was raised to 0.05% and the Sephadex columns were disassembled and washed with 50% ethanol, followed by several rinses with fresh eluant, before each experiment.

Samples were pumped from a syringe into a line leading to the bottom of the Sephadex column, using a Buchler peristalic pump. Eluant followed each sample. The flow rate was 1.1 ml/min. 5 ml samples were collected with a Gilson aliquogel fractionator and collector.

The Sephadex columns were calibrated, using blue dextran to determine void volume, and $^{32}PO_4$ to determine the elution volume of orthophosphate. ^{32}P activity was measured on a Picker Nuclear scintillation counter using Cerenkov counting (Brown, 1971).

Results

$^{32}PO_4$ added to epilimnion water samples incubated in beakers in the laboratory, and to in situ columns of epilimnion water, rapidly was converted to sestonic ^{32}P. In the laboratory-incubated samples, only 0.6 to 3% of the ^{32}P activity remained in the 0.45μ filtrate after 2 hours. The net uptake rate approached zero at this time, but, usually, a slow rise in filtrate ^{32}P activity followed (Figure 2).

After one day of incubation, the two in situ columns in Lake 302 S had 15 to 27% more of their ^{32}P activity in the filtrate than did the corresponding laboratory-incubated samples (Table 1). During the remainder of the in situ incubation, the fraction of ^{32}P activity in the remainder of the in situ incubation, the fraction of ^{32}P activity in the filtrate decreased (Figure 2 A).

In the Lake 227 columns, the fraction of ^{32}P activity in the filtrate after 24 hours was similar to that in the corresponding laboratory-incubated samples. Filtrate ^{32}P activity slowly increased throughout the remainder of the incubation (Figure 2 B).

None of the columns remained in the lake long enough for an equilibrium distribution of ^{32}P to be attained. A comparison of the specific activities of the filter retainable phosphorus and filtrate phosphorus fractions was made on the final day of each incubation (Table 1). If a state of isotopic equilibrium had been reached, the specific activities of the two fractions would have been equal. Instead, the specific activity

of the filter retainable fraction consistently was greater than that of the filtrate fraction. In view of the large discrepancy between the specific activities of the two phosphorus fractions, it is questionable whether the filtrate phosphorus in these columns ever would have been completely labeled.

The results of Sephadex fractionations performed on water from the in situ columns after one or more days of incubation are shown in Figure 3. The total filtrate ^{32}P activity of the Lake 227 samples was so low that the activity of each fraction was barely above background. The Lake 302 S results are more reliable. During each fractionation almost all of the ^{32}P label appeared at void volume, as colloidal phosphorus. The $^{32}PO_4$ peak either was so low as to be undetectable or was obscured by the elution of other low molecular weight ^{32}P compounds.

Although the in situ columns did not provide orthophosphate concentration estimates, they did permit the calculation of phosphorus sedimentation rates from the epilimnion. The rate constant for this process was assumed to be equal to the difference between the rate constant for total ^{32}P loss from a column and the rate constant for radioactive decay. The average TP concentration during each incubation was multiplied by the sedimentation rate constant to yield an estimate of phosphorus flux to the sediments. The results of these calculations are presented in Table 2. The sedimentation rates calculated for Lake 227 are too great (over three times the phosphorus loading). This suggests that the specific activities of readily sedimented particles exceeded

Figure 2. The percent of total ^{32}P activity present in the filtrate fraction of lake water at various times after the addition of $^{32}PO_4$.

The solid lines refer to water incubated with $^{32}PO_4$ in in situ epilimnion columns, and the broken lines to samples which were returned to the laboratory before $^{32}PO_4$ addition. The latter samples were collected on the days when the columns were "spiked".

2A Lake 302 S

The incubations began on 6 June, 1973 (●——●) and 16 July, 1973 (o——o).

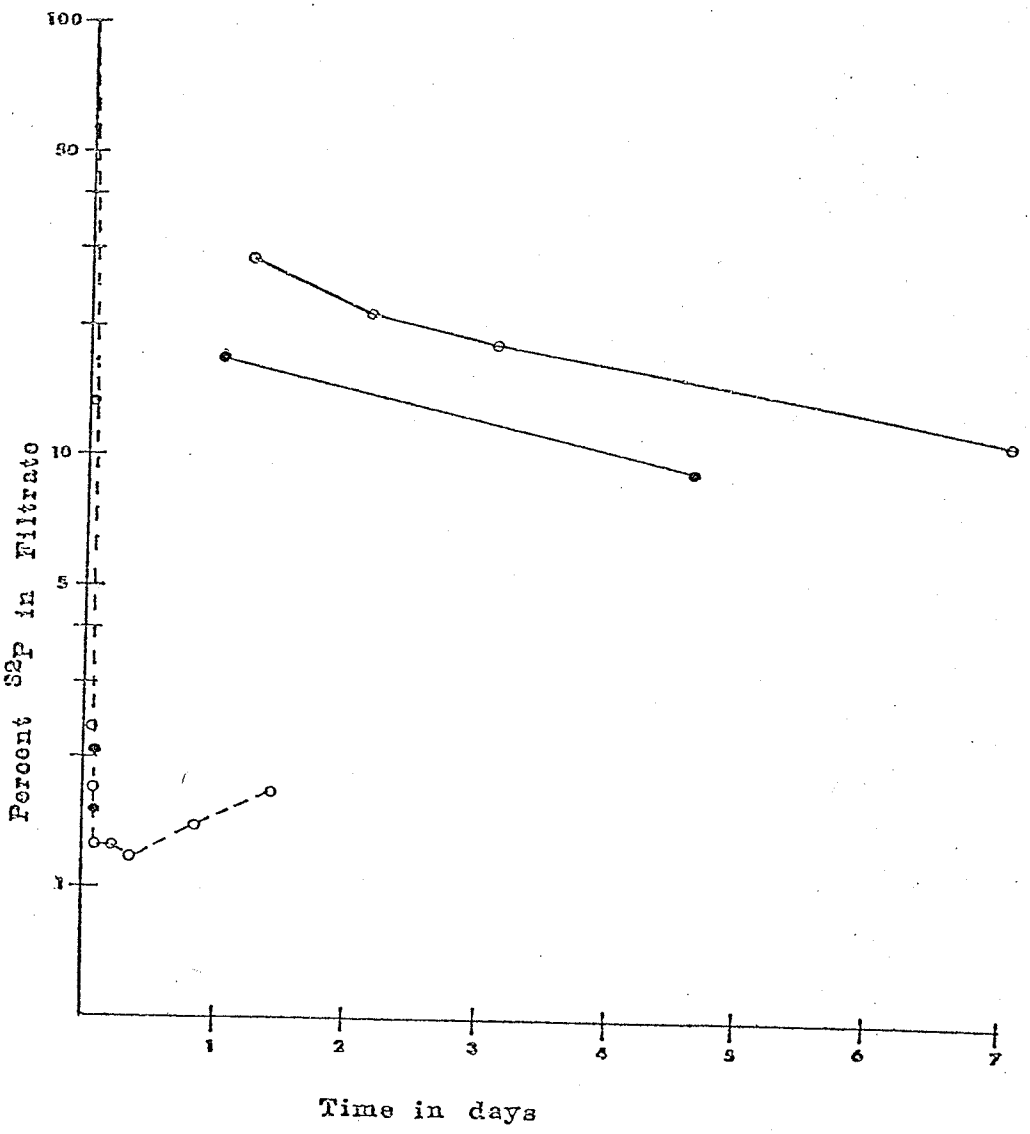

100
50
10
5
1
Percent 32P in Filtrate
1
2
3
4
5
6
7
Time in days

Figure 2 B. Lake 227.

The incubations began on 7 June, 1973 (●——●), 9 July, 1973 (○——○), and 7 August, 1973 (△——△).

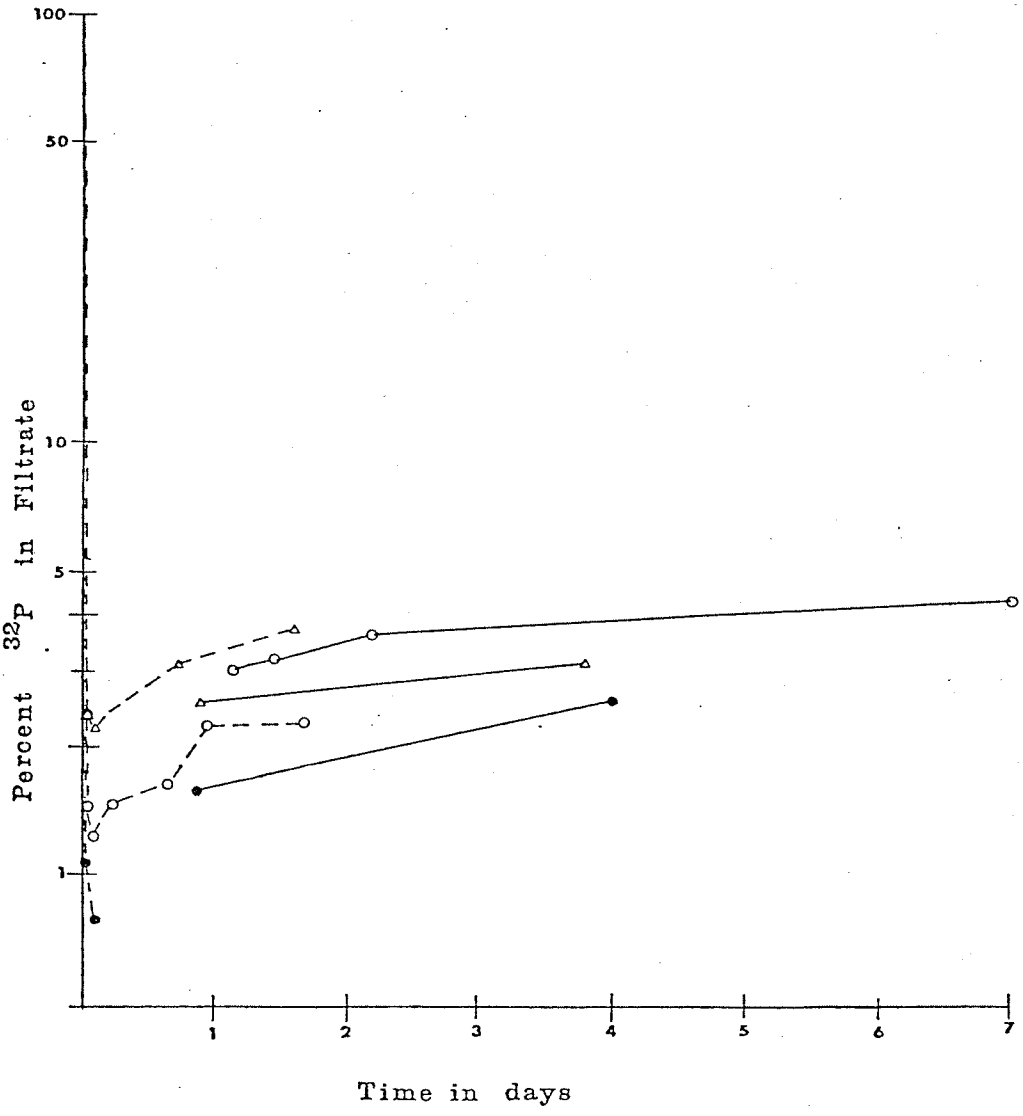

100
50
10
5
1
Percent ^{32}P in Filtrate
1
2
3
4
5
6
7
Time in days

Figure 3. G-25 Sephadex fractionation of the 0.45μ filtrate of ^{32}P-labeled lake water filtered at various times after the addition of $^{32}PO_4$. The water was incubated in *in situ* epilimnion columns.

V_o denotes void volume (compounds with molecular weights greater than about 5000) and PO_4-P represents the elution volume for orthophosphate.

3A Lake 302 S

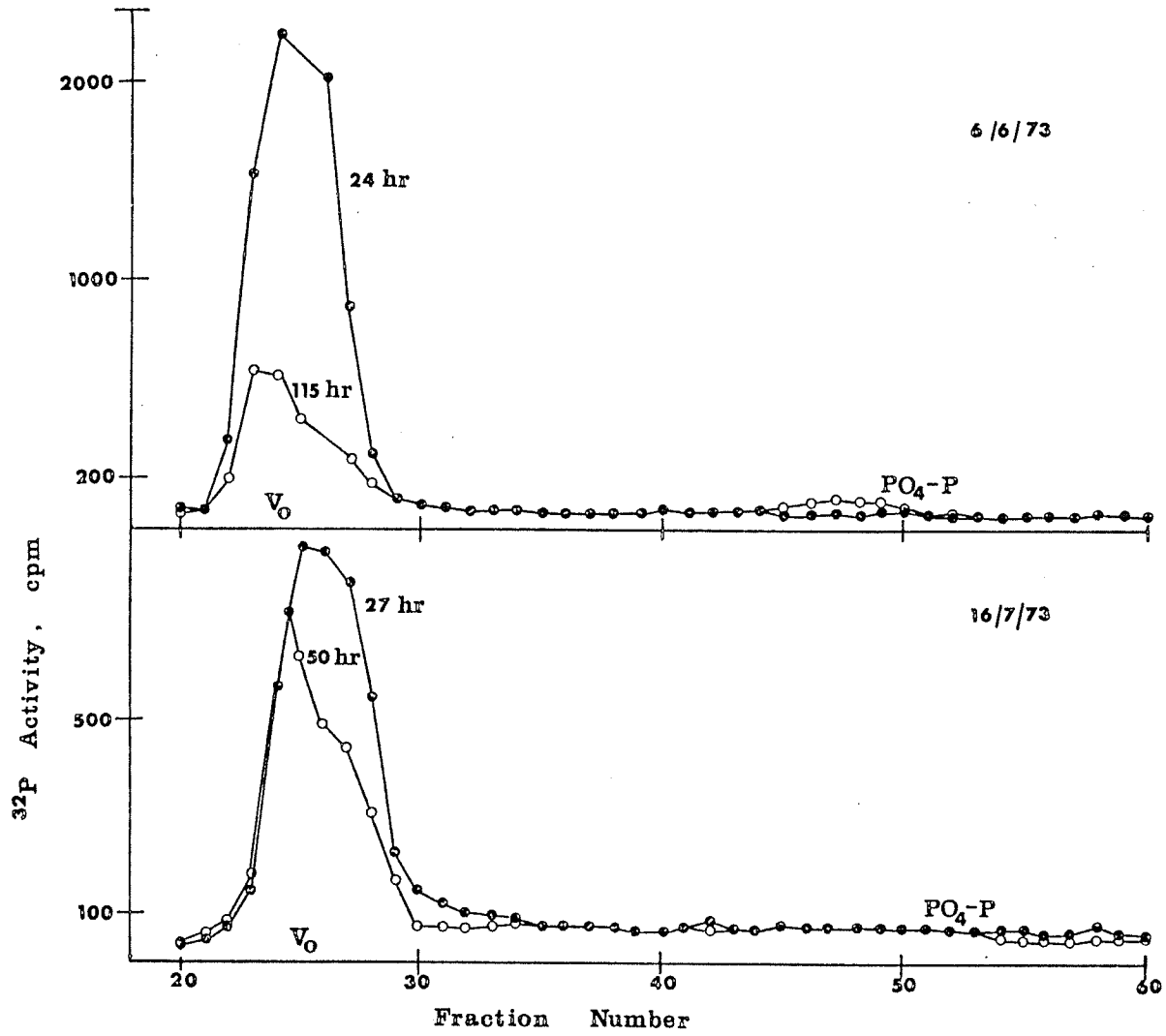

2000
1000
200
24 hr
115 hr
V_o
PO_4-P
6/6/73
500
100
27 hr
50 hr
V_o
PO_4-P
16/7/73
^{32}P Activity, cpm
20
30
40
50
60
Fraction Number

Figure 3B Lake 227

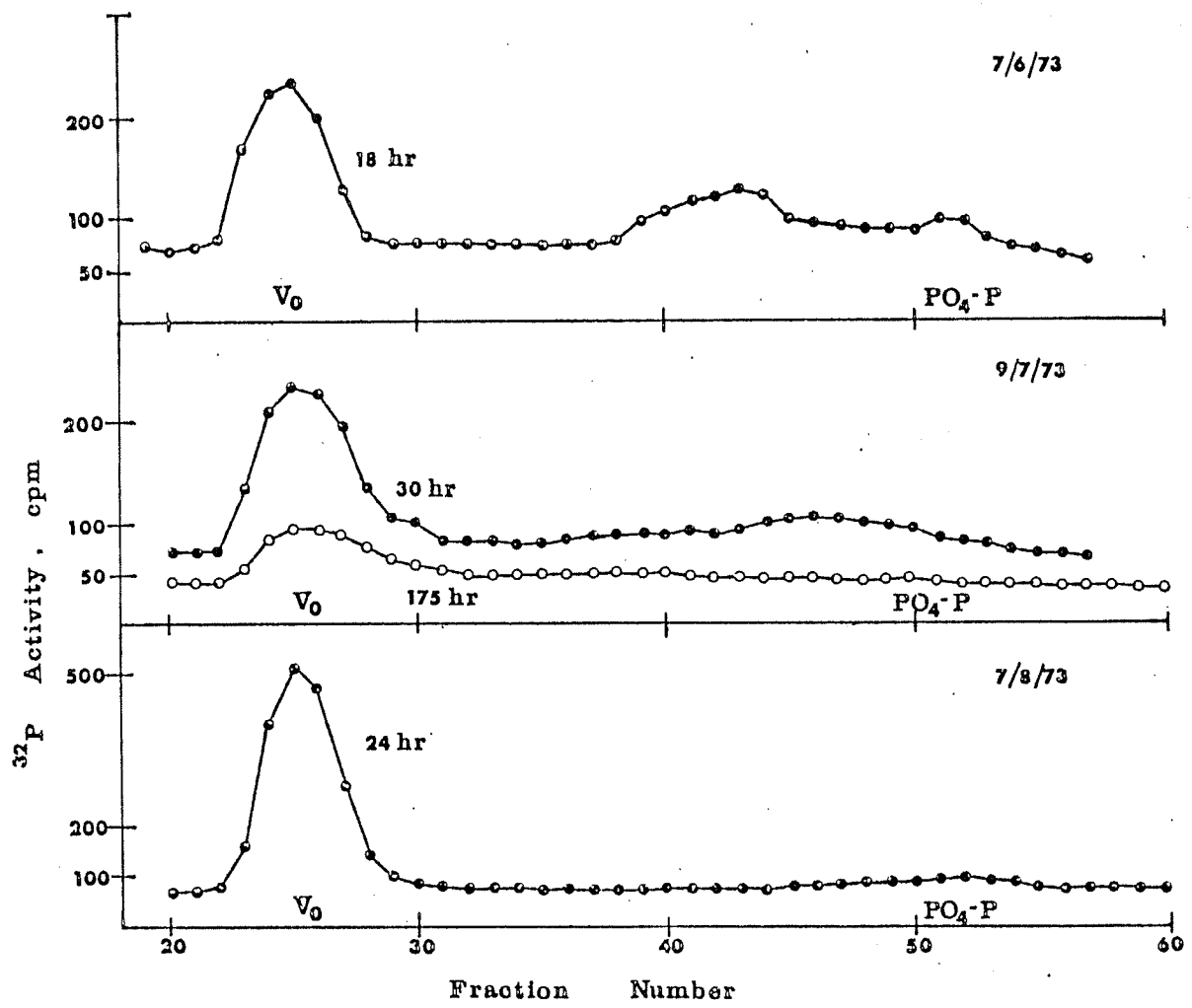

7/6/73
18 hr
200
100
50
V_0
PO_4-P
9/7/73
30 hr
175 hr
200
100
50
V_0
PO_4-P
7/8/73
24 hr
500
200
100
V_0
PO_4-P
^{32}P Activity, cpm
20
30
40
50
60
Fraction Number

TABLE 1. The partition of ^{32}P between the filter retainable and filtrate fractions of lake water incubated in in situ epilimnion columns for from 4 to 7 days after $^{32}PO_4$ addition.

$^{32}PO_4$ was added to in situ epilimnion columns, and its rate of uptake was followed for from 4 to 7 days. The specific activities of the filtrate P and filter retainable P fractions then were compared to determine whether an equilibrium distribution of ^{32}P had been reached.

Lake	Date of $^{32}PO_4$ addition	Length of incubation (days)	% of ^{32}P activity in the filtrate after 24 hrs:		% of ^{32}P activity in the filtrate at the end of the in situ incubation	Specific activity of the filter retainable P on the final day. (nCi/µg)	Specific activity of the filtrate P on the final day. (nCi/µg)
			In the columns	In laboratory incubated samples			
227	7/6/73	4	1.6	(.8 at 2 hrs)	2.5	7.58	0.592
	9/7/73	7	3.1	2.2	4.2	3.38	0.368
	7/8/73	4	2.5	3.2	3.2	1.63	0.188
302 S	6/6/73	5	16.7	(1.5 at 2 hrs)	9.3	27.8	5.73
	16/7/73	7	28.3	1.5	11.0	34.3	7.73

those of other phosphorus forms.

HZO Method

Another method considered worthy of investigation was to concentrate orthophosphate from a known volume of lake water on hydrous zirconium oxide (HZO) crystals, add NaOH to the crystals to extract the orthophosphate, and then use the molybdate blue method to measure the orthophosphate concentration in the NaOH solution. The sensitivity of this method might be increased by using ^{32}P-labeled lake water. If the specific activities of the phosphorus forms present are equal, the fraction of the total activity adsorbed should be equivalent to the fraction of TDP which is PO_4-P. This method assumes that orthophosphate is the only form of phosphorus adsorbed by HZO.

Methods

Hydrous zirconium oxide was obtained from Bio-Rad Laboratories. To clean the powder, distilled water was added and the smaller particles decanted. Five washings with 10% NaOH, and five with distilled water, followed. This suspension was brought to neutrality with 0.1 N HCl and phenolpthalein.

The efficiency of orthophosphate adsorption on the powder was tested by adding 5 to 10 g of HZO to 100 ml of a $^{32}PO_4$ solution. Before the addition, a 5 ml aliquot of the solution was withdrawn, filtered, and its ^{32}P activity counted. After addition of HZO and 30 minutes of agitation on a Burrell wrist-action shaker, a second 5 ml aliquot was filtered and its ^{32}P activity determined.

TABLE 2. Calculation of phosphorus flux to the sediments from the rate of ^{32}P loss from epilimnion columns.

Phosphorus flux to the sediments is equal to the rate constant for sedimentation, the difference between the rate constant for ^{32}P disappearance from the columns and the rate constant for ^{32}P radioactive decay (0.048/day), multiplied by the total phosphorus concentration within the column.

Lake	Date	Rate constant for ^{32}P loss from the column (1/day)	Rate constant for sedimentation (1/day)	Mean TP during the incubation (µg/l)	Phosphorus flux to the sediments (µg/l/day)
227	7/6/73	0.256	0.208	44	9.2
	9/7/73	0.204	0.156	62	9.7
	7/8/73	0.210	0.162	59	9.6
302 S	6/6/73	0.057	0.009	9	0.08
	16/7/73	0.157	0.109	6	0.65

The efficiency of the extraction of orthophosphate from HZO by NaOH then was examined. Ten percent NaOH was added. After 30 minutes of agitation, a 5 ml aliquot of the NaOH solution was withdrawn, filtered and its ^{32}P activity measured.

Finally, the removal of orthophosphate from lake water samples was investigated. Whole lake water was incubated with $^{32}PO_4$ for 24 hours. 100 ml of this water was poured into a BOD bottle (A). Another 100 ml was filtered through an 0.45μ filter and poured into a second bottle (B). $^{32}PO_4$ and unlabeled 0.45μ lake water filtrate were added to a third bottle (C), as a control. About 10 gm of HZO were added to each bottle. If ^{32}P adsorption by HZO were equal in bottles A and B, orthophosphate concentration might be estimated without a filtration step and the hazards of cell breakage and filter adsorption thus avoided.

The bottles were agitated as before. 5 ml aliquots were withdrawn from each bottle at approximately 30 min intervals for from 2 to 3.5 hours and centrifuged at 4000 rpm until all visible suspended HZO had settled. ^{32}P activity in the supernatant then was counted.

Results

The efficiency of orthophosphate removal by HZO suspensions was found to be high. 98% of the $^{32}PO_4$ in distilled water was removed from solution after 30 minutes of agitation. The addition of 10% NaOH to the labeled HZO and 30 minutes of shaking brought 90% of the ^{32}P activity which had been adsorbed by the HZO back into solution. Thus, 88% of the total added $^{32}PO_4$ was adsorbed by and then recovered from the HZO. $^{32}PO_4$ removal from lake water which was filtered prior to $^{32}PO_4$ addition proved to be less efficient. Two hours were required to remove 88% and 3.5 hours

to remove 93% (Figure 4). Apparently, other anions, probably negatively charged colloidal compounds (Rigler, 1968) were competing with orthophosphate for uptake sites.

During the first 50 minutes of shaking, HZO removed ^{32}P from bottle B at a rate equal to the removal of $^{32}PO_4$ from the control bottle (C). The rate then fell to a very low value. Over 60% of the ^{32}P in Bottle B had been adsorbed by the HZO before this time, however. In Bottle A, the adsorption was somewhat slower than it was in Bottle B. After two hours of shaking, more than 50% of the ^{32}P remained in the supernatant phase of a centrifuged aliquot. Before the experiment began, the fraction of ^{32}P activity in the filtrate of the labeled whole lake water (which later was added to Bottle A) was measured as 4.3%. Because the seston were sedimented along with HZO crystals during centrifugation, cell damage or extracellular release must have occurred in Bottle A during agitation.

Rigler Bioassay

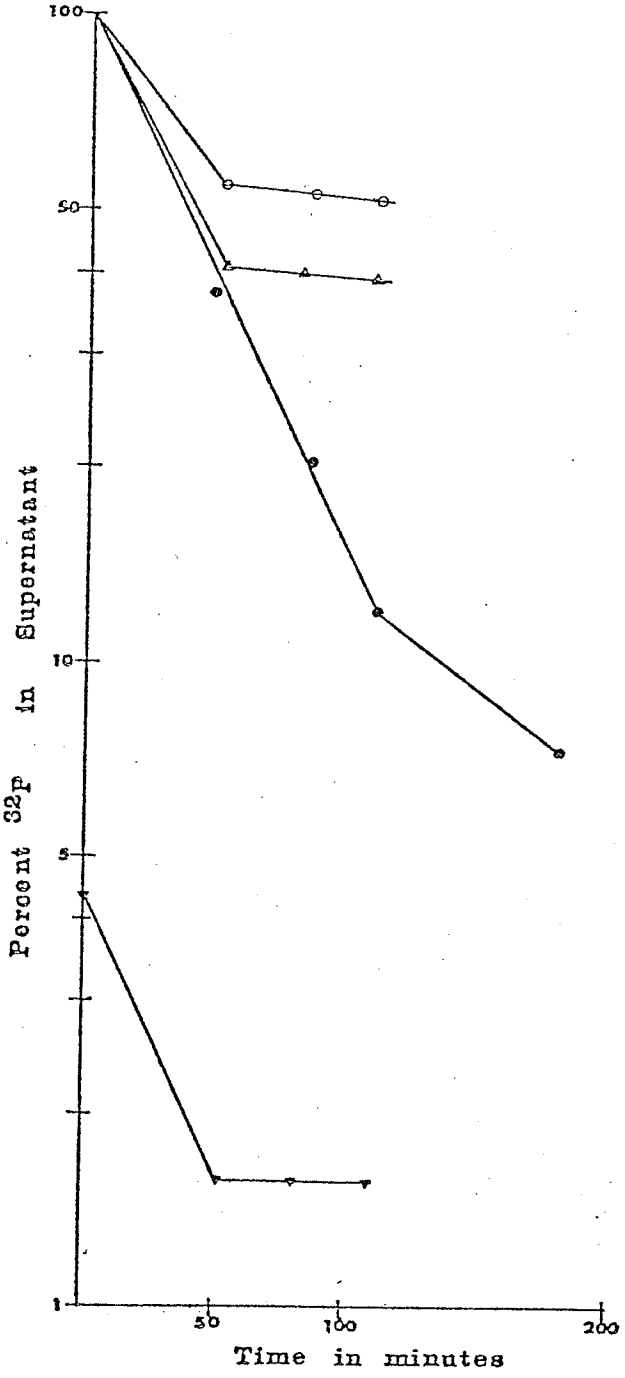
The final group of methods considered were radiobioassays. The first, Rigler's (1966) bioassay (described in the introduction), is sensitive to low orthophosphate concentrations, but is very time consuming, and yields only maximum concentration estimates.

Methods

Rigler bioassays were begun within two hours of water collection. The water was maintained at <u>in situ</u> temperature at all times, and was stirred with a magnetic stirring rod while $^{32}PO_4$ uptake was followed.

Figure 4. The percent of total ^{32}P activity in the supernatant of samples of lake water and lake water filtrate at various times after HZO addition.

○——○ refers to whole lake water which was incubated with $^{32}PO_4$ for 24 hours before HZO addition (Bottle A). △——△ refers to a portion of the above water which was filtered through an 0.45 filter immediately before HZO addition (Bottle B). ▽——▽ denotes the same mixture as △——△ , but is expressed as the percent of total whole-lake water ^{32}P activity, rather than as the percent of total filtrate activity. ●——● refers to lake water which was filtered before $^{32}PO_4$ was added (Bottle C).


100
50
10
5
1
Percent 32P in Supernatant
50
100
200
Time in minutes

The $^{32}PO_4$ and unlabeled orthophosphate solutions were prepared either immediately before each experiment (1973) or before departure for the lake (1974). In the latter case, the solutions were irradiated in quartz tubes with ultraviolet light until needed. Filter retention and Sephadex results indicated that, unless sterilized in this or some other manner, solutions often are converted to colloidal phosphorus within a few days (or even hours) of preparation, even when stored at 4°C.

The added orthophosphate-phosphorus concentrations generally ranged between 0.01 and 2.5 µg/l in summer and between 0.5 and 50 µg/l in winter. One hundred to 200 ml of lake water were used for each concentration tested. The unlabeled orthophosphate and a tracer amount of $^{32}PO_4$ were added simultaneously with Eppendorf pipettes. At regular time intervals, 5 ml aliquots of labeled lake water were withdrawn with a Cornwall syringe and passed through 0.45µ Sartorius filters at a vacuum of 250 mm Hg. Before July, 1974, one aliquot was left unfiltered so that total, and thus the initial filtrate, activity could be determined. After July, 1974, all filters were retained and counted in distilled water. Total activity then was calculated as the sum of the filter and filtrate activities.

The time needed for filtration ranged between 12 and 60 sec. Because this time was relatively constant for all aliquots of any one sample, the time of injection onto the filter was used as the time of filtration in calculations. The rate constant for orthophosphate uptake is concerned with change with time rather than with actual time values, and, therefore, was not affected by this procedure.

Computer programs were written to calculate rate constants (through the exponential regression of % ^{32}P activity in the filtrate on time), and to plot uptake velocity versus concentration at various assumed original concentrations. The latter program printed out slope (for the linear portion, or for the two lowest points, of the velocity versus concentration plot) and y-intercept values, and multiplied the "no addition" rate constant by the maximum estimated concentration to give the flux of orthophosphate-phosphorus to seston.

Uptake velocity calculations were estimated to be no more reliable than $\pm$ 0.001 µg/l/min. Therefore, any assumed original concentration which produced a positive slope and a y-intercept of 0.001 µg/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 0.001 µg/l/min, the maximum orthophosphate-phosphorus concentration was taken to be the assumed original concentration which produced a slope of 0.000.

Results

Rigler's bioassay is capable of estimating orthophosphate concentration only if the rate constant for orthophosphate uptake in any one sample decreases in a regular manner with increasing orthophosphate concentration. The assumption that this requirement is met in natural waters was found to be valid. The replicability of K generally was good (Figure 5). For some of the early bioassays (and on a few days since) inconsistencies in the relationship between K and concentration occurred at low orthophosphate concentrations. Figure 6 demonstrates the problem which occurred during the summer.

Figure 5. Lake 302 S, 30 September, 1973.

Typical plots of ln % ^{32}P remaining in the 0.45μ filtrate with time at different concentrations of added orthophosphate-phosphorus. The rate constant for orthophosphate uptake, K, is the absolute value of the slope of each plot. Diphasic uptake was not noticeable within the period allotted for each sample.

Replicates are shown for each concentration added. One added concentration which was tested, 0.05 μg/l, is not included because the plots made after its addition were so similar to the "no addition" plots that the two sets overlapped.

The additions tested were: "no addition" (●—●), 0.5μg/l (△—△), 1.25 μg/l (■—■), and 5 μg/l (○—○).

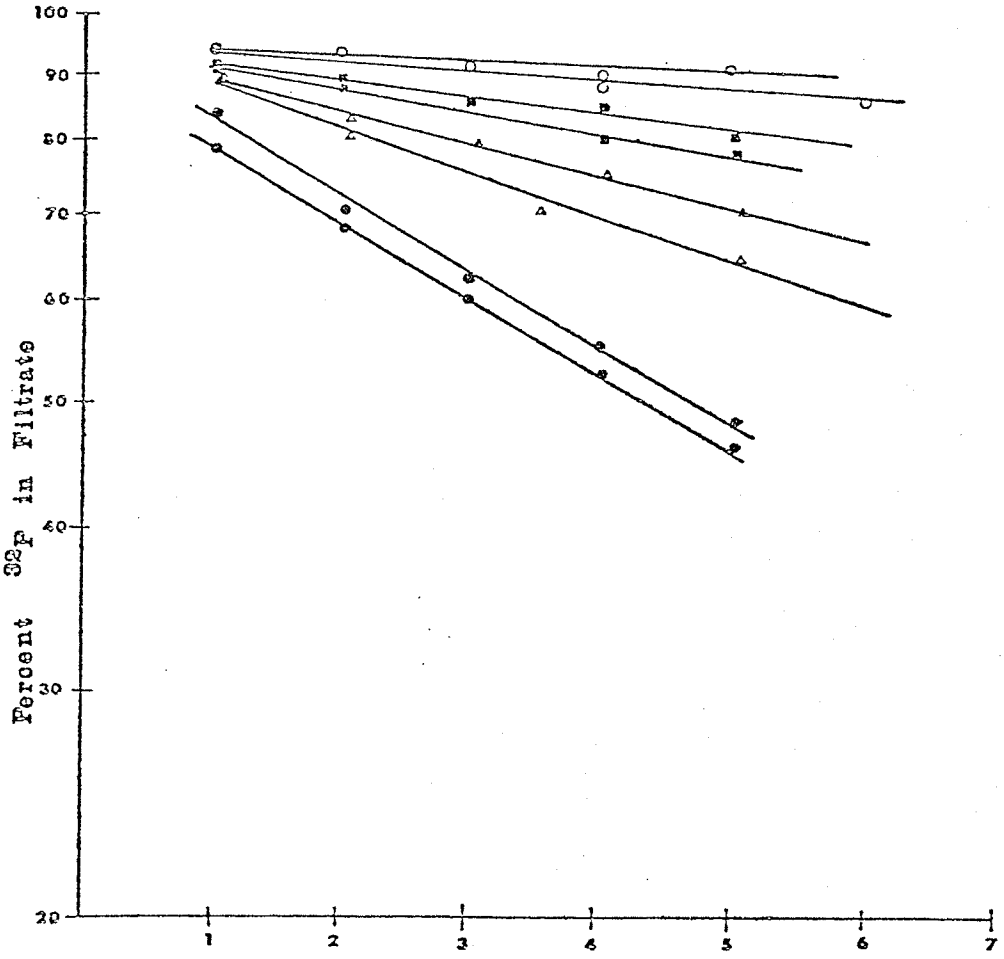
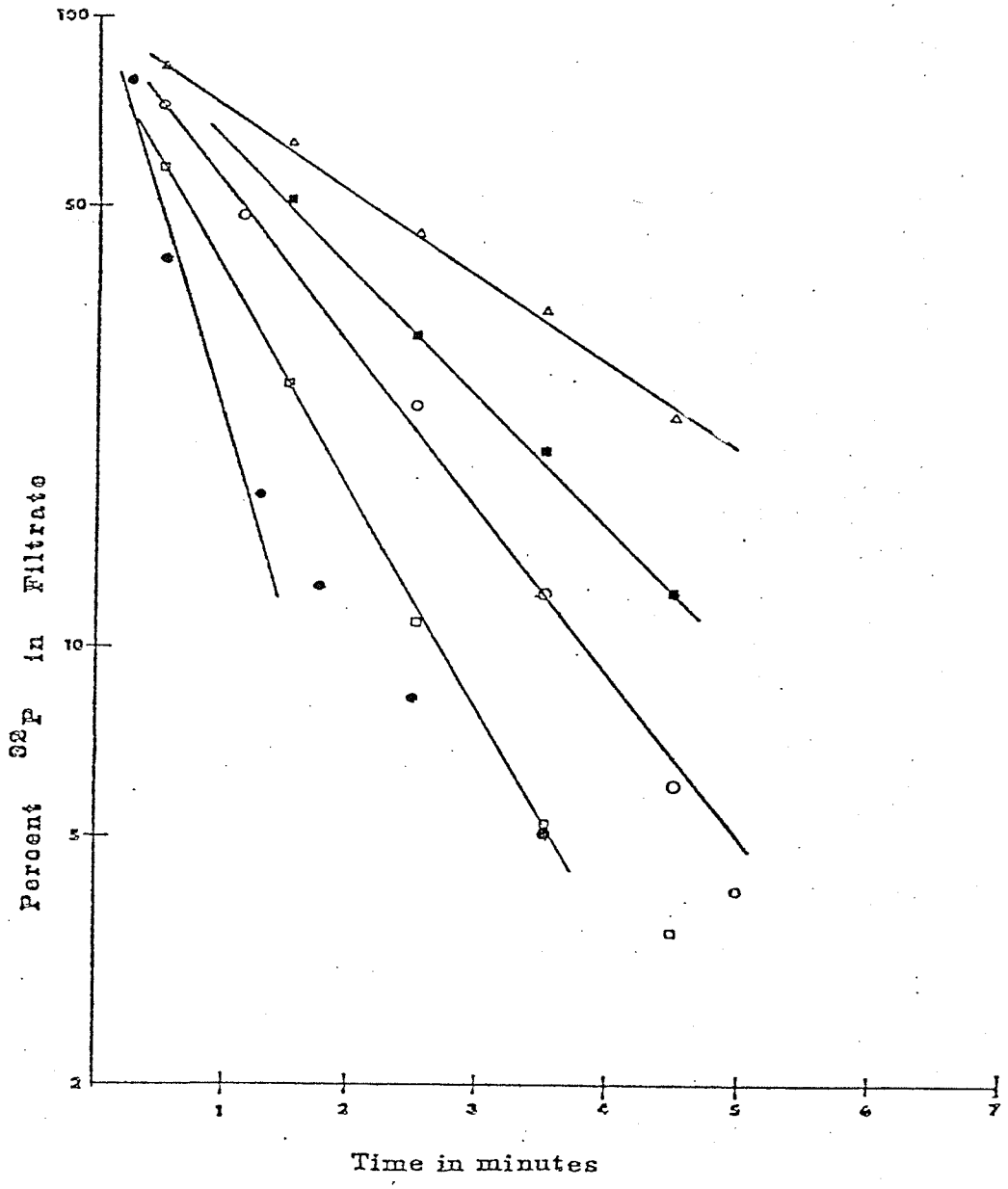

100
90
80
70
60
50
40
30
20
Percent 32P in Filtrate
1
2
3
4
5
6
7
Time in minutes

Figure 6. Lake 227, 7 August, 1973.

A graph of ln % ^{32}P remaining in the filtrate with time at different concentrations of added orthophosphate-phosphorus "no addition" (●—●), 0.25 µg/l (□—□), 1.25 µg/l (○—○), 5 µg/l (■——■) and 10 µg/l (△——△). Three added concentrations which were tested (0.05 µg/l, 0.50 µg/l, and 2.5 µg/l) were omitted to reduce clutter. This graph demonstrates the markedly diphasic uptake often observed at low orthophosphate-phosphorus concentrations. If sampling had begun at 1 minute, the K calculated from the "no addition" plot would have been less than that calculated for an addition of 5 µg/l.


100
50
10
5
2
Percent ^{32}P in Filtrate
1
2
3
4
5
6
7
Time in minutes

The "no addition" curve shown is diphasic; whereas, the curves for the larger orthophosphate additions are monophasic. If the first aliquot of the "no addition" sample had been filtered as late as one minute after $^{32}PO_4$ addition, the rate constant calculated from the remaining filtrations would have been less than that recorded for an orthophosphate addition of 5 µg/l!

Diphasic uptake (generally occurring over a longer period than in the example given) was noted at low added orthophosphate concentrations during all but two or three epilimnion bioassays between mid-May and late August, 1973, and between early June and mid-September, 1974. During these periods, the first aliquot generally was filtered 0.5 min after $^{32}PO_4$ addition.

During the winter, inconsistencies in the relationship between K and concentration sometimes occurred because the rate constants for samples to which no orthophosphate was added and the rate constants for samples to which relatively little orthophosphate was added were so similar that experimental error became important. The use of higher concentrations of added orthophosphate alleviated this problem.

The results of the bioassays are presented in Section II.

Schindler Bioassay

A second bioassay was suggested by Schindler (1974, personal communication). This method, like Rigler's, (Rigler, 1966) was based on the strong correlation between the orthophosphate concentration of a sample and the rate constant for orthophosphate uptake, K. Rather than add orthophosphate directly to the unknown sample, however, a standard plot of K versus known orthophosphate-

phosphorus concentration (or of ln K vs ln (PO_4-P concentration)) was made for water from a eutrophic lake (an algal culture could be used) (see Figure 7). The water was chosen to assure that the curve would have a maximum slope. A small amount of the unknown sample was added to the water and the uptake rate constant obtained was matched with its corresponding concentration value on the standard K versus concentration curve. The orthophosphate concentration of the added sample then was calculated.

Methods

For Schindler bioassays, K versus concentration plots were constructed using epilimnion water from the eutrophic Lakes 227 and 226 (northeast basin).

The experimental procedure was similar to that of the Rigler bioassay. Lower concentrations of added orthophosphate-phosphorus were tested, however. The usual range was between 0 and 0.5 µg/l, with many of the concentrations being less than 0.05 µg/l. Between 0.1 and 10 ml of filtered sample were added to 100 ml of the calibrated water. To increase the accuracy of the rate constant determination, ^{32}P activity on the filter, as well as in the filtrate, was counted.

To be certain that the decrease in K with sample addition was due to an increased concentration of orthophosphate, and not to the addition of some other compound, a portion of a lake water sample (from the hypolimnion of Lake 227) was allowed to run through a column of HZO to remove orthophosphate. An aliquot of the HZO-treated water was added to 100 ml of calibrated epilimnion water. An aliquot of the remaining, untreated sample was added to another 100 ml of calibrated water. Rate constants were determined for both samples.

Results

Figure 7 is an example of the plot used to determine orthophosphate concentration during a typical Schindler bioassay.

To reduce the risk of altering K through the addition of compounds other than orthophosphate, normally only small amounts of sample were added to bioassay water. This practice required that very low concentrations of orthophosphate be used in calibrating the K versus concentration plot. Within the range of the orthophosphate concentrations added (many less than 0.1 µg/l) the scatter of the K versus concentration plot was substantial. When replicates were performed, the ranges of the replicates for each concentration tested tended to overlap. The calibration curve for K versus concentration, therefore, was drawn rather arbitrarily.

Diphasic uptake curves were common and largely were responsible for aberrant K values.

The Schindler bioassay was not sensitive enough to estimate orthophosphate concentration in epilimnion water (the change in the orthophosphate concentration of the calibrated water produced by the addition of 2 ml of an average E.L.A. sample is only about 0.0003 µg/l during the summer). Concentration estimates for hypolimnion and sediment interstitial water samples were obtained (Table 3).

The PO_4-P concentration estimates for hypolimnion samples which were obtained from Schindler bioassays generally were greater than the estimates obtained with Rigler bioassays (see Section II, and Table A13). On one occasion the estimates actually were higher than the concentrations of "total dissolved phosphorus" in the samples. This phenomenon suggested that compounds other than orthophosphate in the added unknown might inhibit orthophosphate uptake in the calibrated bioassay water.

Figure 7. Samples from Lake 302 S added to 100 ml aliquots of Lake 227 epilimnion water. 3 September, 1974. An example of the plot used during the Schindler bioassay to determine how much orthophosphate-phosphorus was present in a sample added to standardized water. The rate constants for orthophosphate uptake are determined for several known added concentrations (replicates are shown, ■). A line then is fitted between the points obtained (this one was drawn by eye). The broken lines represent the rate constants calculated after sample addition (... is for a 2 ml sample from 10 m, _ _ _ _ _ _ is for a 10 ml sample from 10 m, and ___ ___ is for a 10 ml sample from 9 m). Replicates are shown. The concentration of orthophosphate-phosphorus added from the sample is the x-coordinate of the interception of K and the fitted line (the extrapolation to the abscissa is shown with arrows).

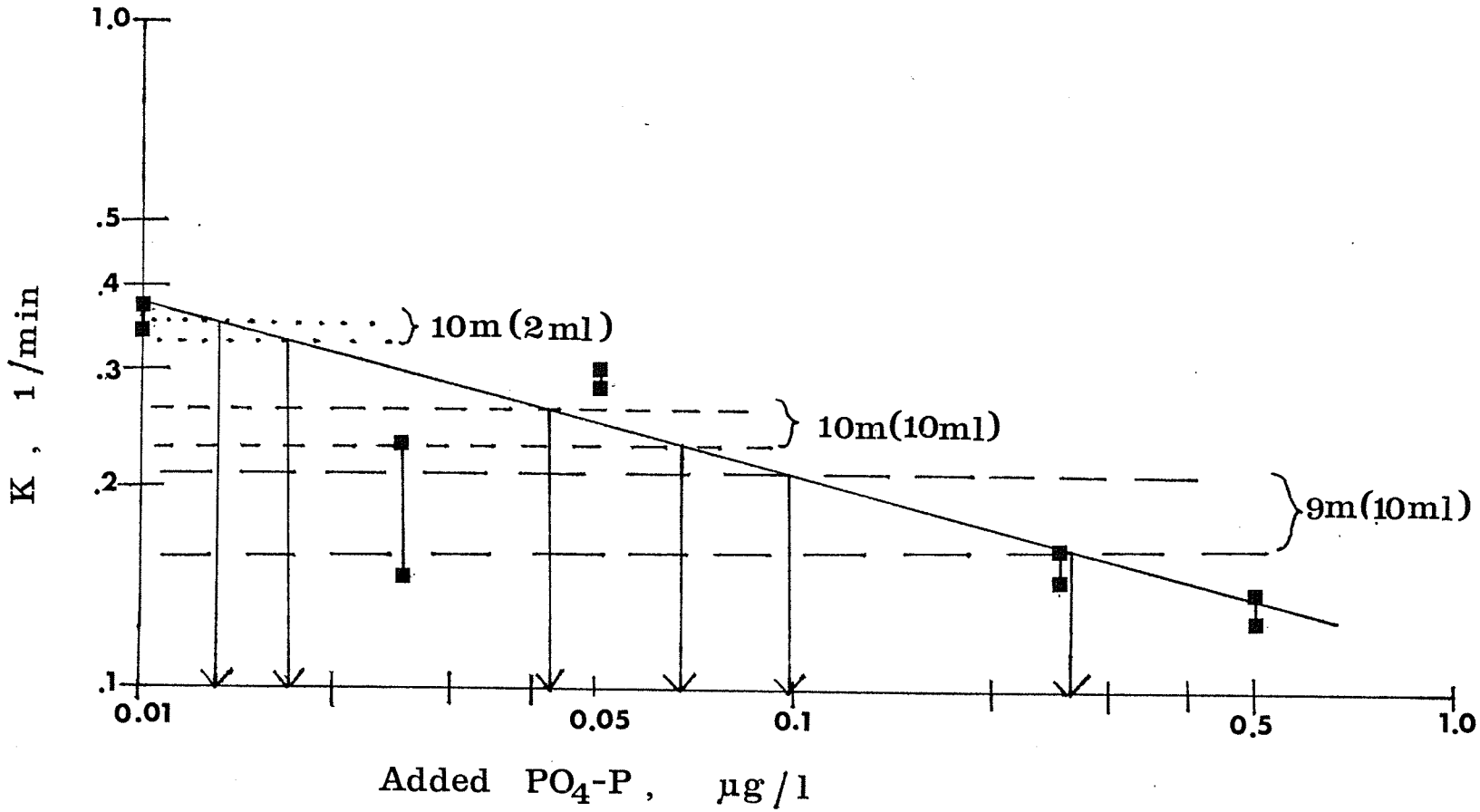

1.0
.5
.4
.3
.2
.1
K , 1/min
10m (2ml)
10m(10ml)
9m(10ml)
0.01
0.05
0.1
0.5
1.0
Added PO4-P , µg/l

TABLE 3. Orthophosphate concentration estimates calculated from Schindler bioassays.

Lake	Depth	Date	Ml of sample per 100 ml of standard water	Estimated orthophosphate concentration (µg/l)
227	5 m	16/8/74	2	1.1
	7 m		2	1.2
	9 m		2	1.3
227	Inter-stitial water from sediments	2/9/74	1	70
302 S	9 m	3/9/74	10	0.95, 2.5
	10 m		2	0.65, 0.75
	10 m		10	0.36, 0.62
227	7 m	20/9/74	4	500,000
	7 m		0.2	850
	9 m		0.2	750
239	Inter-stitial water from sediments			
	0-2 cm		1	35, 37
	6-8 cm		1	11, 14

When hypolimnion water was treated with HZO to remove most of its orthophosphate before it was added to the bioassay medium, the measured K was not significantly different from the K obtained with the untreated hypolimnion water (Table 4). This confirmed inhibition by non-orthophosphate compounds. The source of inhibition might be common hypolimnion compounds which are poisonous to plankton before or after they are oxidized (i.e. HS^- or NH_4^+), or the ferrous iron in the sample may have been oxidized to the ferric form and, then, complexed with the $^{32}PO_4$, preventing its uptake.

TABLE 4. Rate constants for $^{32}PO_4$ uptake in a control, in a sample to which 0.1 ml of Lake 227 hypolimnion water was added, and in a sample to which 0.1 ml of hypolimnion water which had been treated with HZO to remove orthophosphate was added. 24 September, 1974.

Addition to Standardized Water	K (1/min)
"No addition"	0.0411
0.1 ml of hypolimnion water added	0.0003
0.1 ml of hypolimnion water added after treatment with HZO	0.0006

Michaelis-Menton Bioassay

A third bioassay, based on Michaelis-Menton kinetics, might have been constructed. Modifications of the Lineweaver-Burke equation, such as those of Hobbie and Wright (1968), can be made so that the x-intercept of the equation is equal to K_M+C_s, where K_M is the half saturation

constant and C_s is the natural substrate concentration. Since K_M cannot be determined from the information available, only a maximum estimate of concentration can be derived.

This approach requires that the many phosphorus-consuming species of a lake behave in a manner similar to a one-enzyme system with respect to orthophosphate uptake. Since the information needed for Michaelis-Menton equations is similar to that used in Rigler bioassays, the appropriateness of using saturation kinetics to describe orthophosphate uptake was considered.

Methods

Data obtained for Rigler bioassays during the summer of 1973 were processed by a computer program which plotted two popular transformations of the Michaelis-Menton equation, the Lineweaver-Burke plot, $1/v$ versus $1/C_s$ (v is initial uptake velocity, and C_s is substrate concentration), and C_s/v versus C_s, which reduces to $1/K$ versus C_s. The program calculated the slope of each line, its r^2 value and the y-intercept, thus providing estimates of V_{max} (the velocity which occurs when all orthophosphate binding sites are "saturated" with orthophosphate) and orthophosphate turnover time (the time required for the entire orthophosphate pool to be replaced with new orthophosphate). V_{max} is equal to the reciprocal of the y-intercept of $1/v$ vs $1/C_s$ and to the reciprocal of the slope of $1/K$ vs C_s. The y-intercept of $1/K$ vs C_s is equal to the turnover time. V_{max} values from the two transformations were compared with values obtained by direct measurement with $^{32}PO_4$.

Results

The ability of Michaelis-Menton equations to describe ortho-

phosphate uptake kinetics varied from day to day (see Appendix II). On a few days, the plots of both transformations boasted r^2 values over 0.900, and the values for V_{max} predicted by the two plots were similar. On these days the orthophosphate turnover time predicted from the y-intercept of 1/K versus C_s coincided closely with the value obtained from the rate of $^{32}PO_4$ uptake.

On over 70% of the days when uptake kinetics were considered, however, the two plots predicted substantially different values for V_{max}, had poor r^2 values, and/or yielded an estimate of turnover time which was not similar to the value obtained from $^{32}PO_4$ uptake.

A paired t-test of the two sets of V_{max} estimates indicated that they were not significantly different ($t = 1.06$, $df = 60$). The 95% confidence limits for the difference between the two sets were -0.237 and 0.767.

A paired t-test of the estimates of orthophosphate turnover time obtained by Michaelis-Menton equations and those calculated from $^{32}PO_4$ disappearance from 0.45μ filtrate indicated that the two methods produce significantly different results ($t = 3.53$, $df = 60$). The 95% confidence limits for the difference between the two sets of data were 952 and 3442.

Both of the t-tests which were performed were influenced strongly by three or four sets of large numbers. This suggests that the differences between means may be smaller than the test statistics indicate.

Discussion

None of the orthophosphate methods tested was found to be very

satisfactory. The major problems encountered were insufficient sensitivity and lack of specificity. Table 5 summarizes the evaluation of these methods and compares them with common chemical methods.

Orthophosphate-phosphorus concentration estimates obtained for Lakes 227 and 302 S with Rigler bioassays were much lower than previously recorded concentrations of SRP (which ranged between " 1" and 6 µg/l during the summer of 1969 (Armstrong and Schindler, 1971)). During the summer of 1973, the orthophosphate-phosphorus concentration in Lake 227 occasionally dropped below the limit of detection of the bioassay, 0.001 µg/l. The average concentration measured during the summer was about 0.015 µg/l in both lakes.

Sephadex fractionation of ^{32}P-labeled lake water from *in situ* epilimnion columns provided further evidence that orthophosphate was scarce. Fractionations performed more than 24 hours after $^{32}PO_4$ was introduced into the columns generally failed to produce a detectable $^{32}PO_4$ peak. A peak with an elution volume somewhat lower than that of orthophosphate occasionally appeared. This peak may have been $X^{32}P$, the low molecular weight excretory product described by Lean (1973). This peak never accounted for more than 10% of the total filtrate ^{32}P activity. Part of the peak could be orthophosphate. To estimate the orthophosphate concentration of a ^{32}P-labeled sample from the results of Sephadex fractionation, one may use the equation:

$$PO_4\text{-}P = \frac{^{32}PO_4\text{-}P}{^{32}P_{filtrate}} \times \frac{^{32}P\ filtrate}{^{32}P\ total} \times \frac{(PP+TDP)}{TDP} \times TDP.$$

TABLE 5. A comparison of the methods for estimating orthophosphate-phosphorus concentration which were discussed in Section 1.

	Method	Limit of Detection	Interference	Additional Problems or Requirements
1.	Molybdate blue colorimetric	1 µg/l, in water (Murphy-Riley version); 0.2 µg/l after extraction into isobutanol	Arsenate; easily hydrolyzed colloidal P	-
2.	Flame photometry with gas chromotography	About 20 µg/l	Unknown	Requires that the sample be converted to tris-(TMS)-phosphate (or phosphine)
3.	Fluorometry, using the aluminum-morin system	About 1 µg/l	H^+ ion (Armstrong, unpublished)	-
4.	Sephadex fractionation of a filtered sample, followed by molybdate blue analysis of the orthophosphate fractions	15 µg/l, in water; 3 µg/l with extraction into isobutanol	Possibly arsenate (The molecular weights of arsenate and orthophosphate differ by 60. The Sephadex elution volumes of the two anions probably overlap); low molecular weight P compounds ("XP").	-
5.	Sephadex fractionation of filtered ^{32}P-labeled lake water; multiplication of the ratio of $^{32}PO_4/^{32}P_{filtrate}$ by TDP.	About 0.05 µg/l (when the TDP concentration is 5 µg/l, and the ^{32}P activity of the filtered sample is 500 cpm/ml). Sensitivity increases with ^{32}P activity and decreases with TDP concentration.	Possibly "$X^{32}P$" or other low molecular weight P compounds	If all P fractions have not obtained equal specific activities, orthophosphate-P concentration will be overestimated. (Equilibrium was not reached within 7 days in E.L.A. lakes.)

TABLE 5. (cont.)

Method	Limit of Detection	Interference	Additional Problems or Requirements
6. Concentration of orthophosphate on HZO crystals, followed by NaOH elution and molybdate blue analysis	Using a 1 liter sample and extracting the orthophosphate with 50 ml of NaOH: about 0.05 µg/l, in water; about 0.01 µg/l, with extraction into isobutanol.	Possibly arsenate; colloidal P	-
7. Concentration of $^{32}PO_4$ from ^{32}P-labeled lake water on HZO; multiplication of the ratio ^{32}P extracted/total filtrate ^{32}P by TDP	When the conditions stated in methods 5 and 6 are met: about 0.002 µg/l. Sensitivity increases with ^{32}P activity and decreases with TDP concentration	Colloidal P	All P fractions must have equal specific activities
8. Rigler bioassay	About 0.001 µg/l. (It is difficult to filter aliquots at close enough intervals to measure the rate constant at lower concentrations.)	None known	A. No minimum limit on the estimate B. Diphasic uptake
9. Schindler bioassay	Using a 5 ml sample added to 95 ml of standard lake water: about 0.2 µg/l when diphasic uptake isn't very pronounced (so that the replicates at the lower known added concentrations are good); more often, about 1 µg/l.	Unknown compounds in hypolimnion water	A. Diphasic uptake B. The small size of the additions contributes to the percent error

TABLE 5. (cont.)

Method	Limit of Detection	Interference	Additional Problems or Requirements
10. Bioassays based on Michaelis-Menton kinetics	Unknown	None known	A. No minimum limit on the estimate. B. Requires that ortho-phosphate uptake follows one reactant, one product saturation kinetics. This study indicates that this requirement often is not met.

This equation assumes, unjustifiably, that the relative proportions of ^{32}P-labeled compounds in the filtrate at the time of filtration are the same as they would be at isotopic equilibrium. If one assumes that the entire peak appearing near the elution volume of orthophosphate is orthophosphate, a maximum estimate of orthophosphate concentration may be calculated. The largest estimate obtained in this manner was 0.2 µg/l (for the final day of the in situ incubation which began on 6/6/73 in Lake 302 S).[1]

The minute amounts of orthophosphate present in E.L.A. lakes prohibit the use of molybdate blue methods (as well as flame photometry and fluorometry) for estimating orthophosphate-phosphate concentration even after orthophosphate has been separated from colloidal phosphorus, unless the samples are concentrated many fold. The limit of detection of the Murphey-Riley version of molybdate blue method is about 1 µg/l (Strickland and Parsons, 1968). If the blue-color complex is extracted into isobutanol before measurement, however, the sensitivity can be increased to about 0.2 µg/l.

The use of Sephadex fractionation to separate orthophosphate from other filtrate phosphorus compounds before molybdate blue analysis is impractical. The sample is diluted with eluant as it is pumped through the Sephadex column and, eventually, is eluted as about 40, 5 ml fractions. Most of the orthophosphate appears in about 5 aliquots, but about 10 additional fractions contain some orthophosphate. The orthophosphate, therefore, is diluted about 15 fold.

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1. The data used in this calculation are available in Tables 1 and A14.

Even concentration of orthophosphate on a resin, such as HZO, before molybdate blue analysis would be impractical for many E.L.A. epilimnion samples. During some parts of the summer a minimum of 10 liters of water per sample would have to be processed (collected, filtered, and brought into contact with HZO or an anion exchange material to produce a measurable amount of orthophosphate in the extract. The volume of the sample would require that the sample be pumped through an anion exchange column or an HZO column. Agitation, therefore, would be minimal and the contact time between the water and resin would be reduced far below the 3.5 hours which were required for HZO to remove 93% of the $^{32}PO_4$ from filtered lake water in this study. If actual orthophosphate concentrations are close to the estimates obtained by Rigler bioassays, then 1 liter or less of water would be sufficient for orthophosphate analysis during much of the year (when the concentration estimates are greater than 0.01 µg/l). Prolonged contact between HZO and the sample and shaking would be possible under these circumstances.

HZO, however, may be a poor choice as an agent for orthophosphate concentration even for waters with relatively high orthophosphate concentrations. In this study, a much larger fraction of the filtrate ^{32}P activity (60%) was adsorbed by HZO in 3 hours than would be predicted from either Sephadex fractionation or Rigler bioassay estimates of orthophosphate concentration. The increase in time needed for extraction of $^{32}PO_4$ from filtered lake water over that needed for extraction of $^{32}PO_4$ from distilled water also suggests that colloidal phosphorus, as well as orthophosphate, is adsorbed by HZO.

Two of the methods considered during this study, one of the proposed HZO methods and the Sephadex fractionation method, allowed an increase in sensitivity by estimating $^{32}PO_4$ in ^{32}P-labeled lake

water rather than measuring unlabeled orthophosphate directly. This practice, however, requires that the specific activities of all phosphorus fractions are equal when the methods are employed. Long-term ^{32}P uptake experiments in in situ columns indicated that this requirement could not be met within a practical incubation period. Three to 7 days after $^{32}PO_4$ addition the specific activities of the filtrate fractions of these columns were only 5 to 28% of their expected "equilibrium" values.

The three radiobioassays which were considered as methods for estimating orthophosphate concentration are not concerned with ^{32}P equilibrium conditions, but with initial $^{32}PO_4$ uptake.

Diphasic uptake at low orthophosphate concentrations weakened the credibility of all three bioassays. It was especially detrimental for interpreting the results of Schindler bioassays because all of the orthophosphate concentrations used in this bioassay were low enough to be affected.

The cause of diphasic uptake is uncertain. Lean (1973) attributed the change in rate to the excretion of $X^{32}P$ by seston. Excretion may be important in experiments which last several minutes or more, but it seems unlikely that $^{32}PO_4$ could be taken up by an organism, converted to an organic compound, and excreted, in less than one minute.

One alternative hypothesis is that PO_4-P uptake is two-stepped. Plankton may employ one mechanism to "catch" or adsorb orthophosphate, and another, less rapid mechanism, to transport the orthophosphate into the cell. At high orthophosphate concentrations, the first mechanism would be saturated almost instantaneously so that only monophasic uptake would be noted. This

arrangement has been observed in Chlorella during zinc (Matzku and Broda, 1970) and potassium (Schaedle and Jacobson, 1965) uptake. If this arrangement exists for orthophosphate uptake, one would want to measure K for the second, limiting, phase during bioassays. After a few minutes of $^{32}PO_4$ uptake, this measurement might become complicated by actual ^{32}P excretion.

Medveczky and Rosenberg (1971) also have observed diphasic uptake in the first two minutes of $^{32}PO_4$ uptake. They contend that two separate mechanisms for PO_4-P uptake exist. Both mechanisms function during the filling of an intracellular inorganic phosphorus pool, but, once this pool is filled, only the slower mechanism is active. Potassium was found to be required during the initial rapid phase of PO_4-P uptake. Magnesium is required during the second phase. Medveczky and Rosenberg were able to isolate a phosphate-binding protein which seems to participate only in the rapid phase of PO_4-P uptake.

Michaelis-Menton equations aptly described orthophosphate uptake on only about 25% of the days when they were considered. Being unsafe theoretically (Williams, 1973) and of no advantage over the Rigler bioassay in producing accurate orthophosphate estimates, Michaelis-Menton methods of estimating concentration were not considered further during this study.

As mentioned earlier, the Schindler bioassay was not sensitive enough for epilimnion samples. It was discarded as a tool for estimating concentrations in hypolimnion and sediment interstitial water after the discovery that non-orthophosphate compounds in these samples could influence the rate of orthophosphate uptake in the calibrated bioassay water.

The Rigler bioassay was chosen as the best available method for estimating orthophosphate concentration, and was used during the seasonal study described in Section II.

SECTION II

SEASONAL STUDIES

Introduction

During the last few decades a model of the movement of phosphorus through the orthophosphate pool of a lake has evolved and become widely accepted (Figure 8). The model is based, in part, on the chemical properties of orthophosphate and on the responses of cultured algae to orthophosphate additions. Its principal foundation, however, is SRP data from lake studies. Because SRP includes phosphorus from biologically produced colloidal phosphorus compounds as well as from orthophosphate, those properties of the model which were derived from measurements of SRP in lake water or in the filtrate of algal cultures may be unsound. The remaining properties of the model deal with orthophosphate, but their ability to describe processes actually occurring within a lake was largely untested before the present study.

Recently, Lean (1973) presented a new model of the phosphorus cycle in the epilimnion during summer stagnation. Whereas, the classical phosphorus model is essentially a static model, concerned with the structure of the phosphorus cycle and with net changes in the components of this structure, Lean's model (Figure 9) is based on ^{32}P uptake kinetics and emphasizes the dynamics of phosphorus exchange between phosphorus compartments. Two modifications of the classical model of PO_4-P cycling are suggested by Lean's model. First, the

Figure 8. The classical model of the orthophosphate-phosphorus cycle in a lake.[1]

In the well oxygenated euphotic zone, algae remove PO_4-P from solution. Orthophosphate regeneration usually is assumed to be insignificant in this region.[2] Below the euphotic zone, decomposers break down organic phosphorus compounds in sedimenting algae and release orthophosphate. PO_4-P concentration in the aphotic zone is regulated largely by the iron cycle.[3] When oxygen is present, ferric iron combines with orthophosphate, forming insoluble ferric phosphate. In addition, some orthophosphate may co-precipitate with $Fe(OH)_3$, although the attraction between iron and phosphate is believed to be stronger than that between iron and hydroxide. If, during stagnation, the oxygen supply of the bottom waters is depleted by decomposers, ferric phosphate at the sediment surface is reduced to ferrous phosphate which, being soluble, enters the water. The barrier preventing diffusion of ferrous phosphate from the deeper, normally reduced sediments then is removed and orthophosphate which has accumulated in the sediments for many years may be released. Some of this orthophosphate may diffuse across the thermocline and, thus become available to algae. If reducing conditions become more intense, H_2S is formed. H_2S combines with ferrous iron to form FeS which

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1. This model is based on studies by Pearsall (1930), Juday and Birge (1931), Einsele (1936, 1938, and 1941), Mortimer (1941-42), and Hutchinson (1941).

2. This assumption has been contested by Ohle (1962). He found that over one half of the organic carbon in the lakes which he studied was mineralized in the epilimnion.

3. In hard water lakes the combination of orthophosphate with calcium and magnesium may be as important as the reaction with iron.

is highly insoluble. Enough iron may be removed by this process to produce an excess of orthophosphate when $Fe(PO_4)_3$ is formed during fall overturn. As the lake mixes, the uncombined orthophosphate is swept into the epilimnion where it often is responsible for algal blooms.

In regions where lakes are covered with ice and snow during the winter, the aphotic zone extends throughout the water column during the winter. Orthophosphate released during decomposition or produced near reduced sediment surfaces during the winter may foster spring algal blooms.

Many limnologists stress the importance of internal PO_4-P cycling (especially between the hypolimnion and epilimnion) to the point that external phosphorus loading is ignored.

The areas of the boxes are roughly proportional to the phosphorus concentrations and the areas of the arrows are proportional to phosphorus fluxes.

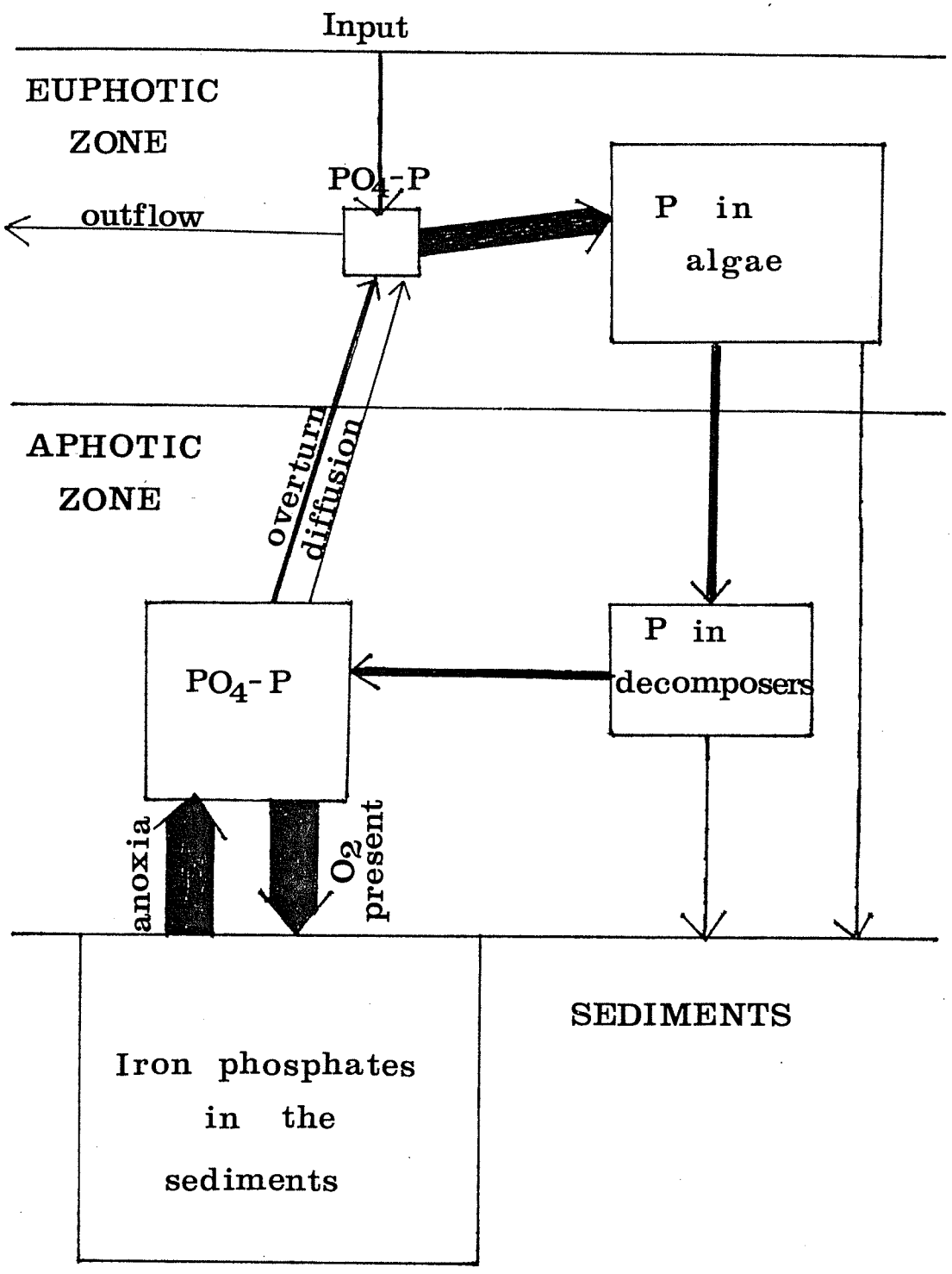
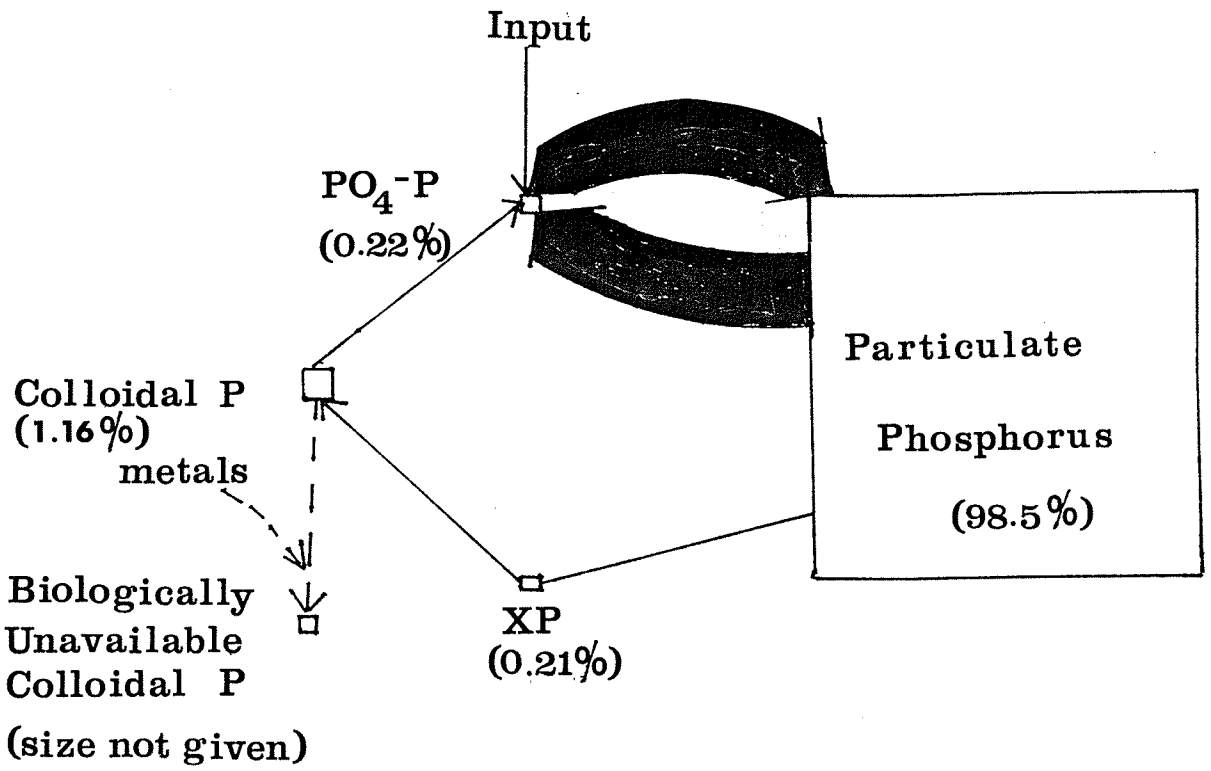

Input
EUPHOTIC ZONE
outflow
PO_4-P
P in algae
APHOTIC ZONE
overturn
diffusion
PO_4-P
P in decomposers
anoxia
O_2 present
SEDIMENTS
Iron phosphates in the sediments

Figure 9. Lean's model of the phosphorus cycle within the epilimnion.

Lean (1973) used ^{32}P tracer and Sephadex fractionation to distinguish the four biologically active phosphorus compartments shown here. In this model, the exchange of phosphorus between particulate phosphorus and PO_4-P dominates phosphorus movement in the epilimnion. Some phosphorus, however, is excreted by the seston as XP, a low molecular weight (about 250) organic compound, XP combines with colloidal phosphorus compounds in lake water, causing them to release PO_4-P. If colloidal phosphorus combines with a metal its ability to participate in this cycle may be destroyed. The rate of inactivation of colloidal phosphorus, however, is believed to be slow.

The relative sizes of the phosphorus compartments were estimated from the ^{32}P distribution in lake water 4.5 hours after $^{32}PO_4$ addition. The phosphorus flux between compartments was determined by multiplying the fraction of labeled phosphorus found in a compartment by the measured rate constant for the movement of ^{32}P to another compartment (except for the flux of phosphorus from PP to orthophosphate which was determined by mass balance).

Phosphorus fluxes are proportional to the areas of the arrows shown. The proportion of the labeled phosphorus found in each compartment is given in parentheses.


Input
$PO_4^{-}P$
(0.22%)
Particulate
Phosphorus
(98.5%)
Colloidal P
(1.16%)
metals
Biologically
Unavailable
Colloidal P
(size not given)
XP
(0.21%)

direct exchange of phosphorus between orthophosphate and organisms is supplemented with a second phosphorus cycle involving orthophosphate, organisms, a low molecular weight excretory product, and free colloidal phosphorus compounds. Second, regeneration of orthophosphate from organisms is postulated to occur within the epilimnion. This flux, in fact, is calculated to be almost equal to PO_4-P uptake (the generation of PO_4-P by the colloidal phosphorus cycle being minor in comparison).[1] Lean's model, however, applies only during the quasi-steady state of summer stagnation. An overall evaluation of the dynamic model of the phosphorus cycle, and its comparison with the classical, static model, cannot be made until seasonal data and data about phosphorus movement within the hypolimnion are available. The present study was undertaken to provide this data for PO_4-P uptake.

Lake Descriptions

Two lakes, Lake 227, and the south basin of Lake 302, were selected for seasonal orthophosphate studies. These lakes are described in more detail in the J. Fish. Res. Board Can. Vol. 28, No. 2, Feb. 1971. Morphometric maps of the lakes appear in Figures 10 and 11. Between the last week in May and the first week in October, Lake 227 receives 0.024 g of phosphorus as H_3PO_4 and 0.31 g of nitrogen as $NaNO_3$ per square meter per week as part of a fertilization experiment

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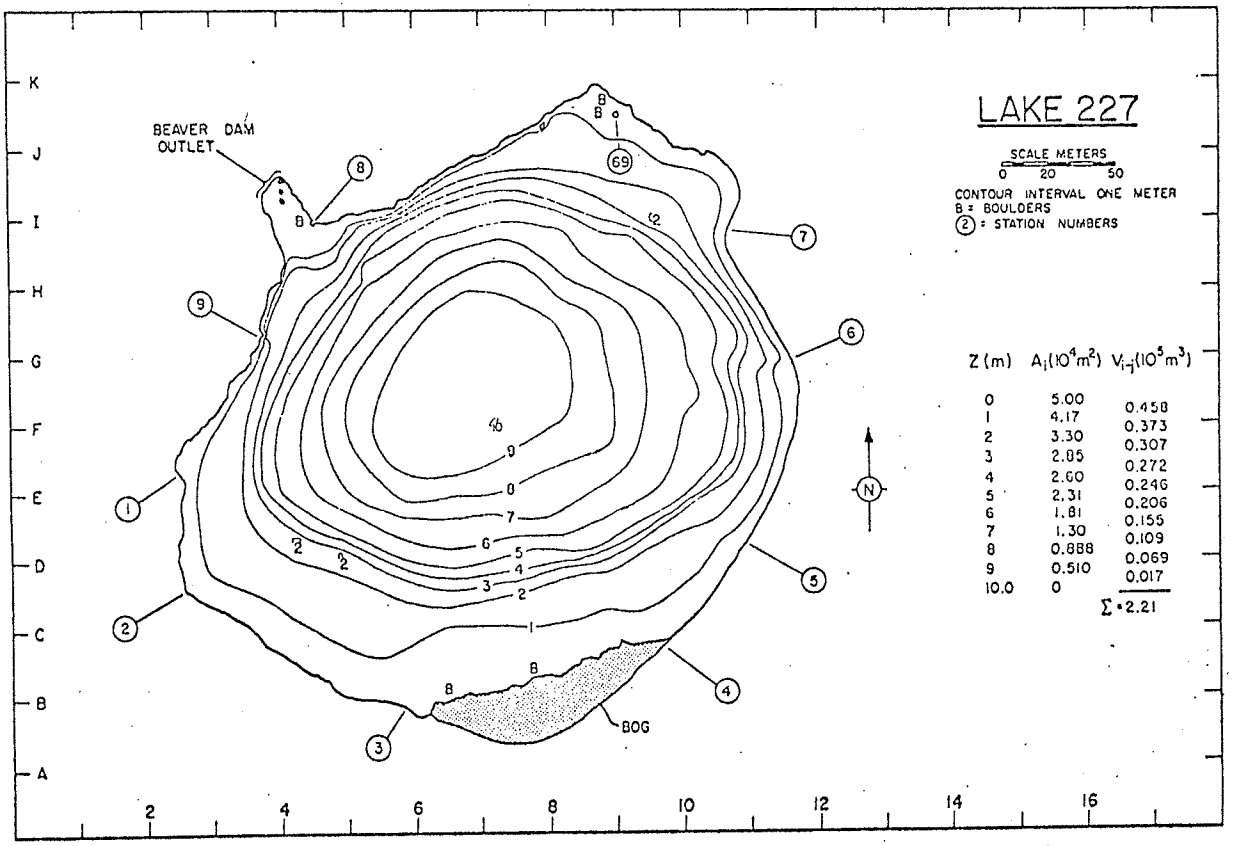
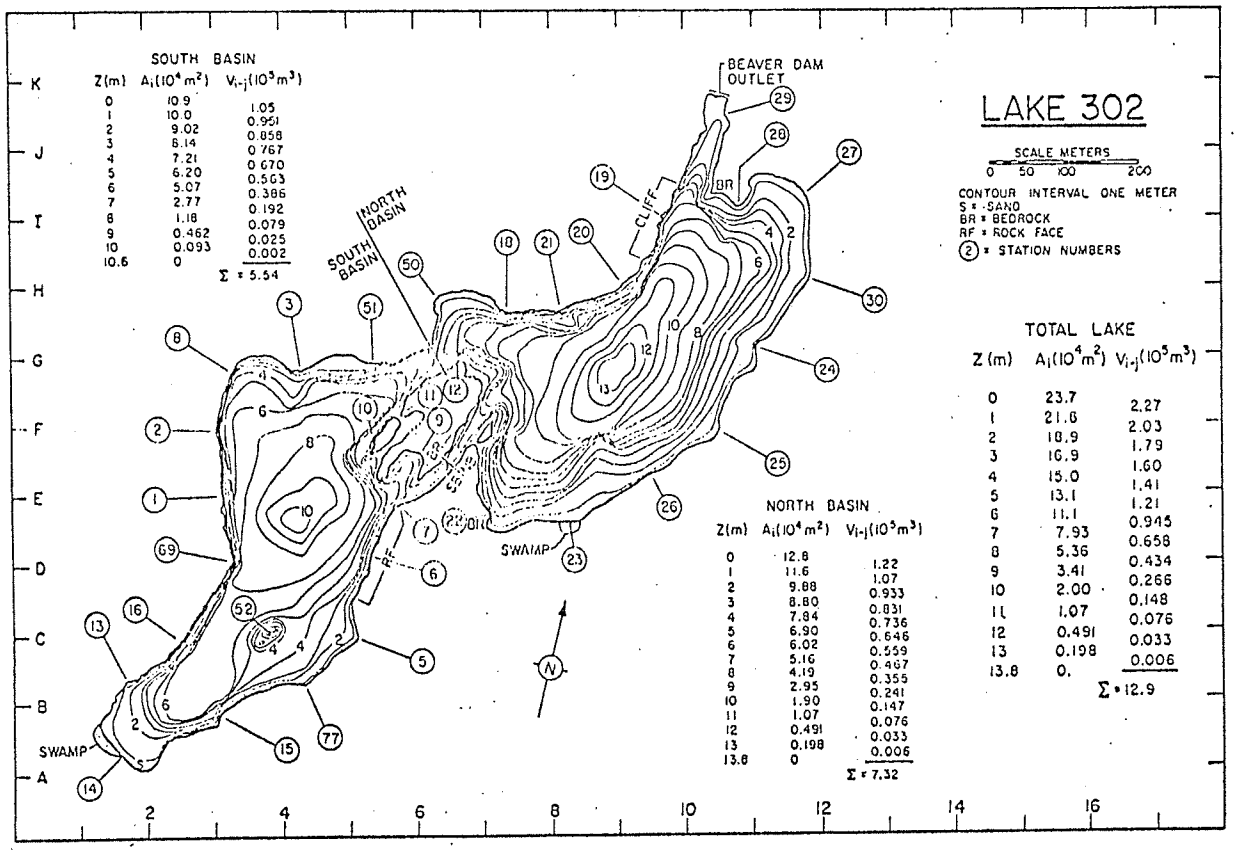
1. Lean assumed, probably justifiably, that the external orthophosphate loading into Heart Lake was negligible compared with the flux of PO_4-P to seston. In the present study phosphorus loading is included in the calculation of phosphorus fluxes.

Figure 10. A morphometric map of Lake 227 from Brunskill and Schindler (1971).



Z (m)	$A_i (10^4 m^2)$	$V_{i-1} (10^5 m^3)$
0	5.00	0.458
1	4.17	0.373
2	3.30	0.307
3	2.85	0.272
4	2.60	0.246
5	2.31	0.206
6	1.81	0.155
7	1.30	0.109
8	0.888	0.069
9	0.510	0.017
10.0	0	
		$\Sigma = 2.21$

Figure 11. A morphometric map of Lake 302 from Brunskill and Schindler (1971).


LAKE 302
SCALE METERS
0 50 100 200
CONTOUR INTERVAL ONE METER
S = SAND
BR = BEDROCK
RF = ROCK FACE
(2) = STATION NUMBERS
BEAVER DAM OUTLET
CLIFF
NORTH BASIN
SOUTH BASIN
SWAMP
SWAMP
SOUTH BASIN
Z(m) Ai(10⁴m²) Vi-j(10³m³)
0 10.9 1.05
1 10.0 0.951
2 9.02 0.858
3 8.14 0.767
4 7.21 0.670
5 6.20 0.563
6 5.07 0.386
7 2.77 0.192
8 1.18 0.079
9 0.462 0.025
10 0.093 0.002
10.6 0
Σ = 5.54
NORTH BASIN
Z(m) Ai(10⁴m²) Vi-j(10³m³)
0 12.8 1.22
1 11.6 1.07
2 9.88 0.933
3 8.80 0.831
4 7.84 0.736
5 6.90 0.646
6 6.02 0.559
7 5.16 0.467
8 4.19 0.355
9 2.95 0.241
10 1.90 0.147
11 1.07 0.076
12 0.491 0.033
13 0.198 0.006
13.8 0
Σ = 7.32
TOTAL LAKE
Z (m) Ai(10⁴m²) Vi-j(10⁵m³)
0 23.7 2.27
1 21.6 2.03
2 18.9 1.79
3 16.9 1.60
4 15.0 1.41
5 13.1 1.21
6 11.1 0.945
7 7.93 0.658
8 5.36 0.434
9 3.41 0.266
10 2.00 0.148
11 1.07 0.076
12 0.491 0.033
13 0.198 0.006
13.8 0.
Σ = 12.9
A B C D E F G H I J K
2 4 6 8 10 12 14 16

The natural phosphorus input into Lake 227 is, on average, 0.11 $g/m^2/yr$ (or about 0.004 g/m^2/week during the ice-free season) (Schindler et al., 1973). The south basin of Lake 302 is unfertilized. (The average natural phosphorus input is 0.05 $g/m^2/yr$ or about 0.002 $g/m^2/wk$ during the ice-free season.) Phosphorus (0.027 $g/m^2/wk$), nitrogen (0.14 $g/m^2/wk$) and carbon (0.19 $g/m^2/wk$), however, are added to the hypolimnion of the north basin during the summer. The north and south basins of Lake 302 are separated by a ridge over which the water is only one to two meters deep. This ridge prevents the mixing of hypolimnion waters from the two basins during summer stagnation. Some transfer of nutrient-rich waters occurred during fall overturn in 1973, however. Beginning during the summer of 1974, a sea curtain was used to separate the two basins and eliminate mixing during overturn.

Both Lake 227 and Lake 302 S are strongly stratified during the summer and in the winter under the ice. Spring overturn generally is incomplete. In 1973, fall overturn also was incomplete. Fall overturn in 1974, however, was somewhat over a month long in both lakes (from mid-October to mid-November). Because the lakes are normally stratified, the deeper waters are anoxic for a significant portion of the year (Figure 23). In the winter of 1973-74 (following the incomplete fall overturn), Lake 227 became anoxic throughout its water column.

Methods

Both epilimnion and hypolimnion[1] samples were examined during this study, but, because the Rigler bioassay requires two or more hours per sample, no attempt was made to construct an orthophosphate concentration-depth profile.

Sample Collection

Epilimnion samples

Epilimnion samples were collected biweekly to monthly during the summers of 1973 and 1974, and monthly to bimonthly during the winters of 1973-74 and 1974-75. The samples were collected two to seven days after fertilization. Because the epilimnion is well mixed chemically, but motile organisms within the epilimnion often are patchily distributed, integrated samples were taken. A 2 m long, 3.25 cm diameter, PVC tube was lowered from the lake surface and stoppered with a rubber bung. It then was quickly raised and drained into a polyethelene bottle. This process was repeated two or three times. The temperature of the water then was recorded, and the water returned to the laboratory. Transport time never exceeded one hour.

Samples were placed in a water bath at *in situ* temperature upon arrival at the laboratory.

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1. To avoid application of different terminology to winter and summer samples, samples collected from the upper two meters of a lake are referred to as epilimnion samples at all times of the year. Similarly, samples collected from a depth of 9 meters are referred to as hypolimnion samples throughout the year.

Hypolimnion samples

Samples were collected from a depth of 9 m approximately bi-monthly from June, 1974 to March, 1975. Integrated hypolimnion samples were not collected because the hypolimnion often is chemically, if not thermally, stratified. A battery-powered pump and tygon tubing were used to draw water from the sampling depth. The line was flushed with this water for 2 to 3 minutes before sample collection. Fifty cc glass syringes then were filled, inspected for bubbles, and, if bubble-free, closed with a three-way valve. Syringes were placed in fitted holes in a styrofoam box to prevent breakage and to keep the water near *in situ* temperature during transport. Water collected for TDP, PP, and ATP analyses was pumped into polyethelene bottles. (No attempt was made to keep this water anoxic.) Finally, a thermometer was inserted into the sampling line and the temperature of the water was recorded.

Analyses

Analyses were conducted for particulate phosphorus (PP) and total dissolved phosphorus (TDP) following the procedure described by Stainton, Capel, and Armstrong (1974). TDP concentration was used as an estimate of colloidal phosphorus concentration. This was justified by the minute amounts of PO_4-P found in the studied lakes (normally less than 1% of the TDP) and by Chamberlain's observation (Chamberlain, 1968) that TDP concentration is reduced by an order of magnitude when lake water is passed through an 0.01μ filter (indicating that most TDP must be colloidal P).

Beginning in June, 1974, adenosine triphosphate (ATP) was measured using the method of Rudd and Hamilton (1973). ATP extractions were performed immediately upon arrival at the laboratory. Sartorius

cellulose filters (25 mm in diameter and with a pore size of 0.45μ) were used to separate the seston from the water.

Dissolved oxygen and chlorophyll-a data were obtained from the E.L.A. analytical laboratory. Because this information was presented for discrete depths, the 1/2, 1, and 2 meter values were averaged to give estimates of the integrated epilimnion concentrations.[1] Some TDP, PP and temperature data from this source are included as well.

Rigler Bioassays

Epilimnion bioassays were performed in the manner described in Section I.

Bioassays for hypolimnion water had to be conducted without introducing oxygen into the samples. For the first bioassay performed for each lake, the $^{32}PO_4$ and the unlabeled orthophosphate were brought to a boil to render them anoxic and then drawn into glass syringes. When needed, the solutions were injected into the sample syringe through its tip. The likelihood of concentrating the accurately measured unlabeled orthophosphate solution by boiling soon was realized. For the second set of samples, only the $^{32}PO_4$ solution was boiled. It was assumed that an insignificant amount of oxygen would be introduced with the small quantity of orthophosphate solution which was added (the maximum addition, 1 ml, would result in a concentration in the syringe

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1. When the epilimnion was well-mixed, this method was fairly reliable. (Temperatures obtained by averaging were close to those measured on integrated samples.) When the "epilimnion" was stratified (under ice), however, the discrepancy between the calculated and measured values became significant (the calculated temperatures for winter samples hovered around 3 or 4°C; whereas, the integrated samples had temperatures of 0.5 to 2°C).

of about 0.009 mg O_2/l, if the solution were air saturated and at room temperature). Beginning in July, all solutions were bubbled with nitrogen rather than boiled during hypolimnion bioassays.

After the injection of $^{32}PO_4$ and unlabeled orthophosphate, the samples were shaken manually for two minutes and occasionally thereafter.

At first water from the syringes was expelled at regular intervals into a 10 ml graduated cylinder which was plugged with a serum bottle stopper and inverted so that 5 ml aliquots could be withdrawn. This time consuming step was eliminated after July. Unmeasured aliquots then were introduced directly into the filtering funnel from the glass syringe. Both filter and filtrate activity were counted and the proportion of filtrate to total ^{32}P activity was used in the analysis.

Results

Epilimnion Samples

PO_4-P Concentration

Although the PO_4-P concentration in Lake 227 was raised to 12 µg/l immediately after fertilization each week during the summer, the average PO_4-P concentration in Lake 227 was no larger than in Lake 302 S when samples were collected (Table 6). In both lakes, PO_4-P concentration was extremely low (from less than 1 to 53 ng/l) during the summer (Figure 12). Only slightly higher concentrations were recorded during spring and fall overturn when orthophosphate from the hypolimnions was mixed into the epilimnions. The largest PO_4-P concentrations (up to 135 times the summer means) occurred during the winter of 1973-74 when the dissolved oxygen concentration in the epilimnions were very low

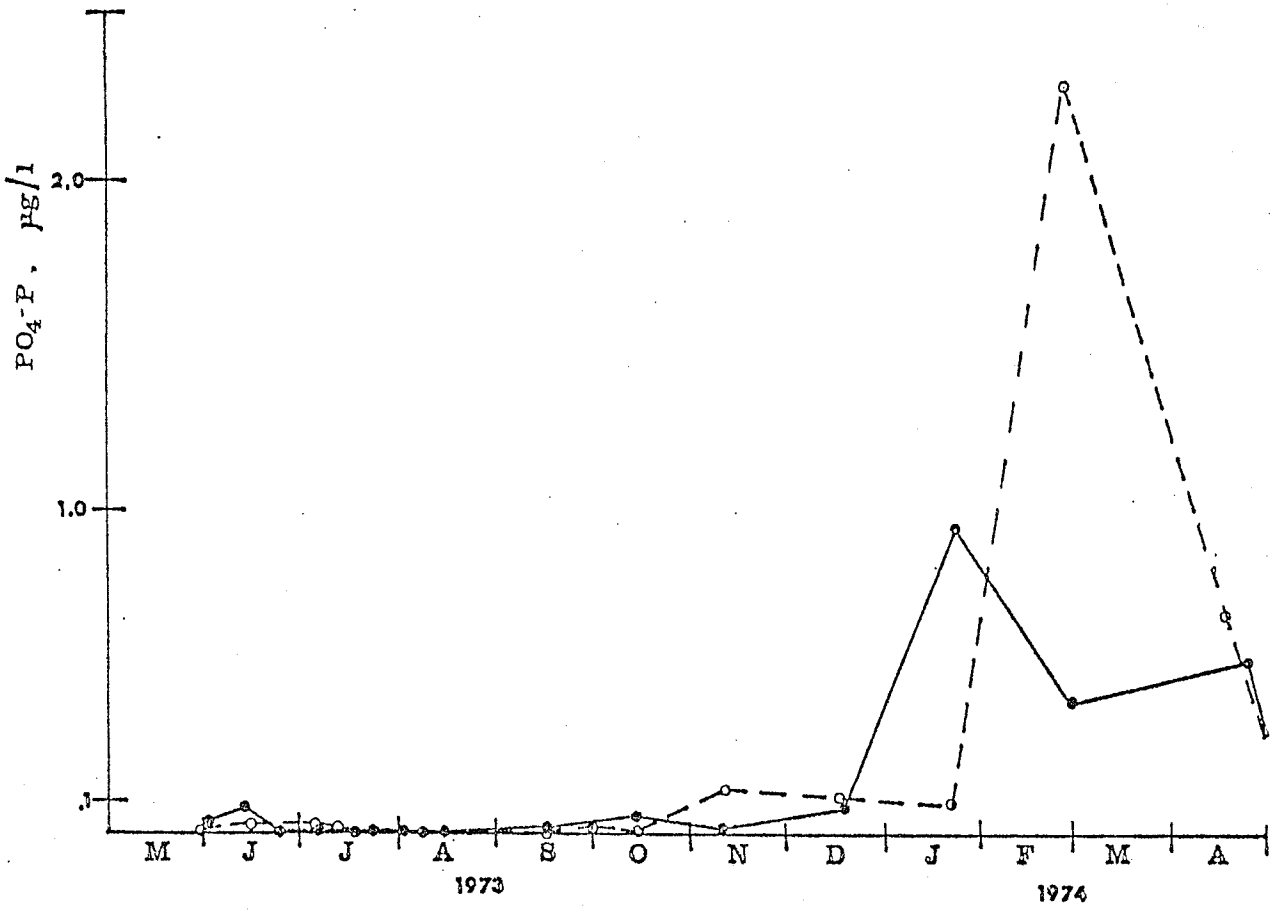
TABLE 6. The mean PO_4-P concentrations in Lakes 227 and 302 S during summer stagnation and in the winter.

Epilimnion samples were collected between May, 1973 and April, 1975 and hypolimnion samples between May, 1974 and April, 1975. Because the DO concentrations in the epilimnion were very different during the two winters, the epilimnion PO_4-P concentrations for the two winters are presented separately.

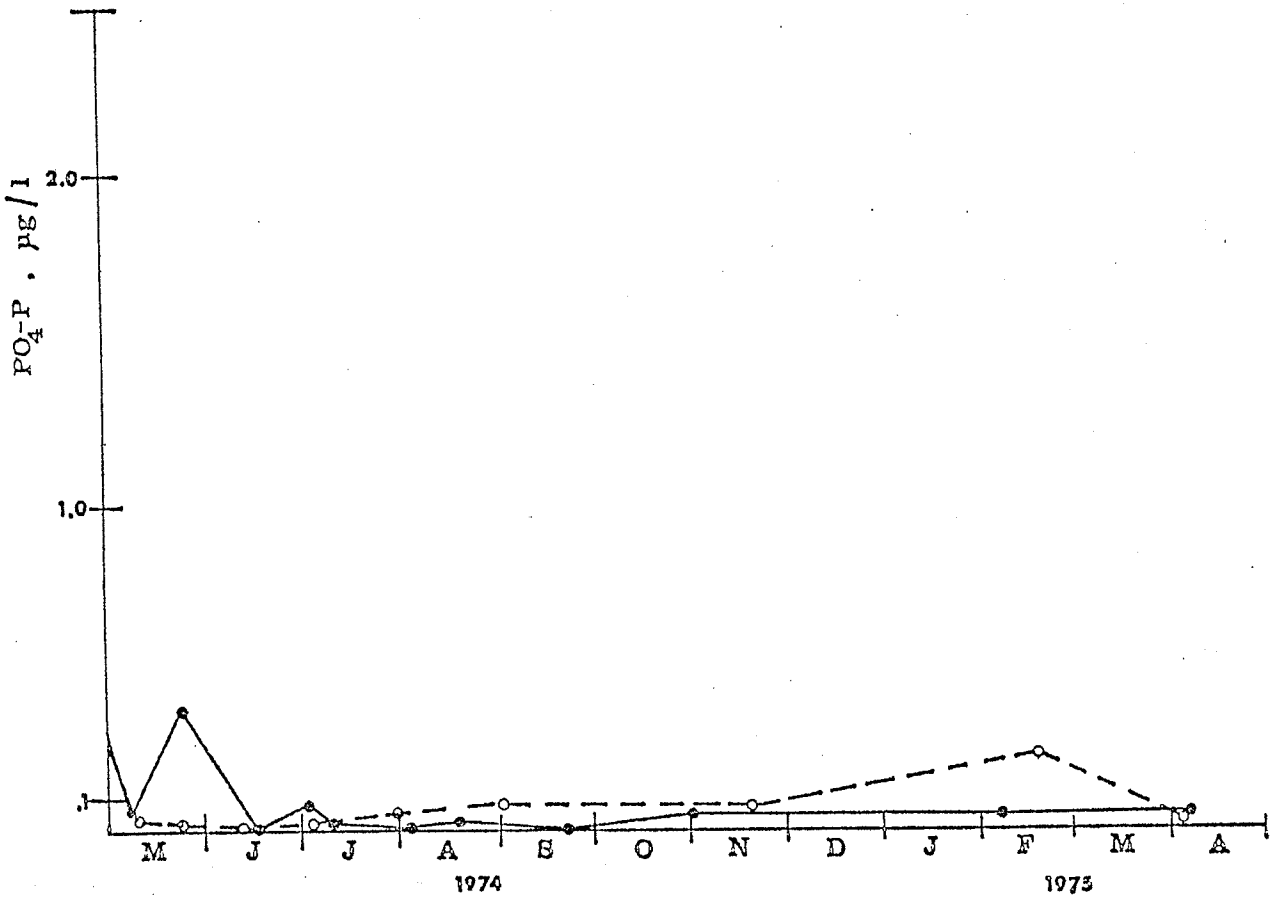
Stratum	Season	Mean PO_4-P Concentration (µg/l)	
		Lake 302 S (oligotrophic)	Lake 227 (eutrophic)
epilimnion	summer	0.017	0.016
	winter (1973-74)	0.53	0.36
	winter (1974075)	0.11	0.041
hypolimnion	summer	0.037	0.070
	winter	0.075	0.018

FIGURE 12. Seasonal changes in orthophosphate-phosphorus concentrations in the epilimnions of Lake 227 (●——●) and Lake 302 S (○--○).

12 A May, 1973 to April, 1974.


PO4-P . µg/l
2.0
1.0
.1
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

12 B May, 1974 to April, 1975.


PO_4-P . µg/l
2.0
1.0
.1
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

(Figure 13). The dissolved oxygen concentration in the epilimnions of the lakes remained high throughout the winter of 1974-75. The PO_4-P concentrations attained during this winter were much lower than the year before (from 1 to 10 times the mean summer values).

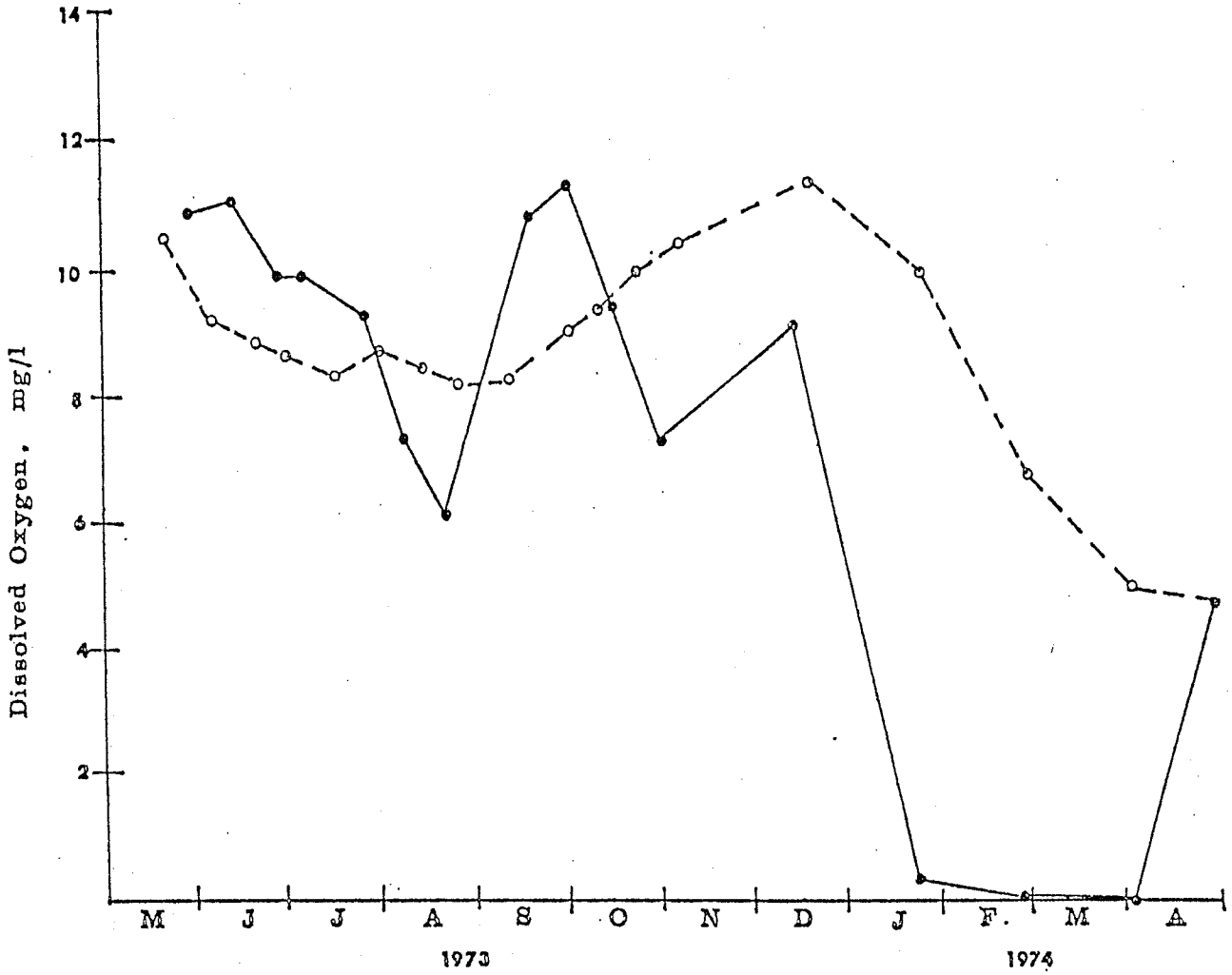
The Rate Constant for PO_4-P Uptake

The rate constant for PO_4-P uptake by seston, K, varied inversely with changes in PO_4-P concentration (Figures 14 and 15). During the summer the turnover time of the PO_4-P was short (usually less than 10 minutes) (Table 7). In the winter, phosphorus moved through the pool more slowly (the turnover time ranged from 13 minutes (immediately after ice-cover) to several hours (when the dissolved oxygen concentration was very low)). A plot of K versus PO_4-P concentration indicated that the relationship between the two parameters is curvilinear (Figure 15). The regression of ln K on PO_4-P concentration was significant for both lakes (r_{227} = 0.723, df = 26) $r_{302\ S}$ = 0.654, df = 20). This relationship suggests that K is a function of the availability of uptake sites. K was significantly correlated with temperature (r_{227} = 0.453, df = 23; $r_{302\ S}$ = 0.702, df = 19)[1], but could not be significantly correlated with PP, chlorophyll-a, or ATP. When biocides or inhibitors were added to samples (Appendix III), K decreased greatly, confirming that most PO_4-P uptake is biological. Most likely, K would be related to biomass if all PO_4-P uptake sites were saturated. Under these conditions, species composition and the adaptation of organisms to certain PO_4-P concentrations also would influence uptake rate. Because the capacity of the organisms to take up PO_4-P was not exhausted at the

1. Seasonal changes in temperature are shown in Figure 16.

FIGURE 13. Seasonal changes in the dissolved oxygen concentrations in the epilimnions of Lake 227 (●——●) and Lake 302 S (○- -○).

13 A May, 1973 to April, 1974.


Dissolved Oxygen, mg/l
14
12
10
8
6
4
2
M
J
J
A
S
O
N
D
J
F.
M
A
1973
1974

13 B May, 1974 to April, 1975.

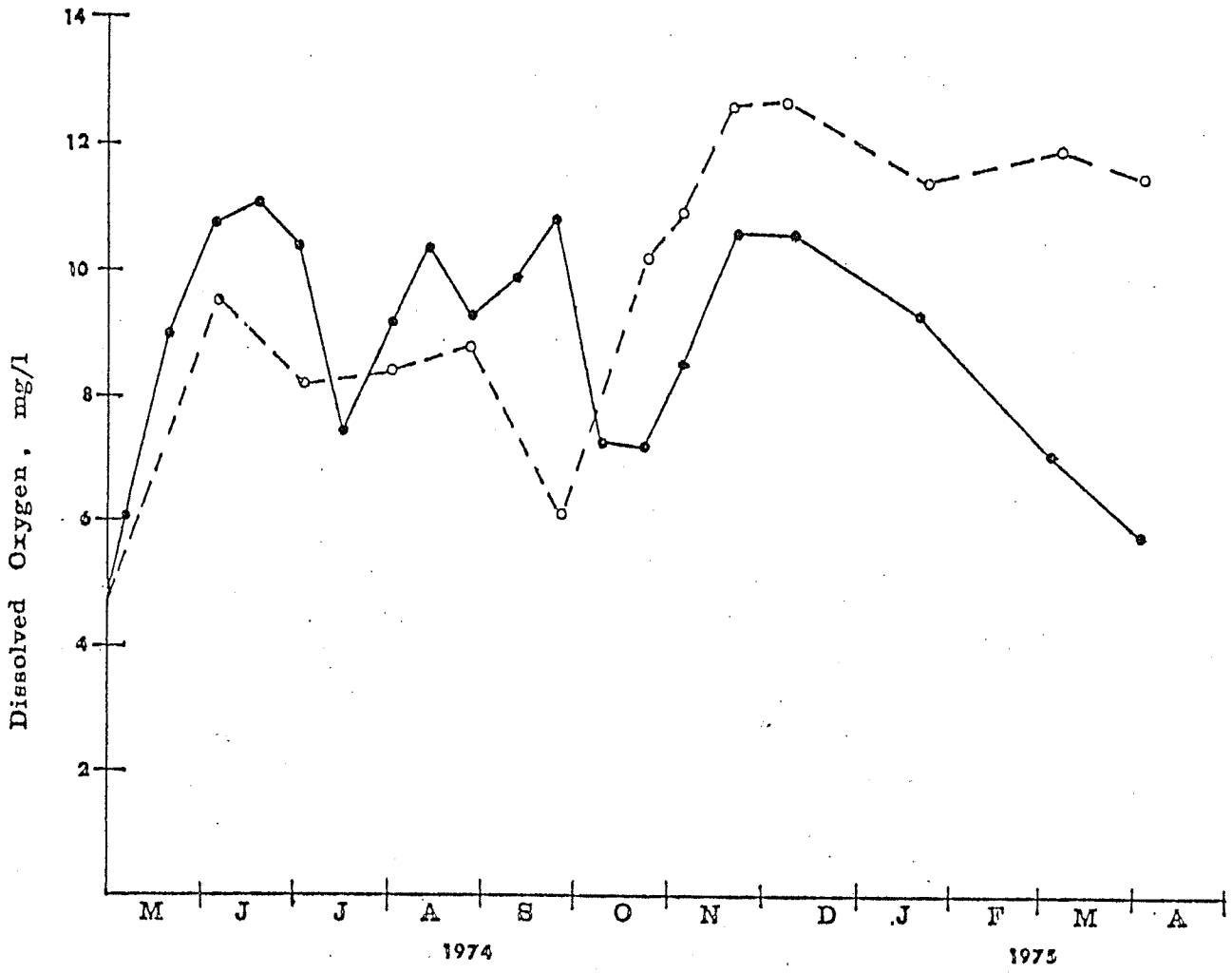

Dissolved Oxygen, mg/l
14
12
10
8
6
4
2
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

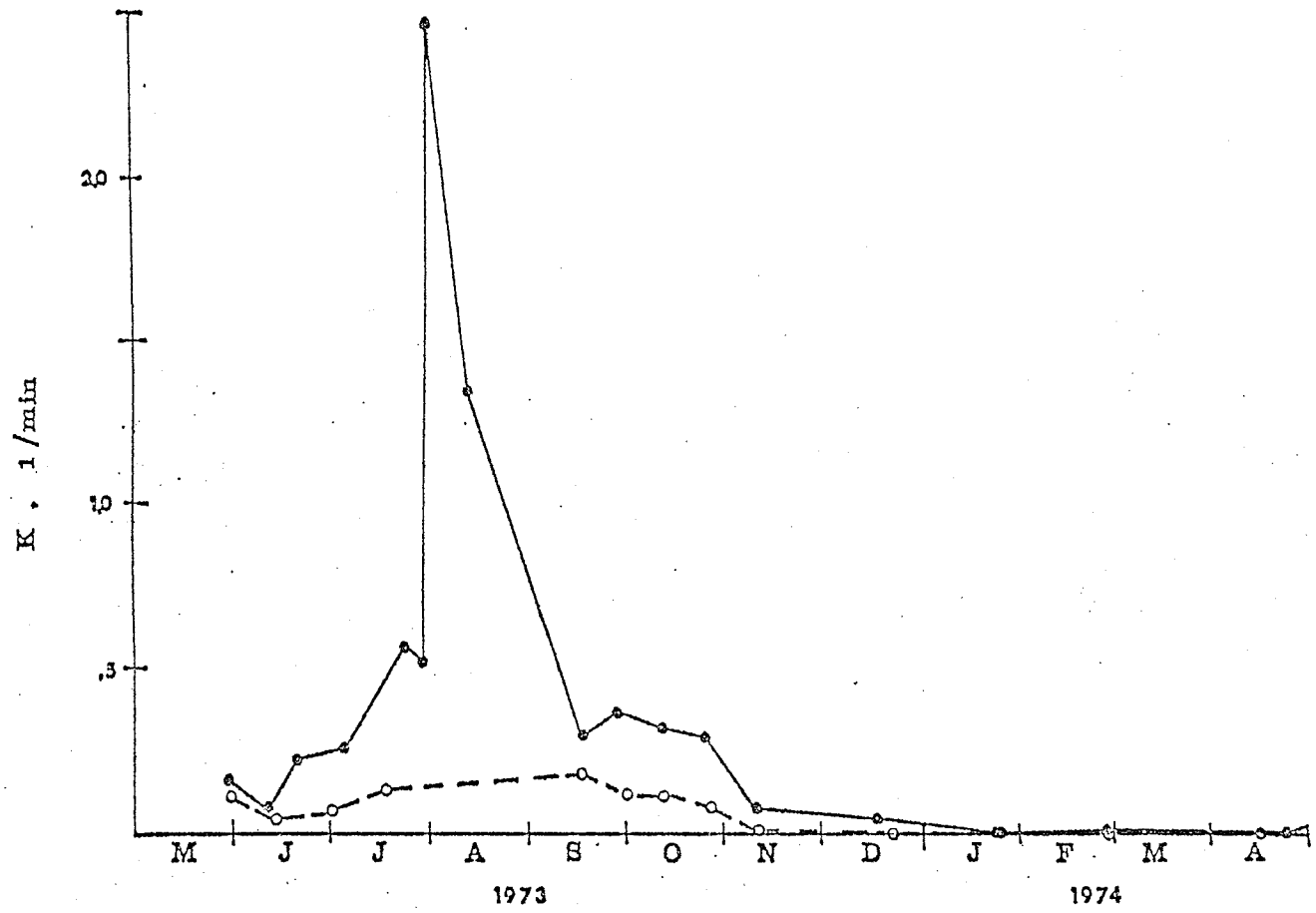
TABLE 7. Mean turnover times of the PO_4-P pools in Lakes 227 and 302 S during summer stagnation and in winter.

Epilimnion samples were collected between May, 1973 and April, 1975 and hypolimnion samples between May, 1974 and April, 1975. Because the DO concentrations in the epilimnion were very different during the two winters, the epilimnion PO_4-P concentrations for the two winters are presented separately.

Stratum	Season	Mean Turnover Time (min)	
		Lake 302 S (oligotrophic)	Lake 227 (eutrophic)
epilimnion	summer	9.09	2.03
	winter (1973-74)	270	83.3
	winter (1974-75)	83.3	2.94
hypolimnion	summer	7.75	14.8
	winter	19.1	8.33

FIGURE 14. Seasonal changes in the rate constants for orthophosphate-phosphorus uptake by seston in the epilimnions of Lake 227 (●——●) and Lake 302 S (○— —○).

14 A May, 1973 to April, 1974.


K . 1/min
2,0
1,0
,5
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

14 B May, 1974 to April, 1975

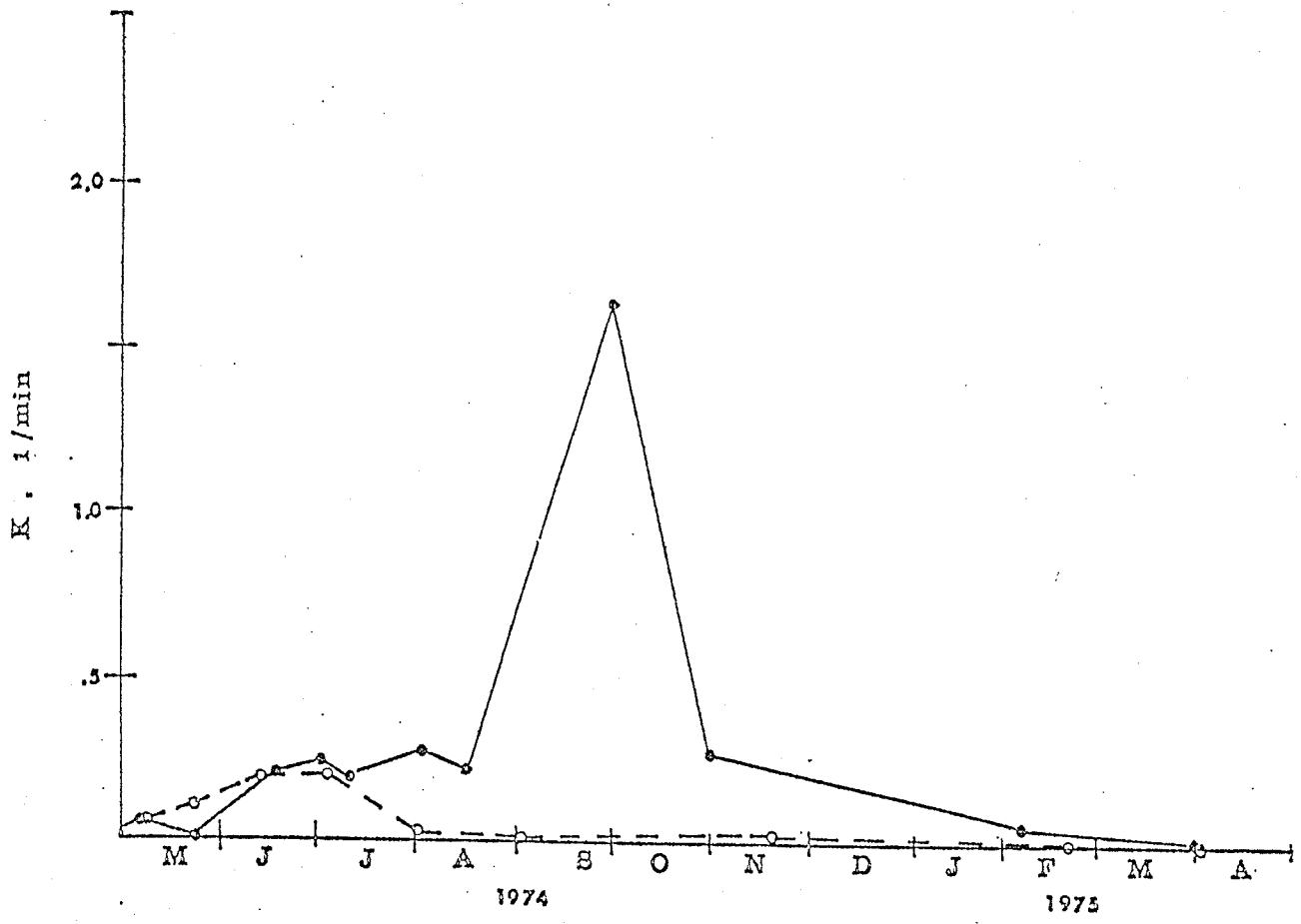

K . 1/min
2.0
1.0
.5
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

FIGURE 15. A plot of rate constant for orthophosphate-phosphorus uptake by seston (K) vs the orthophosphate-phosphorus concentration. Data from both Lake 227 (epilimnion: ○ ; hypolimnion: ●) and Lake 302 S (epilimnion: △ ; hypolimnion: ▲) are included. Three points with rate constants greater than 0.4/min and two points with concentrations greater than 0.18 µg/l are not shown. These points lie very close to the y and x axes respectively.

The exponential regression of K on orthophosphate-phosphorus concentration was highly significant (r^2 = 0.636, df = 64, all data included).

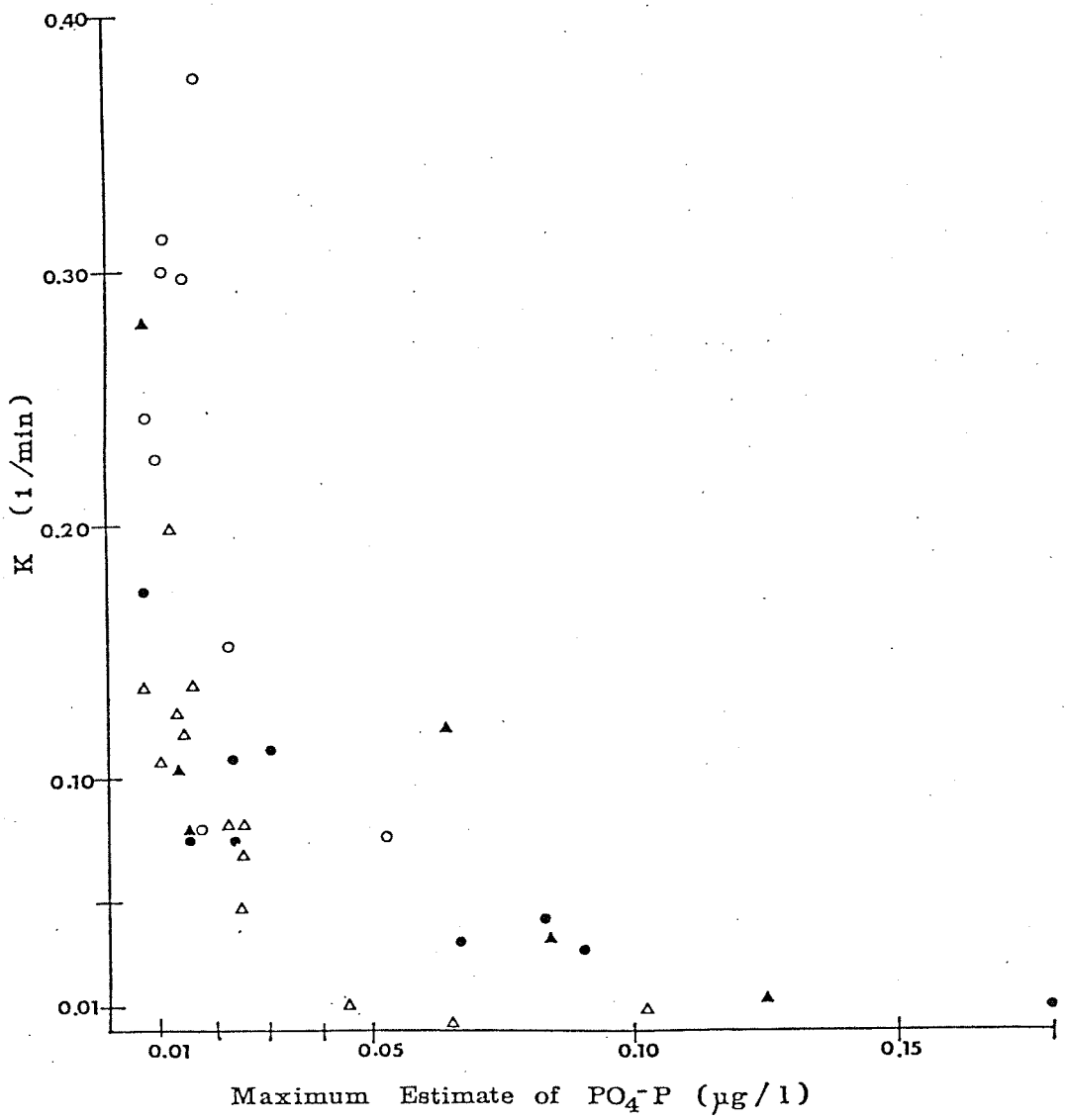
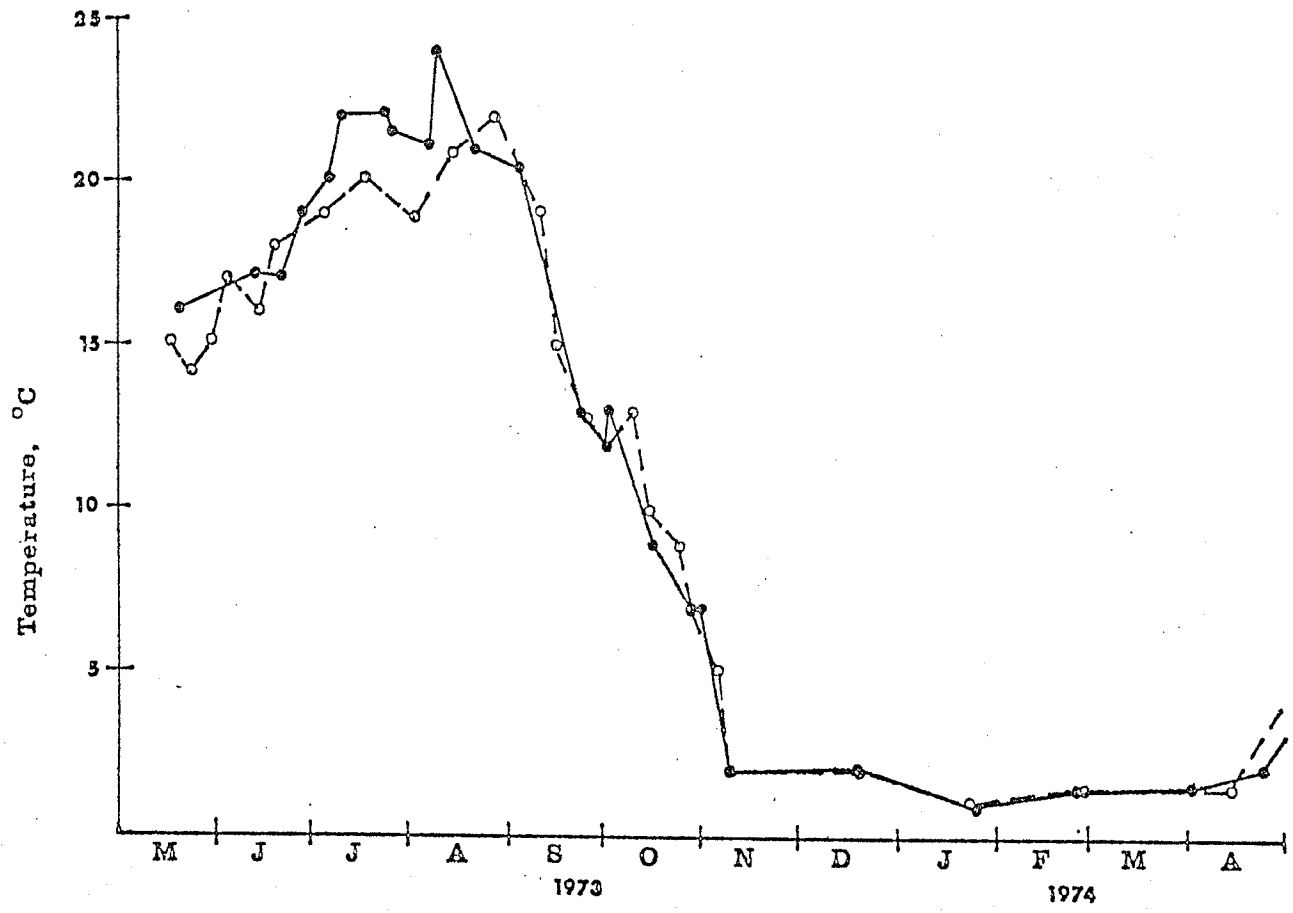
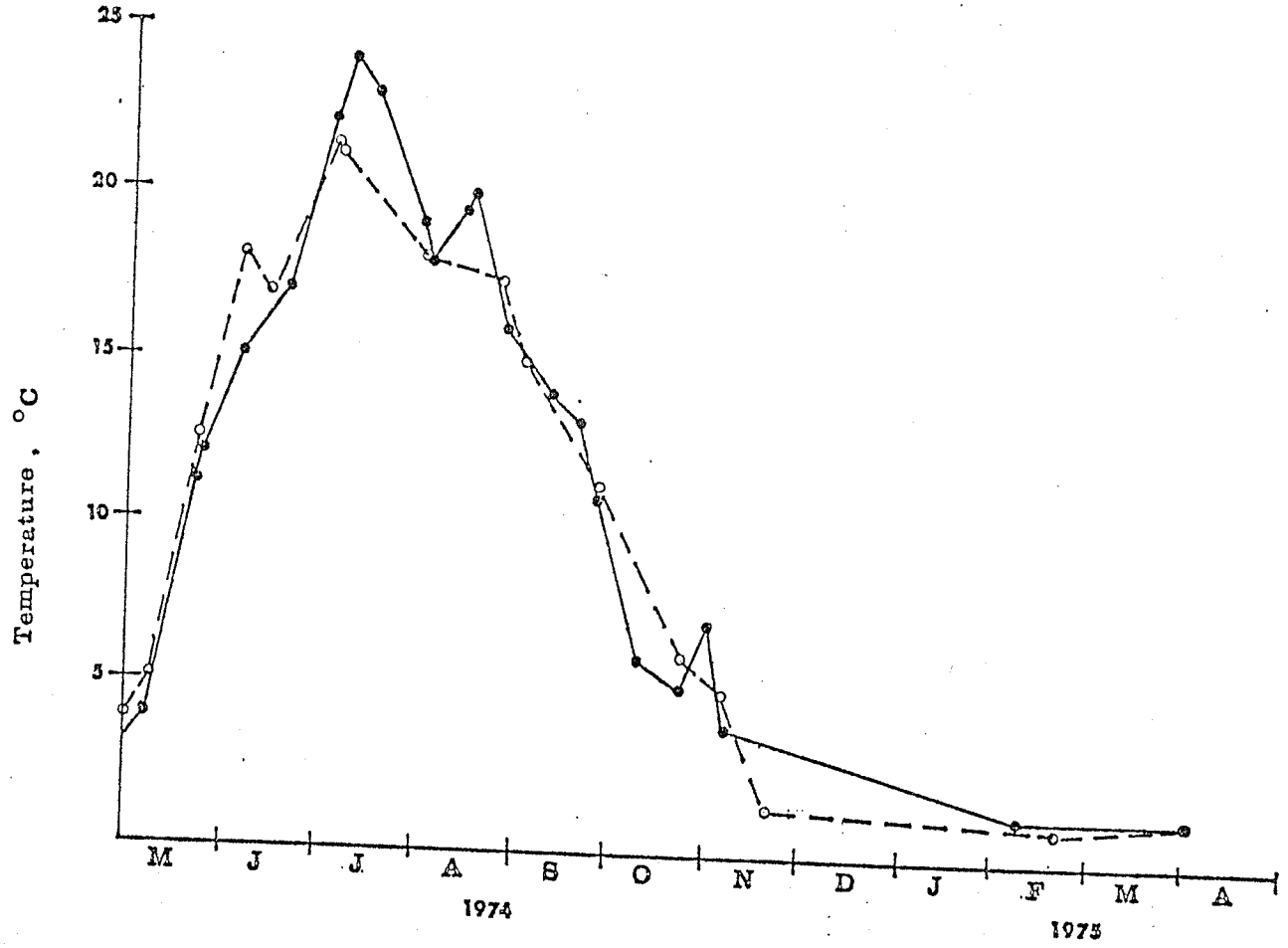

0.40
0.30
0.20
0.10
0.01
K (1/min)
0.01
0.05
0.10
0.15
Maximum Estimate of PO_4^-P (μg/l)

FIGURE 16. Seasonal changes in the temperature of the epilimnions of Lake 227 (●—●) and Lake 302 S (○- -○).

16 A May, 1973 to April, 1974.


25
20
15
10
5
Temperature, °C
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

16 B May, 1974 to April, 1975.


25
20
15
10
5
Temperature, °C
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

concentrations of PO_4-P which were present, K was a function of PO_4-P concentration, rather than of biomass.

PO_4-P Flux to Seston

Because large fluctuations in PO_4-P concentration were matched with nearly opposite changes in K, seasonal variations in the PO_4-P flux to seston were relatively small (Figure 17, Table 8). In Lake 302 S, PO_4-P flux tended to be minimal during late winter. Otherwise there was no seasonal trend in PO_4-P flux in this lake. Highest flux values in Lake 227 were recorded during fall overturn and immediately following a forest fire in July, 1974 (less than 5% of the watershed of Lake 227 was burned and some ash was blown into the lake). These peaks in flux were short-lived and probably are analogous to the elevation in PO_4-P flux which must occur in Lake 227 each week after the lake is fertilized. If PO_4-P flux did not rise temporarily after fertilization, the PO_4-P concentrations which were measured two or three days after fertilization would have been considerably higher than they were. Like K, PO_4-P flux was not significantly correlated with any of the biomass parameters (ATP, chlorophyll-a, and PP) which were measured.

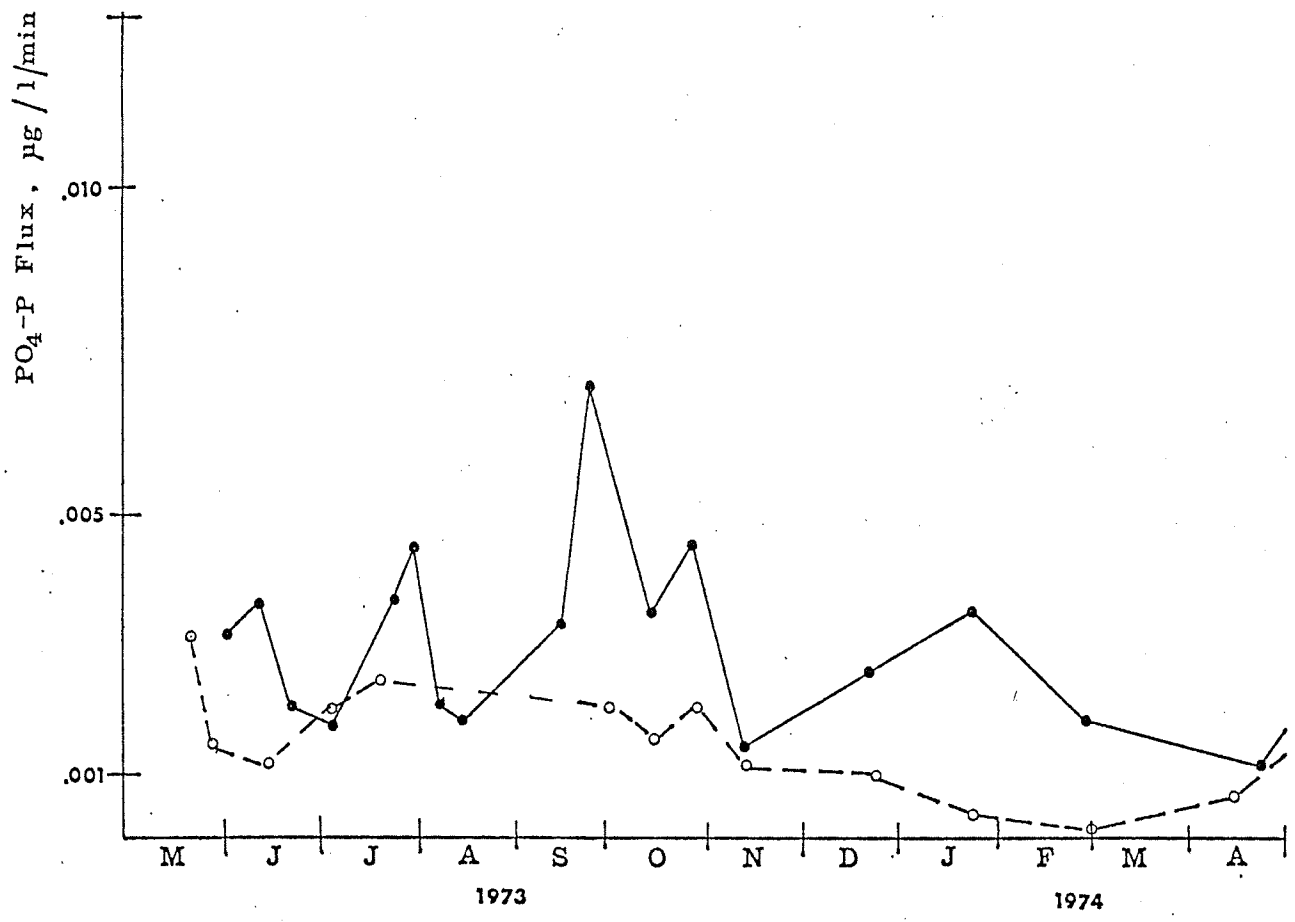
Particulate Phosphorus

Much of the phosphorus added to Lake 227 as PO_4-P appeared as PP when samples were collected (Table 9). The mean summer PP concentration in Lake 227 was seven times the summer mean in Lake 302 S. In the winter, when no phosphorus entered the lakes, this ratio dropped to 2.

Compared with the seasonal change in phosphorus loading, changes in PP concentration were not pronounced (Figure 18). In Lake 302 S, PP

FIGURE 17. Seasonal changes in the orthophosphate-phosphorus flux into seston in the epilimnions of Lake 227 (●——●) and Lake 302 S (○– –○).

17 A May, 1973 to April, 1974.


PO_4-P Flux, µg / l/min
.010
.005
.001
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

17 B May, 1974 to April, 1975.

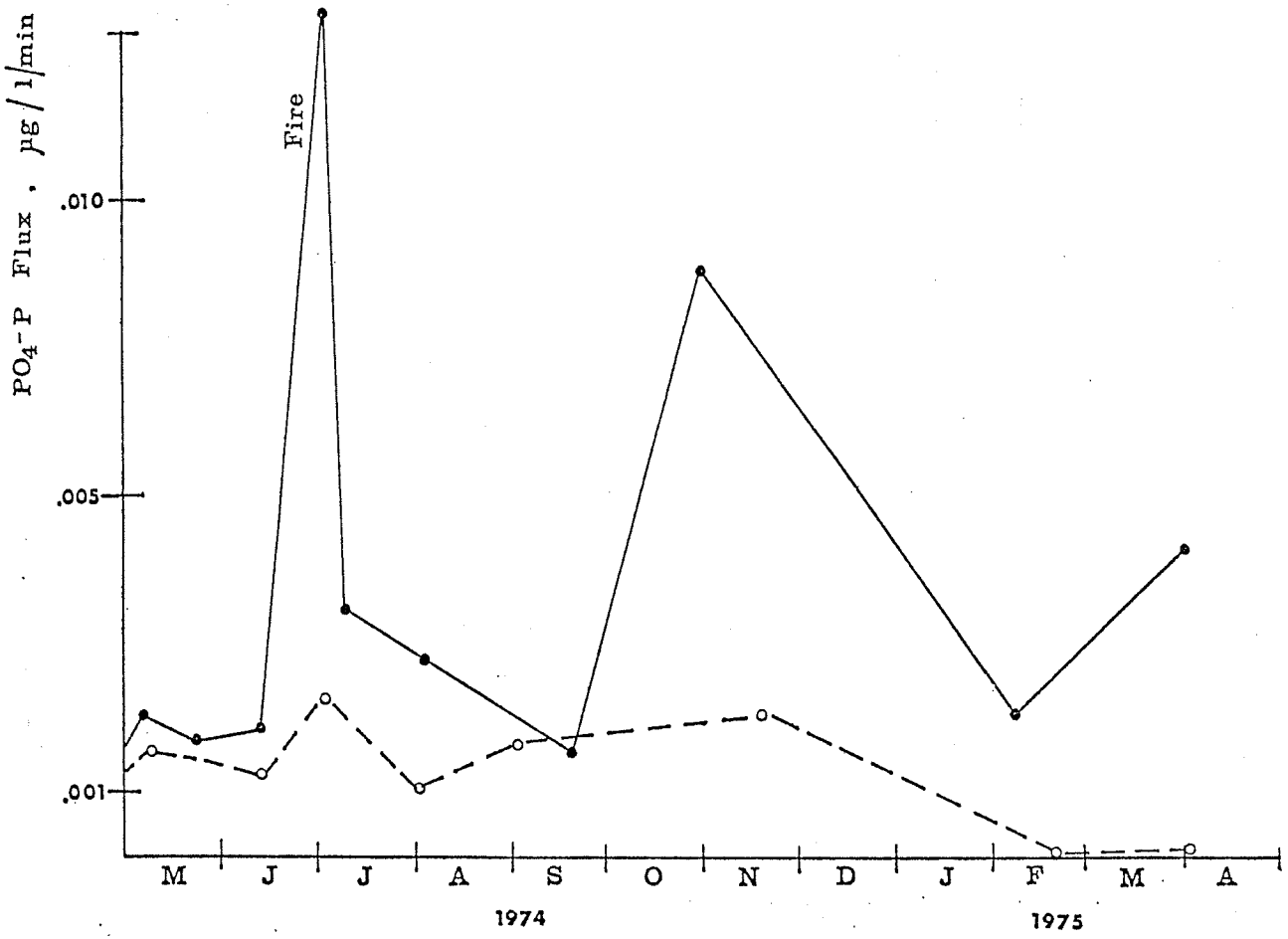

PO4-P Flux, µg/l/min
.010
.005
.001
Fire
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

TABLE 8. The mean PO_4-P flux to seston in Lakes 227 and 302 S during summer stagnation and in the winter.

Epilimnion samples were collected between May, 1973 and April, 1975 and hypolimnion samples between May, 1974 and April, 1975. Because the DO concentrations in the epilimnion were very different during the two winters, the epilimnion PO_4-P concentrations for the two winters are presented separately.

Stratum	Season	Mean PO_4-P flux (µg/l/min)	
		Lake 302 S (oligotrophic)	Lake 227 (eutrophic)
epilimnion	summer	0.0017	0.0023
	winter (1973-74)	0.0011	0.0019
	winter (1974-75)	0.0078[a]	0.0035
hypolimnion	summer	0.0033	0.0024
	winter	0.0022	0.0020

a. The mean value was highly influenced by one early sample.

TABLE 9. Mean TDP, PP, ATP and chlorophyll-a concentrations in the epilimnions and hypolimnions of Lakes 227 and 302 S during summer stagnation and in the winter (under ice).[1]

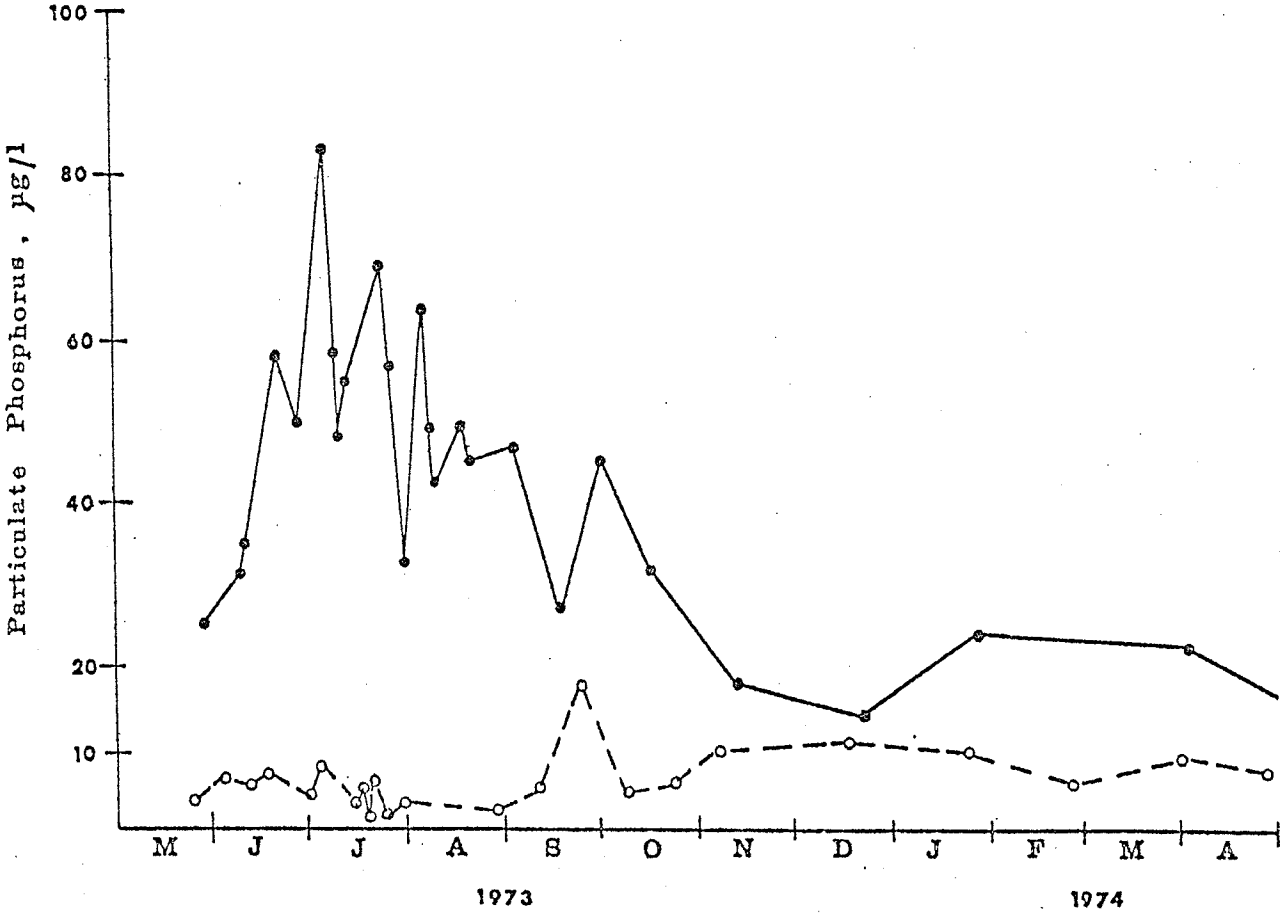
The epilimnion samples were collected between May, 1973 and April, 1975, and the hypolimnion samples between May, 1974 and April, 1975. ATP concentration was not measured until May, 1974.

Lake	Stratum	Season	Mean PP (µg/l)	Mean TDP (µg/l)	Mean ATP (µg/l)	Mean Chlorophyll-a (µg/l)
227	epilimnion	summer	42	11	0.59	50.0
227	epilimnion	winter	20	8	0.31	16.5
227	hypolimnion	summer	102	16	0.70	108
227	hypolimnion	winter	70	11	0.74	23.8
302 S	epilimnion	summer	6	3	0.15	4.2
302 S	epilimnion	winter	9	5	0.23	5.2
302 S	hypolimnion	summer	30	6	0.21	53.6
302 S	hypolimnion	winter	18	7	0.33	10.8

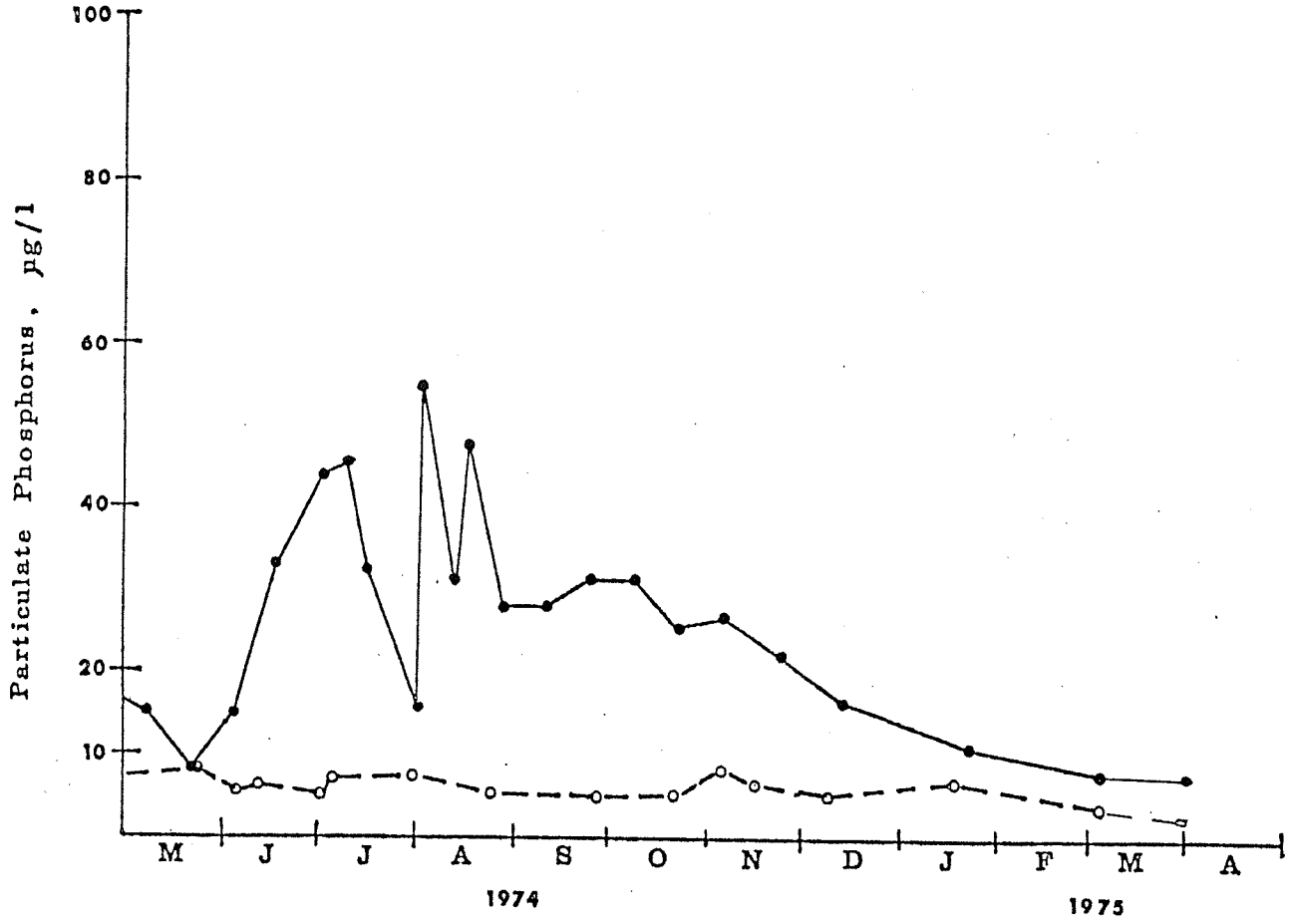
1. Because spring and fall overturn were brief, values for samples collected during these periods were not included in these calculations. The term "summer", therefore, covers the period between late May and about mid-October and "winter" is the period when the lake is covered with ice.

FIGURE 18. Seasonal changes in the particulate phosphorus concentrations in the epilimnions of Lake 227 (●——●) and Lake 302 S (○- -○).

18 A May, 1973 to April, 1974.


Particulate Phosphorus, µg/l
100
80
60
40
20
10
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

18 B May, 1974 to April, 1975.


Particulate Phosphorus, µg/l
100
80
60
40
20
10
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

concentration was higher during fall overturn and throughout the winter than it was during the summer. This pattern occurred because most primary production takes place in the hypolimnion (at about 6 m) in Lake 302 S (E.J. Fee, unpublished data). PP builds up in this region over the summer and is swept into the epilimnion during fall overturn. In Lake 227, high turbidity limits the euphotic zone to the epilimnion. Nevertheless, PP concentrations in Lake 227 were only twice as high during the summer as they were in the winter.

Total Dissolved Phosphorus

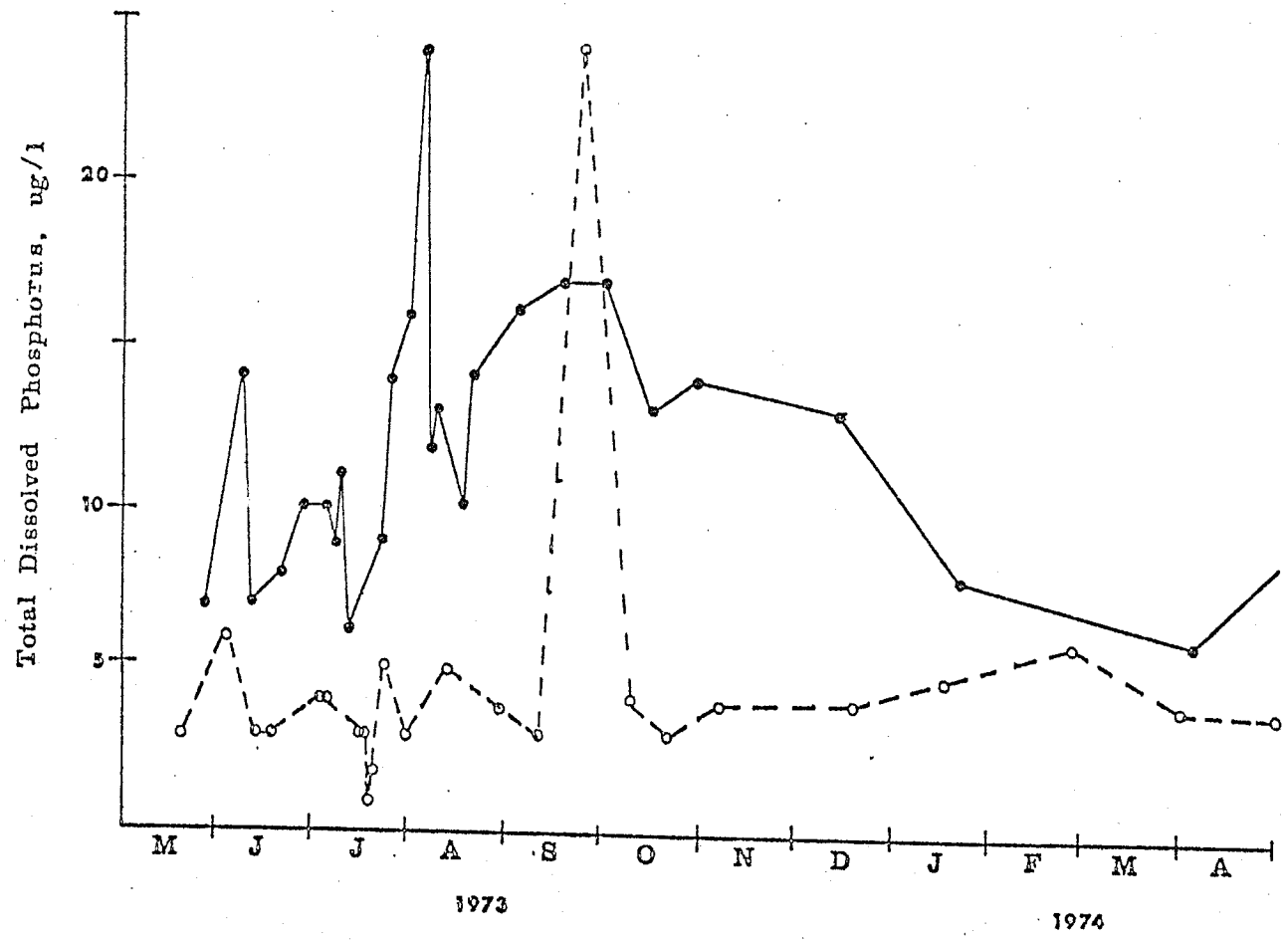
Total dissolved phosphorus concentration also increased in response to fertilization, but not as strongly as the PP concentration did. The TDP concentration in the epilimnion of Lake 227 was, on average, only three times that in the epilimnion of Lake 302 S during the summer (Table 9). Because nearly all TDP is colloidal phosphorus, this suggests either that a large fraction of colloidal phosphorus is allochthonous or that the fraction of seston involved in the colloidal phosphorus cycle was smaller in the fertilized lake than in the unfertilized lake. Unlike PP concentration, TDP concentration did not decrease greatly when fertilization was stopped for the winter. The winter TDP concentration in Lake 227 was about twice that in Lake 302 S. This attests to the relative inertness of colloidal phosphorus compared with other forms of phosphorus in lake water. TDP concentration did not vary greatly with season in either lake (Figure 19). The maximum concentrations coincided with or followed peaks in biomass.

ATP and Chlorophyll-a

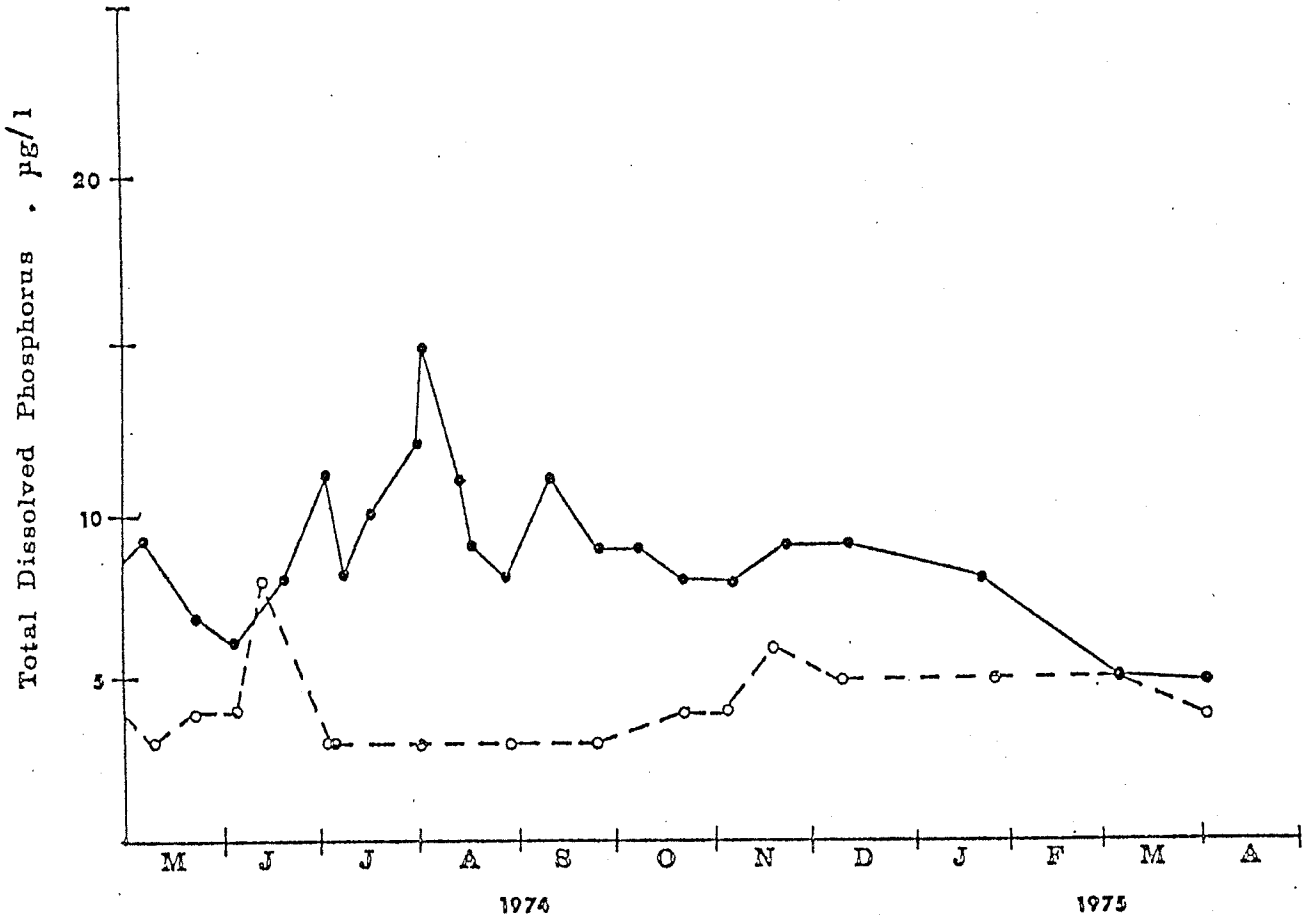
ATP and chlorophyll-a were measured so that changes in the distribution of phosphorus between the phosphorus fractions could be

FIGURE 19. Seasonal changes in the total dissolved phosphorus concentrations in the epilimnions of Lake 227 (●——●) and Lake 302 S (○— —○).

19 A May, 1973 to April, 1974.


Total Dissolved Phosphorus, ug/l
20
10
5
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

19 B May, 1974 to April, 1975.


Total Dissolved Phosphorus . µg/l
20
10
5
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

related to changes in total biomass and in algal biomass respectively.

As a result of fertilization, the mean summer ATP concentration in Lake 227 was four times the mean for Lake 302 S (Table 9). Thus, ATP does not seem to have responded as strongly to phosphorus loading as PP did. Nevertheless, ATP concentration was significantly correlated with PP concentration in both lakes ($r = 0.808$, $df = 9$).[1] This suggests that part of the increase in PP concentration was due to phosphorus in dead suspended material. ATP concentration was twice as high in the summer as in the winter in Lake 227 and slightly higher during the winter than during the summer in Lake 302 S (Figure 20).[2]

Chlorophyll-a responded strongly to phosphorus loading. The mean summer chlorophyll-a concentration in Lake 227 was 12 times that in Lake 302 S (Table 9). In winter, the mean chlorophyll-a concentration in Lake 227 exceeded that in Lake 302 S by only threefold.

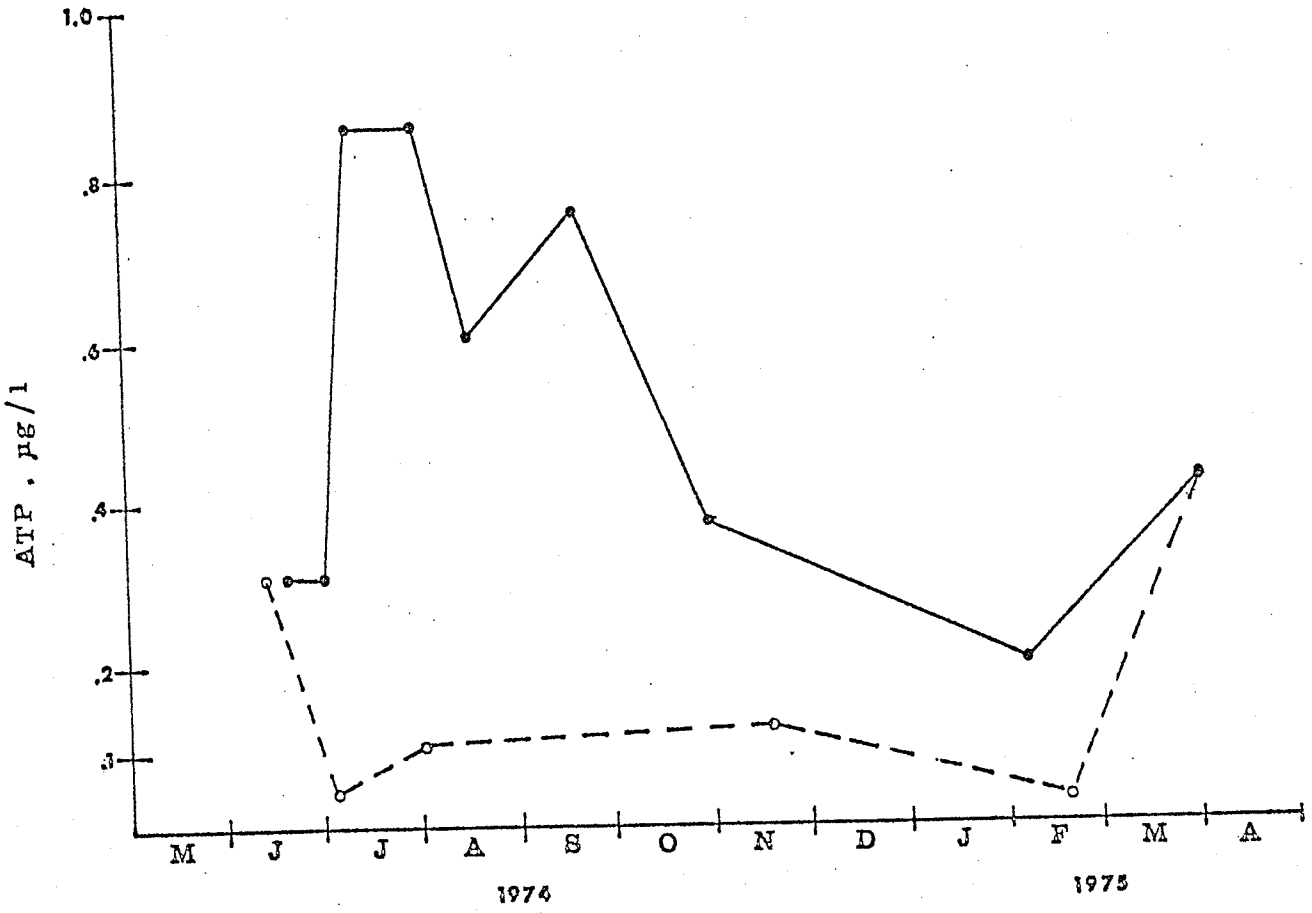
Although there was insufficient light for photosynthesis under the ice, the mean winter chlorophyll-a concentration in Lake 227 was one-third the mean summer concentration. The relatively high winter chlorophyll-a concentrations, as well as the high ATP and PP concentrations suggest that many algae and bacteria survive through the winter in the epilimnion. The phosphorus cycle, therefore, is controlled by organisms throughout the year. In Lake 302 S., chlorophyll-a concentration was

---

1. Data from both lakes were included in one analysis because not enough information was available to justify doing a separate analysis for each lake.

2. The replicability of the determination of ATP concentration often was poor (Appendix VI). (The mean standard deviation was $\pm$ 0.10 µg/l. The median standard deviation was better, $\pm$ 0.05 µg/l). The results, however, were good enough to demonstrate basic trends.

FIGURE 20. Seasonal changes in the ATP concentrations in the epilimnions of Lake 227 (●——●) and Lake 302 S (○— —○). May, 1974 to April, 1975.


1.0
.8
.6
.4
.2
.1
ATP , µg/l
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

maximum during fall overturn and early winter (Figure 21). Like PP, chlorophyll-a was high during this period because large concentrations of algae from the hypolimnion were introduced into the epilimnion. By the end of January, the chlorophyll-a concentration fell to the minimum values recorded. The regression of PP on chlorophyll-a revealed a highly significant correlation between the two parameters in Lake 227 ($r = 0.836$, $df = 31$), but not in Lake 302 S ($r = 0.151$, $df = 25$).

Hypolimnion samples

PO_4-P Concentration

During the summer, the PO_4-P concentration in the hypolimnion was three to four times that in the epilimnion. Occasionally, order of magnitude differences in concentration developed between the two regions. These concentrations, however, were still very low (from 7 to 180 ng/l). During the winter, PO_4-P concentration was lower in the hypolimnion than in the epilimnion.

As mentioned in the methods section, the validity of the data from the first two hypolimnion bioassays is questionable. If these values are accepted, the observed seasonal trend in PO_4-P concentration was a gradual increase over the summer and a decrease during fall overturn (Figure 22).[1] The PO_4-P concentration in the hypolimnion of Lake 302 S increased during winter stagnation. By late winter, the PO_4-P concentration in this lake exceeded that measured during the summer. In Lake 227, PO_4-P concentration continued to

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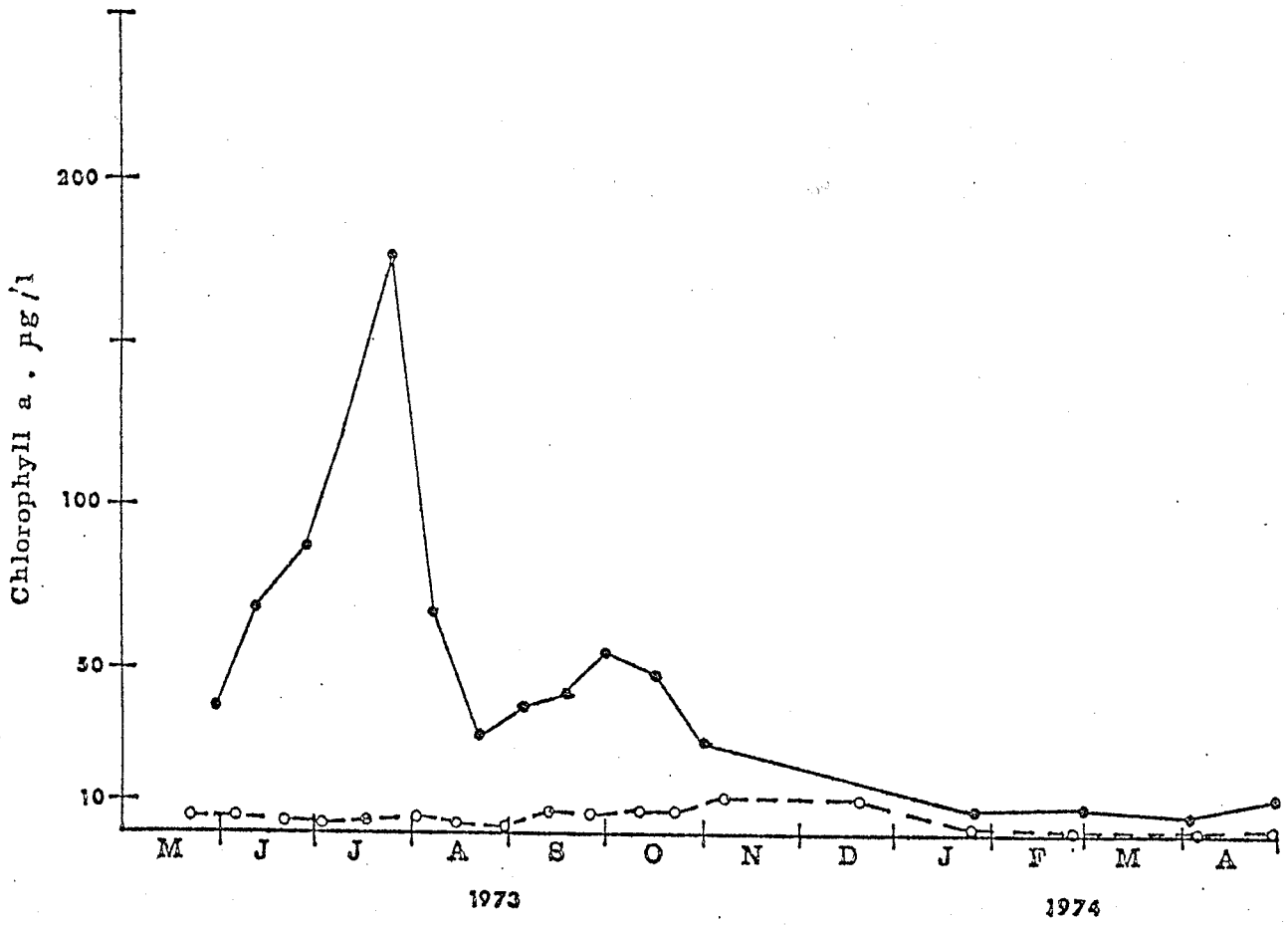
1. Because bioassays were not conducted during August or early September, the possibility that PO_4-P concentration began to decline before fall overturn cannot be dismissed.

FIGURE 21. Seasonal changes in the concentrations of chlorophyll-a in the epilimnions of Lake 227 (●—●) and Lake 302 S (○— —○).

21 A May, 1973 to April, 1974.


Chlorophyll a . µg/l
200
100
50
10
M
J
J
A
S
O
N
D
J
F
M
A
1973
1974

21 B May, 1974 to April, 1975.

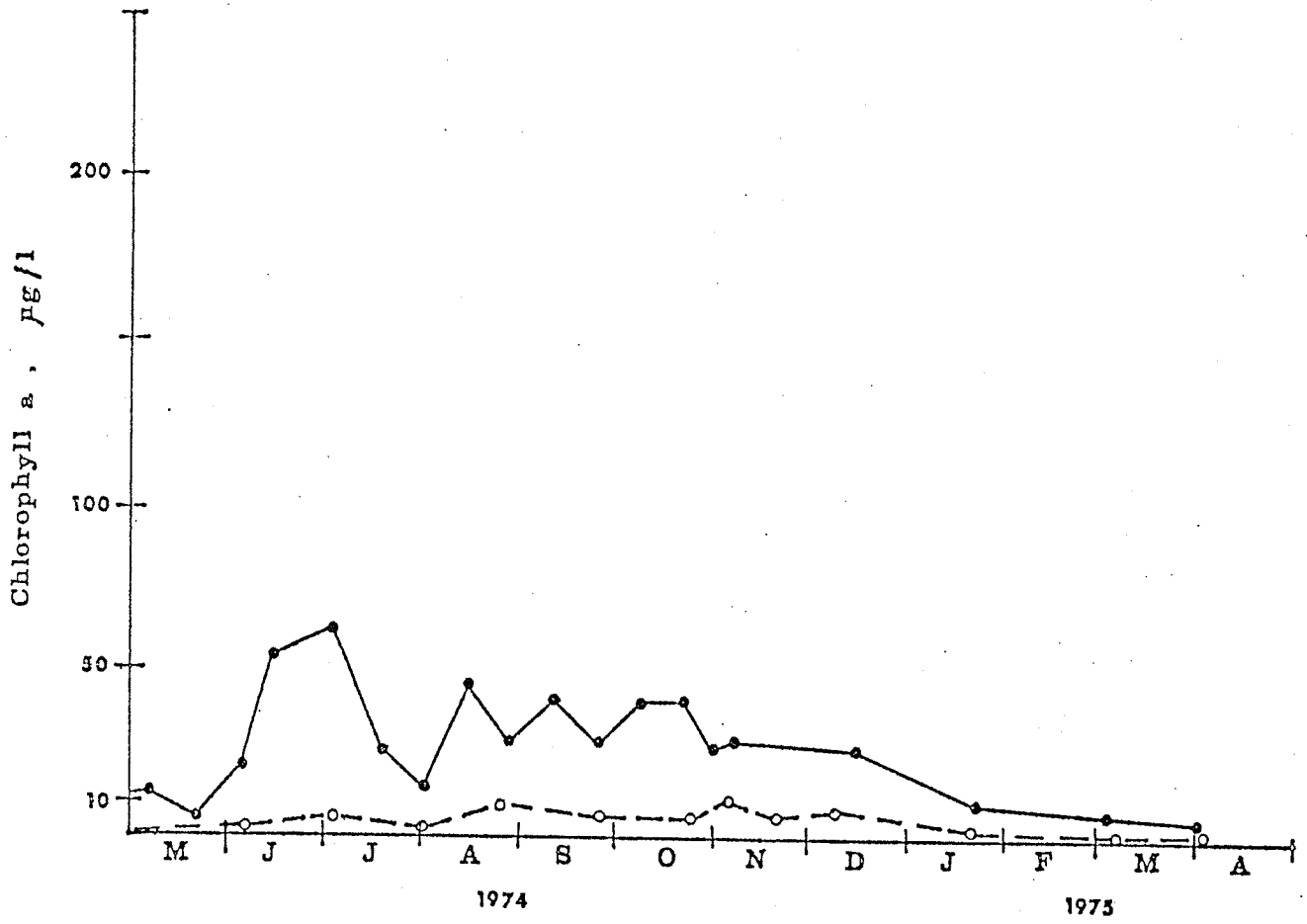
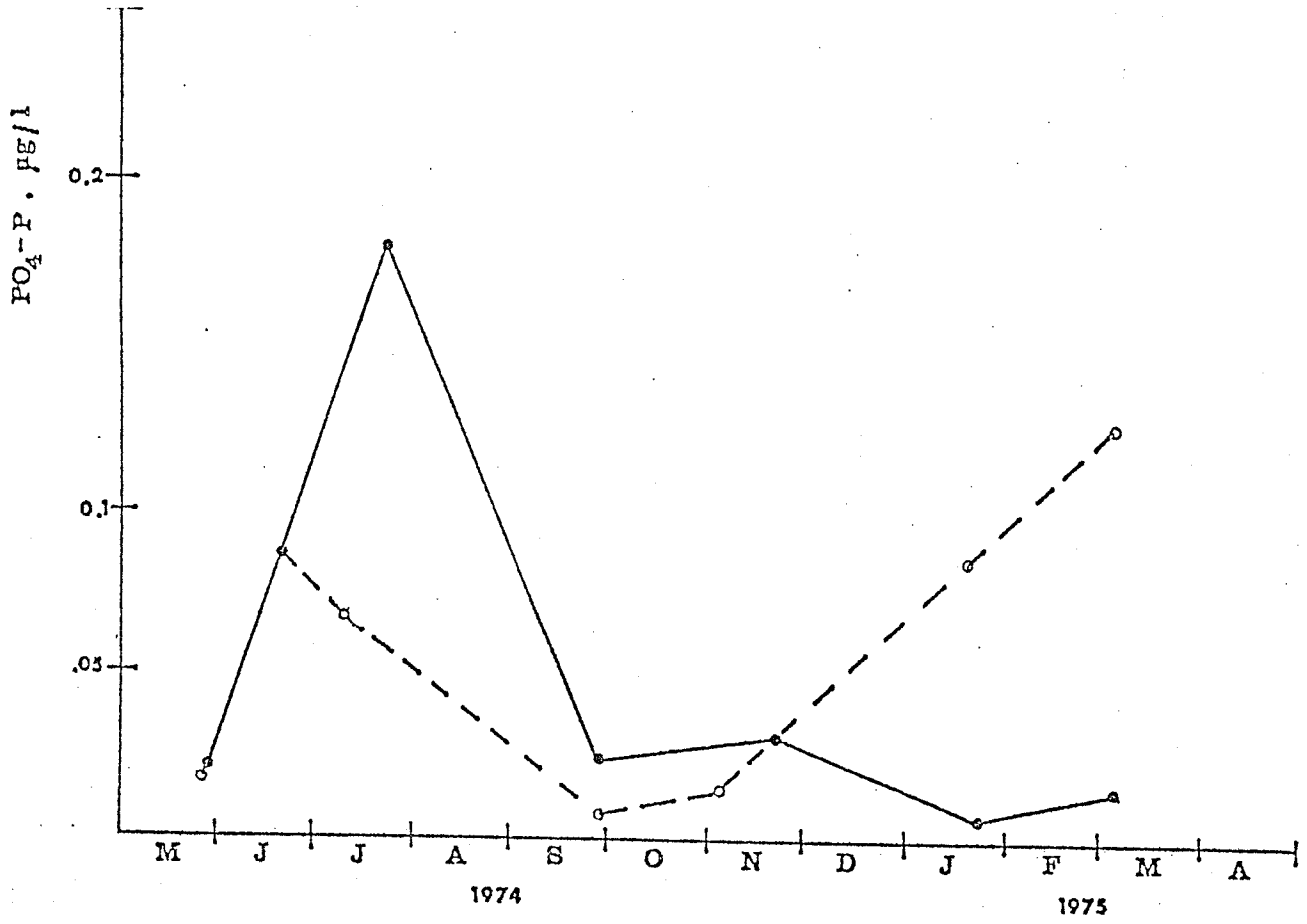

Chlorophyll a . µg/l
200
100
50
10
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

FIGURE 22. Seasonal changes in the orthophosphate-phosphorus concentrations in the hypolimnions of Lake 227 (●—●) and Lake 302 S (○— —○).
May, 1974 to March, 1975.

The January samples were taken from 9.5 m rather than 9.0 m.


PO_4-P . µg/l
0.2
0.1
.05
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

decline after fall overturn. This was expected because the phosphorus loading was terminated.

According to classical theory, the PO_4-P concentration in the hypolimnion of Lake 227 should be many times that in Lake 302 S because the hypolimnion of Lake 227 is anoxic for 10 months of the year, while the hypolimnion of Lake 302 S is anoxic only during two brief periods (1 to 3 months) in late winter and in late summer (Figure 23). The mean summer PO_4-P concentration in the hypolimnion of Lake 227, however, was only twice that in the hypolimnion of Lake 302 S and the mean winter PO_4-P concentration was less than that in Lake 302 S (Table 9).

The Rate Constant for PO_4-P Uptake

As in the epilimnion, changes in the rate constant for PO_4-P uptake by seston in the hypolimnion were nearly opposite to changes in PO_4-P concentration (Figure 24). The regression of ln K on PO_4-P concentration was significant (r_{227} = 0.803, df = 5; $r_{302\ S}$ = 0.870, df = 5), as was the regression of K on temperature (r_{227} = 0.763, df = 5; $r_{302\ S}$ = 0.880, df = 5).[2] K could not be significantly correlated with PP concentration or with ATP concentration.

PO_4-P Flux to Seston

PO_4-P flux to seston was of the same order of magnitude in the hypolimnion as it was in the epilimnion and was similar in the hypolimnions of the two lakes (Table 9). As in the epilimnion, the largest fluxes which were measured occurred in the summer in Lake 302 S and in the fall in Lake 227 (Figure 26).

---

2. Seasonal changes in temperature are shown in Figure 25.

FIGURE 23. Seasonal changes in the dissolved oxygen concentrations in the hypolimnions of Lake 227 (●——●) and Lake 302 S (○– –○). May, 1974 to April, 1975.

The January samples were taken from 9.5 m, rather than from 9.0 m.

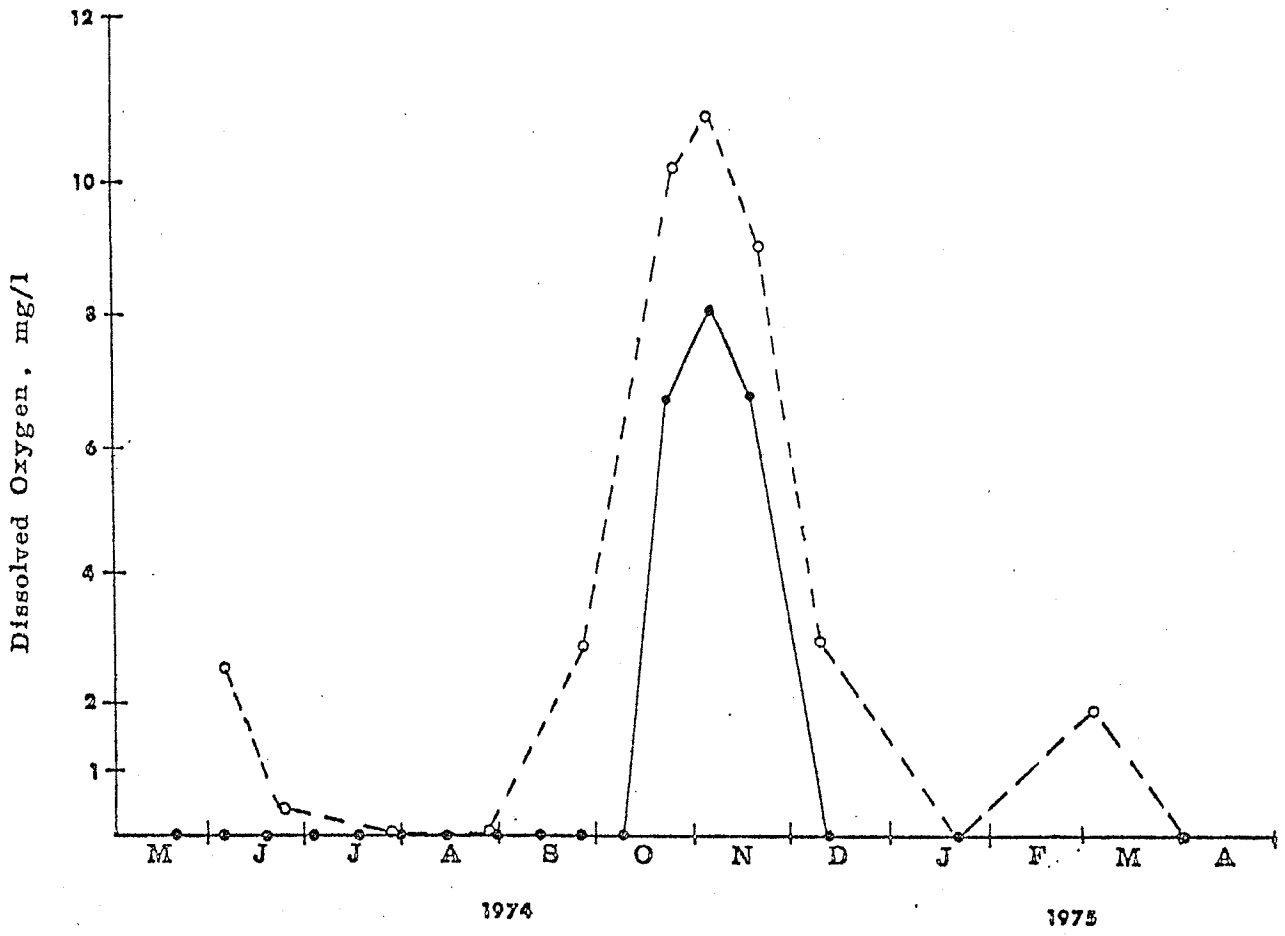

Dissolved Oxygen, mg/l
12
10
8
6
4
2
1
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

FIGURE 24. Seasonal changes in the rate constants for orthophosphate-phosphorus uptake by seston in the hypolimnions of Lake 227 (●——●) and 302 S (○— —○). May, 1974 to April, 1975.

The January samples were taken from 9.5 m rather than 9.0 m.

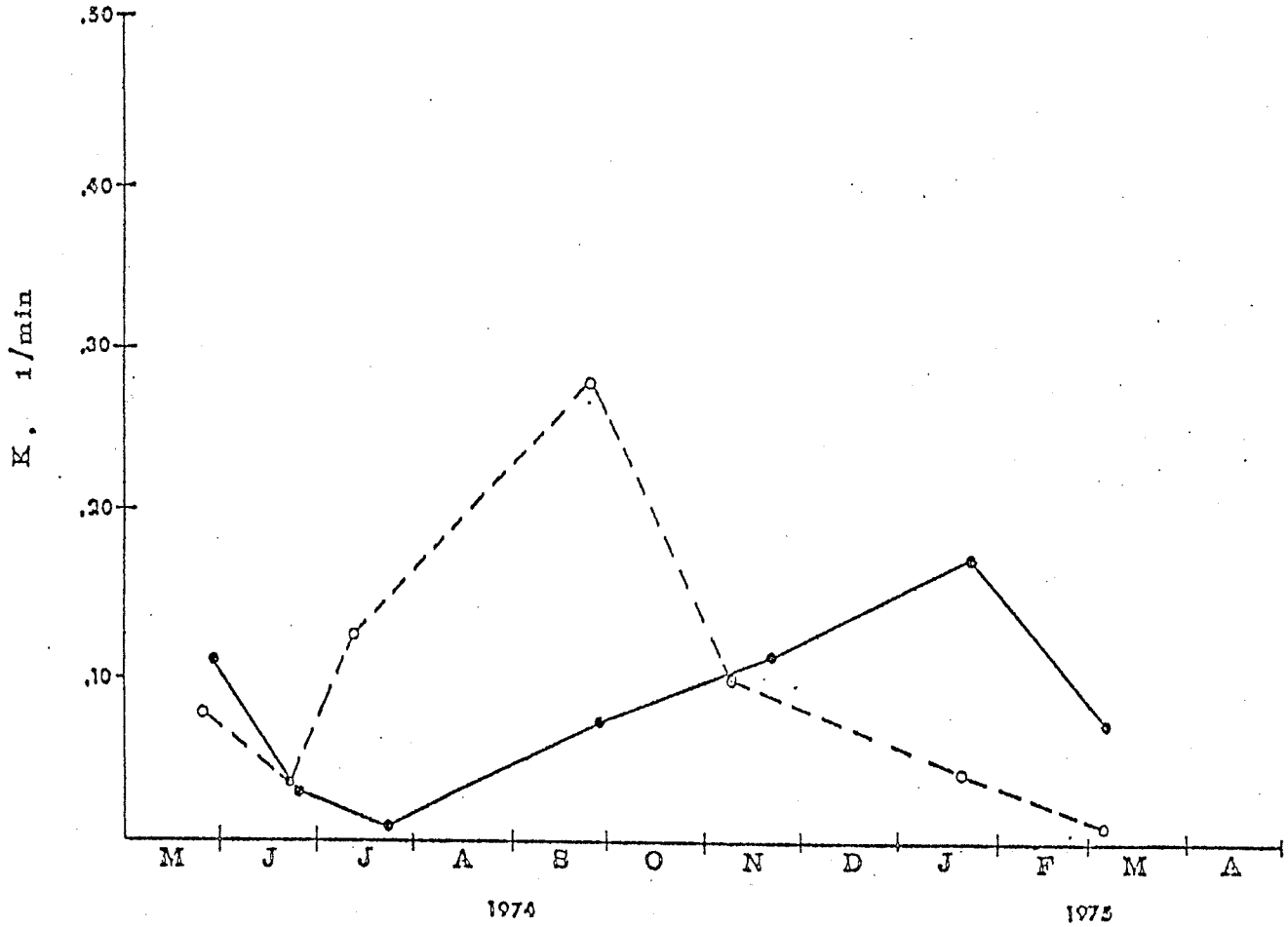

.50
.40
.30
.20
.10
K. 1/min
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

FIGURE 25. The seasonal changes in the temperatures of the hypolimnions of Lake 227 (●——●) and Lake 302 S (○– –○).
May, 1974 to April, 1975.

The January samples were taken from 9.5 m, rather than 9,0 m.

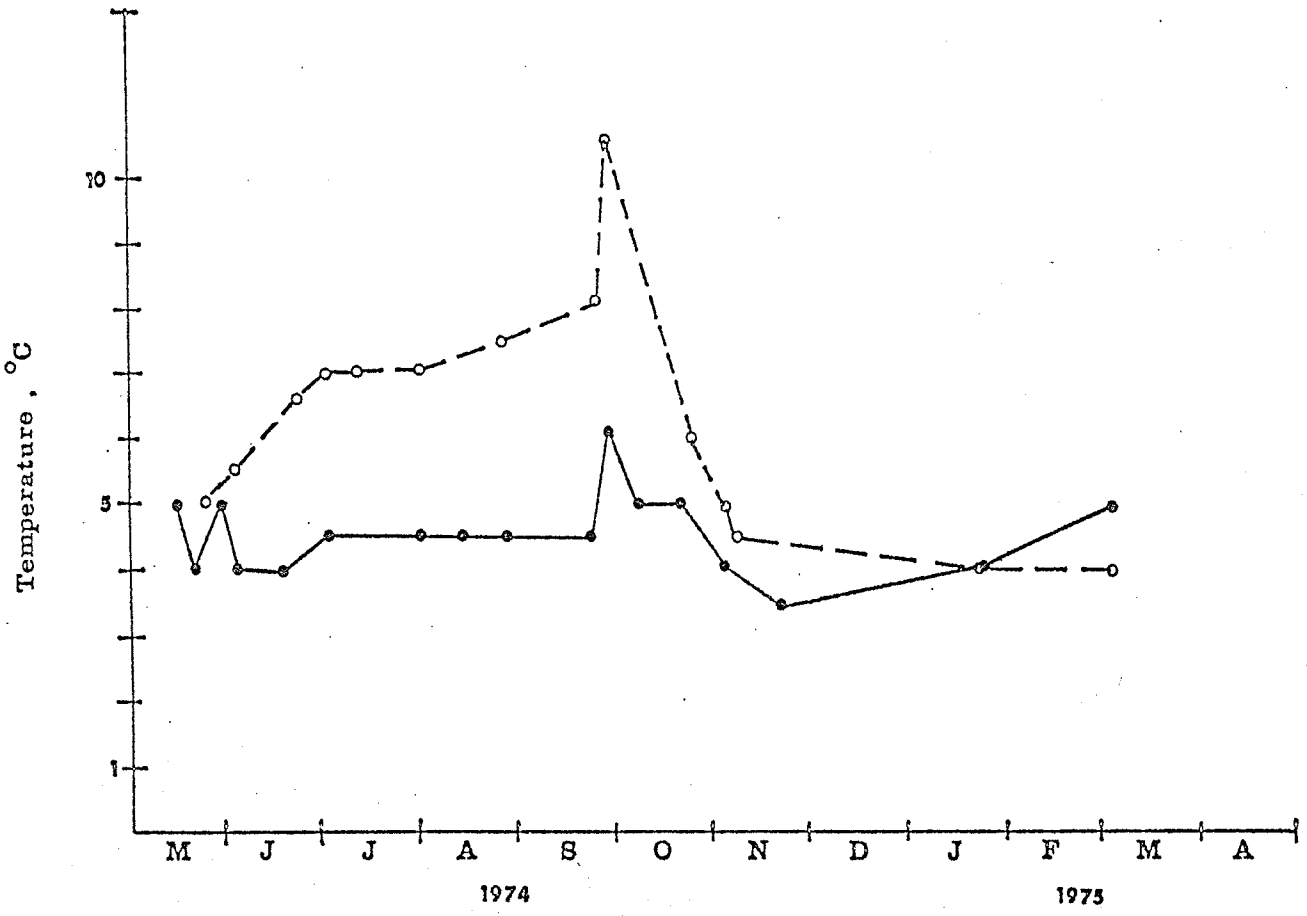
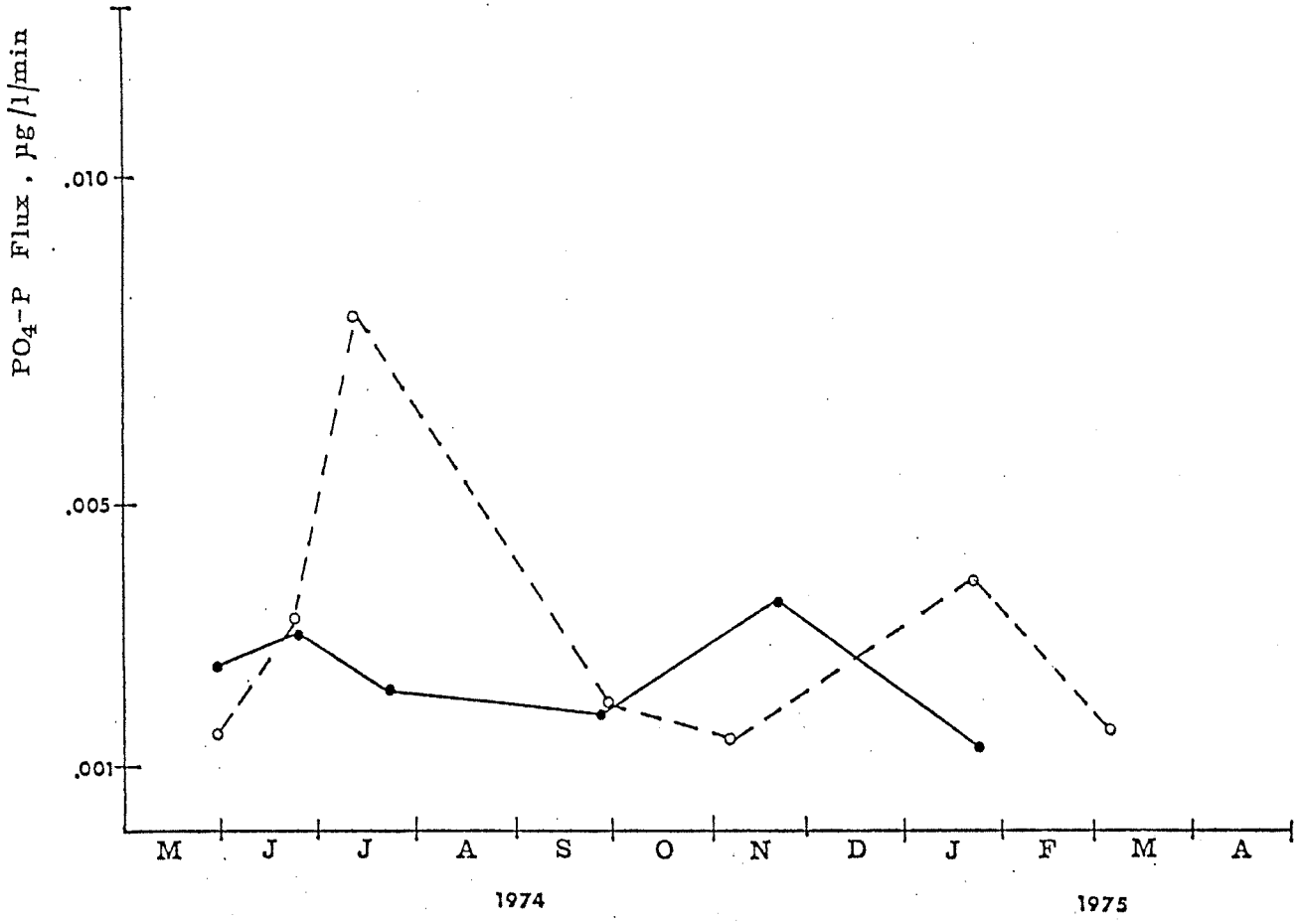

Temperature, °C
10
5
1
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

FIGURE 26. Seasonal changes in orthophosphate-phosphorus flux to seston in the hypolimnions of Lake 227 (●——●) and Lake 302 S (○— —○).
May, 1974 to March, 1975.

The January samples were taken from 9.5 m rather than 9.0 m.


PO_4-P Flux, µg/l/min
.010
.005
.001
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

Particulate Phosphorus

Most of the phosphorus in the hypolimnion was in particulate form. Because the rate of sedimentation of phosphorus from the epilimnion is high, much of the PP in the hypolimnion is detrital. Changes in the PP concentration of the hypolimnion, therefore, integrate changes occurring throughout the water column. As a result of fertilization, the summer mean PP concentration was three times higher in Lake 227 than in Lake 302 S (Table 9). This ratio was higher in the epilimnion (7:1), suggesting that the impact of increased phosphorus loading on biomass is concentrated largely within the epilimnion.

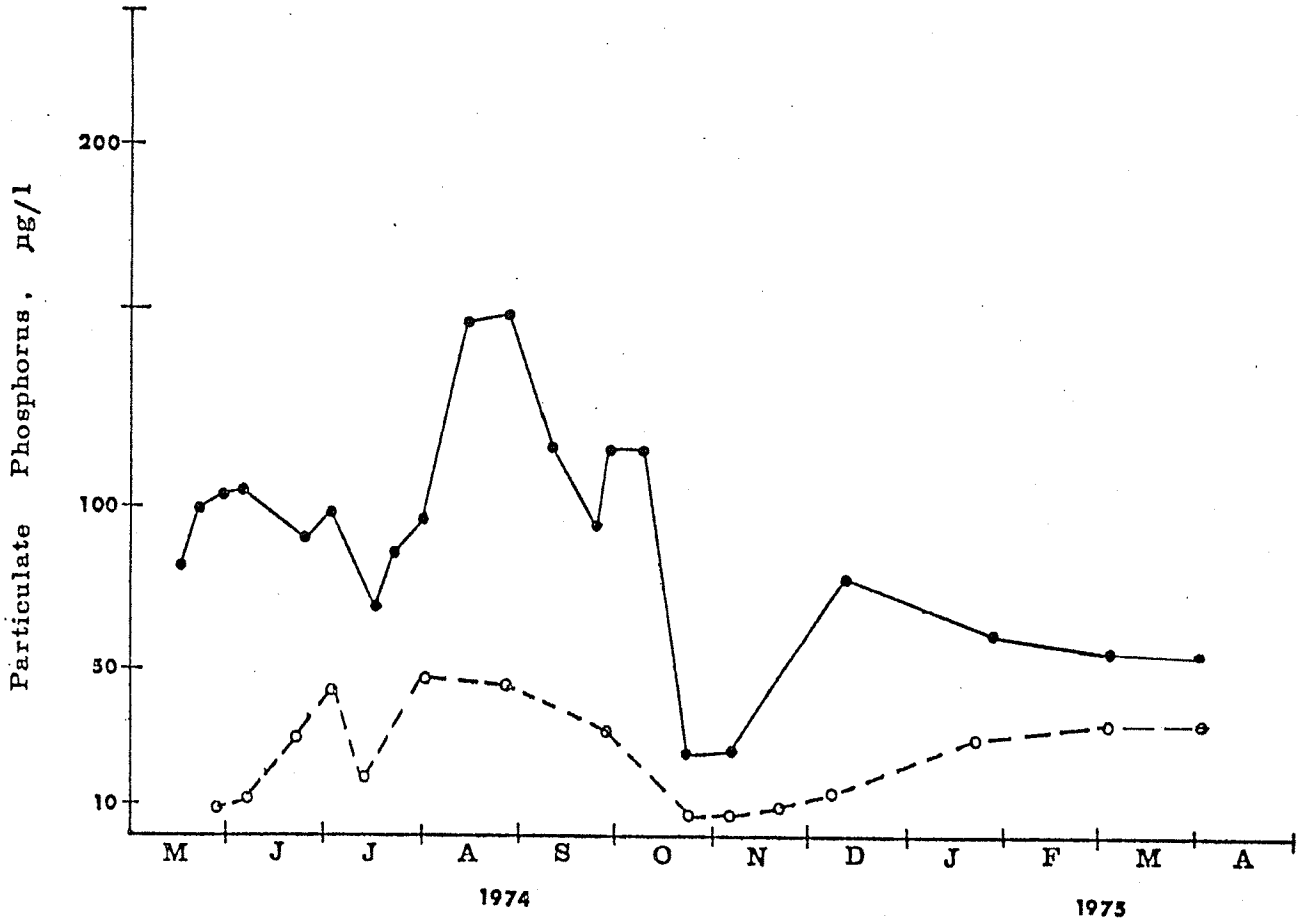
As in the epilimnion, seasonal variations in the size of the PP pool were small (the difference between the winter and summer means was less than twofold) (Figure 27). In both lakes, the peak concentrations occurred in late summer and the minimum concentrations during fall overturn. Since the concentration of PP in the epilimnion of Lake 302 S was lowest during the summer, the high hypolimnion PP concentrations attained during this period confirm that the bulk of the biomass in Lake 302 S is in the hypolimnion.

Total Dissolved Phosphorus

The total dissolved phosphorus concentration in the hypolimnion was only slightly greater than the concentration in the epilimnion (Table 9). This was expected because dissolved substances and colloids do not sediment and, therefore, are not physically concentrated in the hypolimnion. The phosphorus from these compounds must be converted to PP (either through uptake by organisms or by colloid aggregation) before it is sedimented. Most of the TDP in the hypolimnion is produced by

FIGURE 27. Seasonal changes in the particulate phosphorus concentrations in the hypolimnions of Lake 227 (●—●) and Lake 302 S (○— —○).
May, 1974 to April, 1975.

The January samples were taken from 9.5 m, rather than 9.0 m.


Particulate Phosphorus, µg/l
200
100
50
10
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

organisms living in that region. The influence of phosphorus loading on the TDP concentration in the hypolimnion was similar to that in the epilimnion. The mean summer TDP concentration in Lake 227 was three times the mean summer concentration in Lake 302 S. There was a one-and-one-half fold difference in winter means. Seasonal variations in TDP concentration were even less pronounced in the hypolimnion than in the epilimnion (Figure 28).

ATP and Chlorophyll-a

Except for a temporary decrease during fall overturn, the ATP concentration in the hypolimnion varied little with season (Figure 29). Winter concentrations tended to be slightly higher than summer concentrations (probably because many pelagic organisms overwinter in the hypolimnion). As in the epilimnion, the regression of PP on ATP was significant ($r = 0.635$, $df = 9$). This correlation seems odd at first because most of the PP in this region is detrital. Bacterial growth, however, may be proportional to the concentration of particulate matter in the water. Hypolimnion ATP concentrations normally were one to two times the epilimnion ATP concentrations (Table 9). The ATP concentration in Lake 227 generally was about three times the concentration in Lake 302 S.

Because there is very little light and, therefore, negligible photosynthesis at 9 m in Lakes 227 and 302 S (E.J. Fee, unpublished data), almost all of the chlorophyll-a at this depth is derived either from the epilimnion or from that portion of the hypolimnion where enough light is available for photosynthesis to occur. The influence of increased phosphorus loading on the algal biomass of the entire

FIGURE 28. Seasonal changes in the total dissolved phosphorus concentrations in the hypolimnions of Lake 227 (●——●) and Lake 302 S (○— —○).
May, 1974 to April, 1975.

The January samples were taken from 9.5 m, rather than 9.0 m.

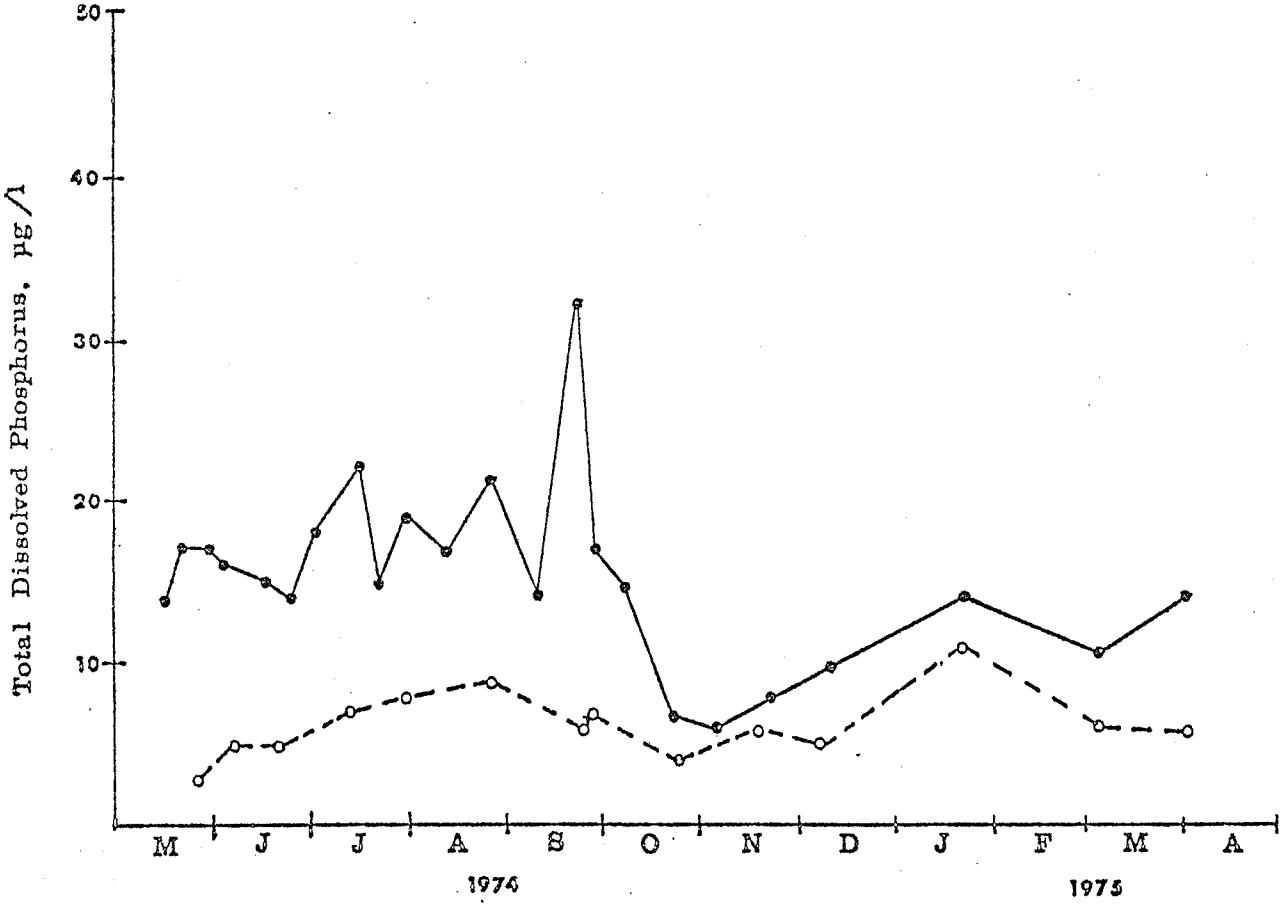
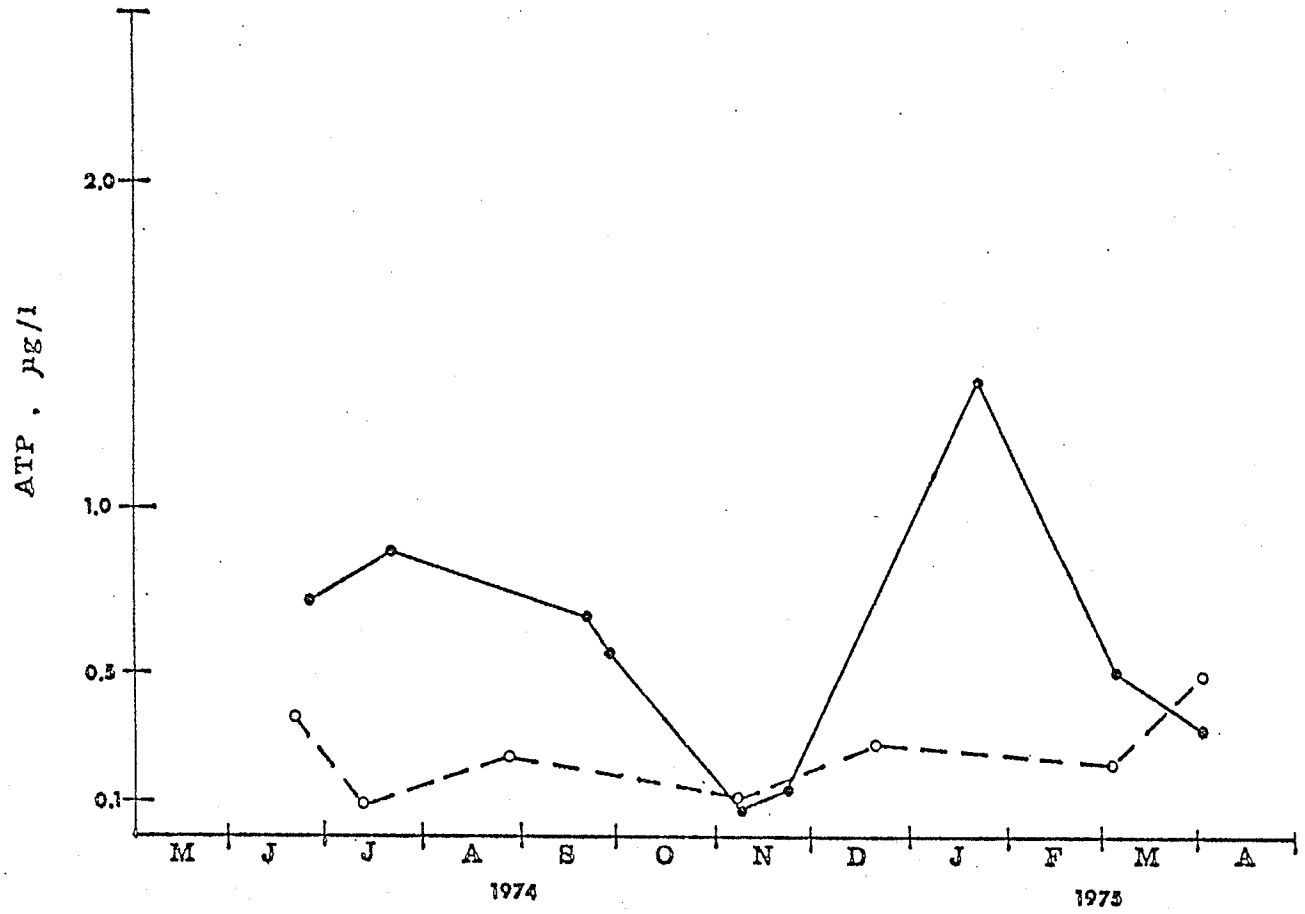

Total Dissolved Phosphorus, µg/l
50
40
30
20
10
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

FIGURE 29. Seasonal changes in the ATP concentrations in the hypolimnions of Lake 227 (●——●) and Lake 302 S (○– –○). May, 1974 to April, 1975.

The January samples were taken from 9.5 m, rather than 9.0 m.


ATP . µg/l
2.0
1.0
0.5
0.1
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

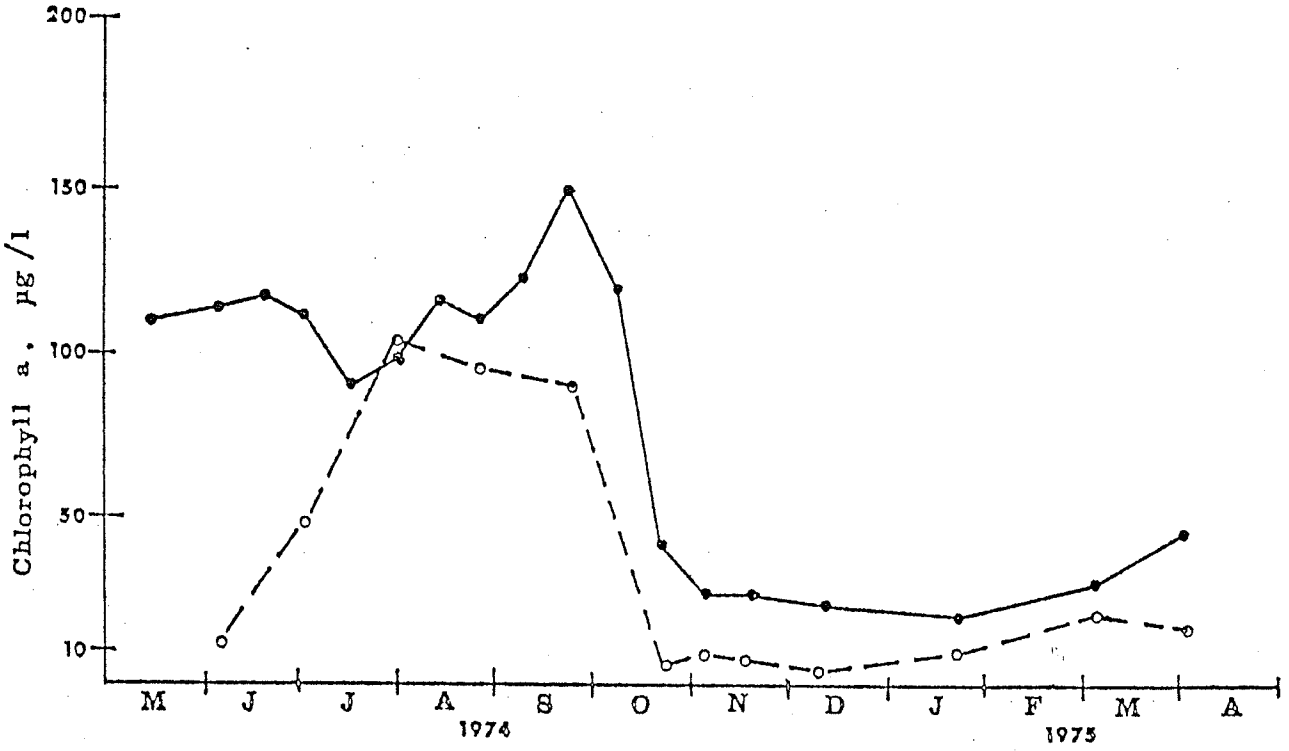
euphotic zone (as reflected by the chlorophyll-a concentration in the lower hypolimnion) was considerably less than that on the epilimnion alone. The mean chlorophyll-a concentration in the hypolimnion of Lake 227 was only twice the mean concentration in Lake 302 S (whereas, the ratio in the epilimnion was 12).

Seasonal variations in chlorophyll-a were of the same magnitude as those in the epilimnion (Figure 30). Throughout the year, the hypolimnion chlorophyll-a concentration in Lake 227 was about twice the epilimnion chlorophyll-a concentration. In Lake 302 S, the epilimnion chlorophyll-a concentration was twice the hypolimnion concentration during the winter. The mean chlorophyll-a concentration in hypolimnion of Lake 302 S during the summer, however, was 13 times the mean epilimnion concentration. This gap reflects the importance of hypolimnion (6 m) algal blooms in this lake.

In summary, very little of the phosphorus in either the epilimnions or hypolimnions of the studied lakes was PO_4-P. Nearly all phosphorus was present as PP or as colloidal P. When the PO_4-P loading was raised (as it was in Lake 227 each week), the size of the PP and colloidal P pools increased (the PP pool expanded more than the colloidal P pool did, however). The PO_4-P concentration was increased temporarily, but within two days of the PO_4-P addition, the PO_4-P pool was the same size in the fertilized and unfertilized lakes. A comparison of the concentrations of PP and chlorophyll-a in the hypolimnions of the two lakes with those in the epilimnions suggested that the impact of PO_4-P loading is greater within the epilimnion than in the lake as a whole. This finding was not surprising since the PO_4-P was added to the epilimnion rather than to the hypolimnion and was largely limited to the epilimnion by the thermocline. Most of the added phosphorus which reached the

FIGURE 30. Seasonal changes in the chlorophyll-a concentrations in the hypolimnions of Lake 227 (●—●) and Lake 302 S (o— —o). May, 1974 to April, 1975.

The January samples were taken from 9.5 m, rather than 9.0 m.


200
150
100
50
10
Chlorophyll a. µg /l
M
J
J
A
S
O
N
D
J
F
M
A
1974
1975

hypolimnion was in particles and, therefore, not as readily available to microorganisms as PO_4-P would have been. The distribution of algae in Lake 227 also was altered by a secondary effect of fertilization, the turbidity of the epilimnion. With large standing crops of algae in the epilimnion, not enough light could penetrate into the hypolimnion to permit primary production in this region.

Discussion

Errors in the Classical Model of PO_4-P Cycling in Lakes

Several errors in the classical model of PO_4-P cycling were uncovered during this study. First, as Lean (1973) postulated, the epilimnion was found to be a region of PO_4-P regeneration as well as of PO_4-P uptake. In the epilimnions of the studied lakes the gross PO_4-P flux to seston (about 3 µg/l/day, on average[1]) normally exceeded phosphorus loading (on average, 0.2 µg/l/day in Lake 302 S and 1.7 µg/l/day in Lake 227), indicating that a cycling of PO_4-P within the epilimnion occurs. At steady state, the phosphorus entering Lake 302 S is recycled 15 times before it is sedimented. In Lake 227, the entering phosphorus is recycled twice.

Second, substantial accumulations of PO_4-P were not found in the hypolimnion of the studied lakes under either oxic or anoxic conditions. (The greatest PO_4-P concentration recorded was 0.18 µg/l.) Measurement of the flux of PO_4-P into seston indicated that PO_4-P concentration is low in the hypolimnion because decomposers rapidly and actively take up PO_4-P. (PO_4-P flux was of the same order of magnitude in the hypolimnion and the epilimnion.) Apparently, decomposers do not fulfill their phosphorus requirements with phosphorus from detritus as the classical model assumes that they do.

In contrast with classical theory, the results of this study indicated that iron has little control over the hypolimnion PO_4-P cycle. Organisms keep the concentration of PO_4-P in lake water so low (although the flux of phosphorus through the orthophosphate pool is relatively large) that very little phosphorus is susceptible to complexing with

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1. This value does not include the flux of PO_4-P to seston immediately following fertilization in Lake 227, this flux was much greater, at least 8 µg/l/day.

iron (or with calcium or magnesium) at any one time. The introduction of oxygen into the hypolimnion at overturn, therefore, does not result in a massive precipitation of ferric phosphate, as the classical model of the PO_4-P cycle predicts. Most iron probably becomes associated with organic compounds in the water or is precipitated as $Fe(OH)_3$. Because little ferric phosphate is formed in the water column, iron-phosphate complexes are not present in the sediments (Coey, Schindler, and Weber 1974). Even if they were, reducing conditions at the sediment surface would not lead to high PO_4-P concentrations in the hypolimnion. Any PO_4-P leaving the sediments would be taken up quickly by organisms near the sediment surface.

Also in contrast with classical theory, the amount of PO_4-P introduced into the epilimnion from the hypolimnion during fall and spring overturn was found to be insufficient to support the algal blooms which for many lakes are characteristic of these periods. The highest hypolimnion PO_4-P concentration which was recorded during this study was two orders of magnitude less than the PP concentration in Lake 227 during the average algal bloom (these blooms occur in the summer while the lake is being fertilized) and an order of magnitude less than the highest PP concentration recorded for Lake 302 S. Some algal blooms (such as those in Lake 302 S) are the remnants of hypolimnion populations which are swept into the epilimnion during overturn. Phosphorus which reaches the epilimnion as PP may participate in the epilimnion phosphorus cycle. In doing so, it would stimulate algal growth. Spring and fall blooms also may be supported by the phosphorus in precipitation (precipitation often is high during spring and fall) or snowmelt.

The diffusion of orthophosphate across the thermocline was found to be an even less significant contribution to the epilimnion phosphorus budget than the introduction of orthophosphate from the hypolimnion during overturn. In addition to small PO_4-P concentration gradients, the eddy diffusion coefficients for the thermoclines of the studied lakes were very low (on the order of 10^{-4} cm^2/sec (Hesslein and Quay, unpublished data)). The PO_4-P flux across the thermocline normally was less than 0.05 $\mu g/m^2/day$.

The Relationship Between Phosphorus Loading and Biomass

Because phosphorus is a limiting nutrient (and because a large portion of the phosphorus in lakes is in organisms), changes in biomass were expected to be proportional to changes in phosphorus loading. Surprisingly, this was not verified in the studied lakes. Although the phosphorus loading into Lake 227 is 16 times higher than in Lake 302 S, none of the biomass parameters was 16 times higher in Lake 227 than in Lake 302 S. In fact, the factor by which the parameters differed in the two lakes varied with the parameter which was measured. Two alternative explanations of this paradox can be offered here.

The first and most reasonable hypothesis is that the apparent differences in the reactions of the biomass parameters to phosphorus loading are, in fact, artifacts of the pulsed nature of the phosphorus additions. In the first few hours following PO_4-P addition, the formation of PP and ATP may be equally stimulated. Chlorophyll-a is formed more slowly than ATP and PP and, therefore, it would attain its maximum concentration later than ATP and PP. As the added PO_4-P is depleted, less phosphorus is available for the formation of new cells. After a few days, the older, phosphorus-rich cells may die. Because ATP is degraded to ADP within minutes after death (Holm-Hansen and Booth, 1966), ATP concentration responds quickly to changes in biomass. Although much of the phosphorus in dead cells is released by autolysis within a few hours of death, some may remain as PP for several days (Golterman, 1960). The response of PP to biomass changes, therefore is slower than that of ATP. Chlorophyll-a not only is degraded slowly, but some of the degradation products cannot be distinguished from chlorophyll-a by standard chlorophyll methods (Moss, 1967). Thus, what seems to be a

discrepancy in the reactions of the three biomass parameters to phosphorus additions may be simply a measure of the residence time of the compounds.

If the effects of pulsed phosphorus loading which are described above do not exist, the data indicate that there is a different relationship between each biomass parameter and phosphorus loading. The proportionally greater increase in chlorophyll-a concentration than in PP concentration or in ATP concentration suggests that algal growth is stimulated more by the addition of orthophosphate than is bacterial growth. Thus, algae gain more control over the phosphorus cycle as the phosphorus loading is increased. In support of this hypothesis, Fuhs et al. (1972) have found that the two species of algae which they grew in phosphorus-limited chemostats had greater affinities for phosphorus (lower Km values) than three species of bacteria. Rhee (1972), however, found that a bacterium outcompetes an alga for phosphorus in mixed cultures. The lesser impact of orthophosphate addition on ATP concentration than on PP concentration can be explained under these circumstances only if the ratio of ATP-P/cellular P is greater in bacteria than in algae. Studies by Holm-Hansen and Booth (1966) suggest that this is not so.

Changes Necessary in Lean's Model of Phosphorus Cycling in the Epilimnion

In addition to changes in the classical model of PO_4-P cycling minor alterations in Lean's (Lean, 1973) model were found to be necessary. The main shortcoming of Lean's model is its failure to incorporate specific activities. Rather than measure the phosphorus concentrations

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1. Holm-Hansen and Booth measured the ratio of ATP to cellular dry weight in several species of algae and bacteria. The values for algae and bacteria overlapped.

in the compartments which he defined, Lean expressed the sizes of the phosphorus compartments as fractions of "biological phosphorus" ("biological phosphorus" is simply the fraction of phosphorus which was labeled at the time of Sephadex fractionation). Lean did not observe what he believed to be significant changes in the asymptote of the plot of ln % ^{32}P in the filtrate versus time between 2 and 48 hours after $^{32}PO_4$ addition. He, therefore, concluded that a state of isotopic equilibrium had been reached. His estimates of the sizes of the phosphorus compartments are from a Sephadex fractionation performed 4.5 hours after $^{32}PO_4$ addition.

If Lean had measured specific activities, he would have noticed that only a small fraction of colloidal phosphorus is labeled in the first few hours after $^{32}PO_4$ addition. In the present study (Section I) the partition of ^{32}P between the filter retainable and filtrate fractions of in situ columns of lake water was followed for several days after PO_4 addition. These long-term incubations revealed that the relatively rapid rate with which colloid acquires ^{32}P after $^{32}PO_4$ is added to lake water (the labeling described by Lean) is followed by a very slow increase in radioactivity. If most colloidal phosphorus is autochthonous,[1] nearly all TDP should be labeled at isotopic equilibrium. When an asymptote equal to TDP/PP x 100% is assumed, however, the plot of ln % ^{32}P in filtrate versus time after $^{32}PO_4$ addition is distinctly diphasic (Figure 31).[2] Diphasic uptake kinetics occur when more than two compart-

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1. A large fraction of the colloidal phosphorus in lake water, however, may be allochthonous and, therefore, not susceptible to labeling with ^{32}P.

2. Even if an asymptote slightly higher than the percent of ^{32}P activity in the filtrate after one week is used, the curve is better described by diphasic than by monophasic kinetics.

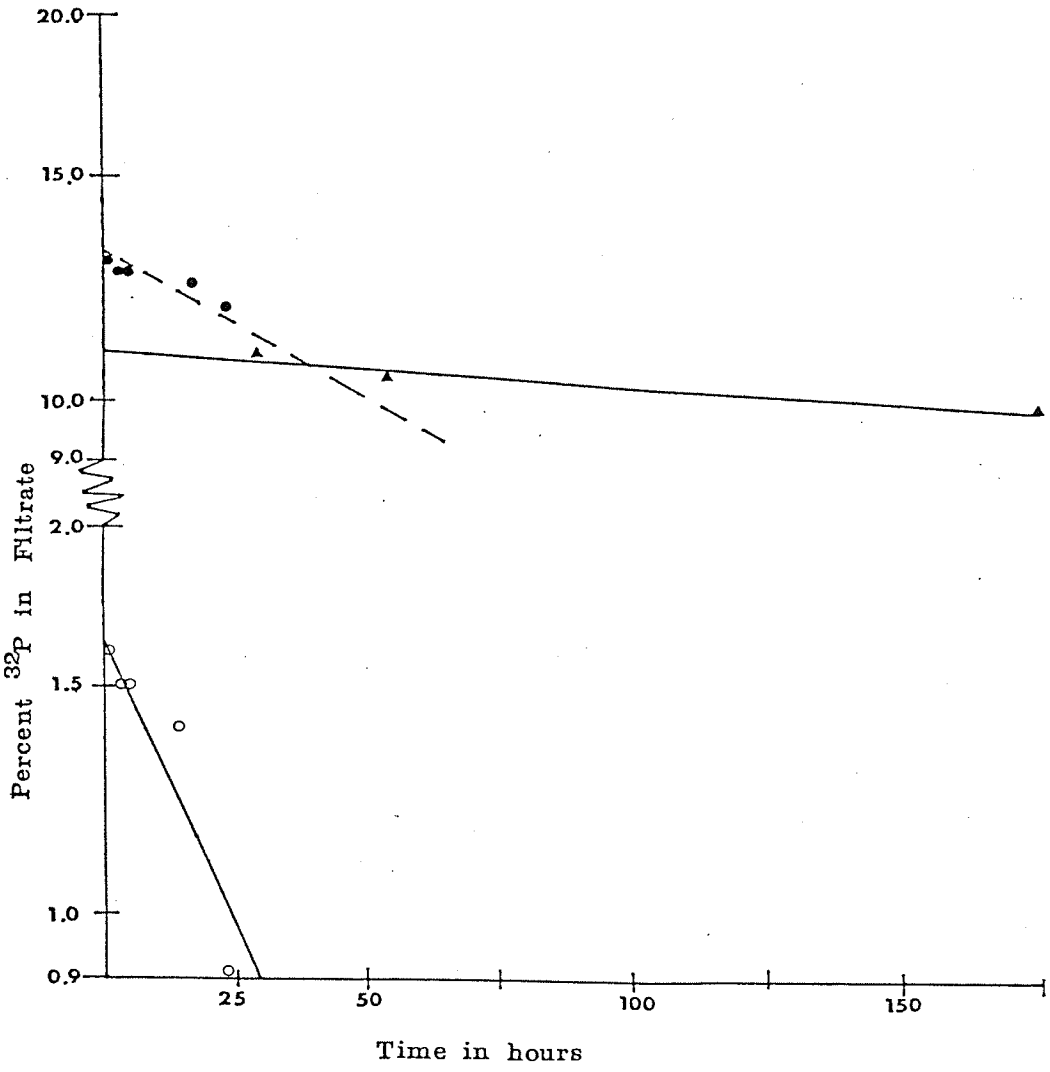
Figure 31. Method used to demonstrate the diphasic nature of colloidal phosphorus formation.[1]

A plot was made of Ln ($P_{c_{asymp}} - P_c$) vs time (where P_c is the percent ^{32}P in the filtrate),[2] using TDP/TP x 100% as the asymptote. If only one colloidal phosphorus compartment and a PP compartment existed the plot would have been a straight line.

The rate constant for the flux of phosphorus into the more slowly labeled colloidal phosphorus compartment is the slope of a line drawn through the more gently sloped portion of the curve (▲—▲). This line is extrapolated to the y-axis and values from the extrapolated portion are subtracted from values of the steeper portion of the original curve (●—●) to obtain a second line (o—o). The slope of the second line is equal to the rate constant of the flux of phosphorus into the more labile colloidal phosphorus compartment.

The data shown here[3] are for Lake 227, 9-16 July, 1973 (see Figure 2). Data from both a laboratory-incubated sample and an in situ epilimnion column are included. The rate constants for the two fluxes are 0.022/hr and 0.00065/hr.

1. From Riggs (1973).
2. Nearly all filtrate ^{32}P is colloidal ^{32}P 3 hours after $^{32}PO_4$ addition (Lean, 1973).
3. Not all points are included in the figure.


20.0
15.0
10.0
9.0
2.0
1.5
1.0
0.9
Percent ^{32}P in Filtrate
25
50
100
150
Time in hours

ments exist.

Recently, Minear (1975) identified a large portion of colloidal phosphorus (as much as 50% of the colloidal phosphorus in the samples which he analyzed) as DNA. DNA is more likely to be released from organisms during death and decay than through excretion. It probably belongs to the slowly-labeled colloidal phosphorus compartment.

Lean's model is not invalidated by the addition of a second colloidal phosphorus compartment. There is enough difference between the rate constants for formation of the two types of colloidal phosphorus so that Lean's measurements apply primarily to the faster compartment.

A New Model of Phosphorus Cycling in Canadian Shield Lakes

Figure 32 is a diagram of the movement of phosphorus through the water column of a Canadian Shield lake during the summer. It incorporates information obtained during the present study with Lean's findings. There are many uncertainties in the diagram, but the basic structure is clear. Phosphorus flows almost irreversibly from sources outside the lake to the sediments. Coupled with this flow is a rapid cycling of phosphorus between orthophosphate and seston and a slower movement of phosphorus through the colloidal phosphorus cycle. The pattern is similar in the epilimnion and hypolimnion. The hypolimnion phosphorus compartments, however, are somewhat larger than the epilimnion compartments (partly because phosphorus is concentrated in this region during sedimentation and partly because more of the introduced phosphorus is in less labile, detrital (PP) forms). Eutrophication expands the size of the phosphorus compartments in both the epilimnion and hypolimnion. If the phosphorus loading is discontinuous (as it is in most lakes), however, the phosphorus

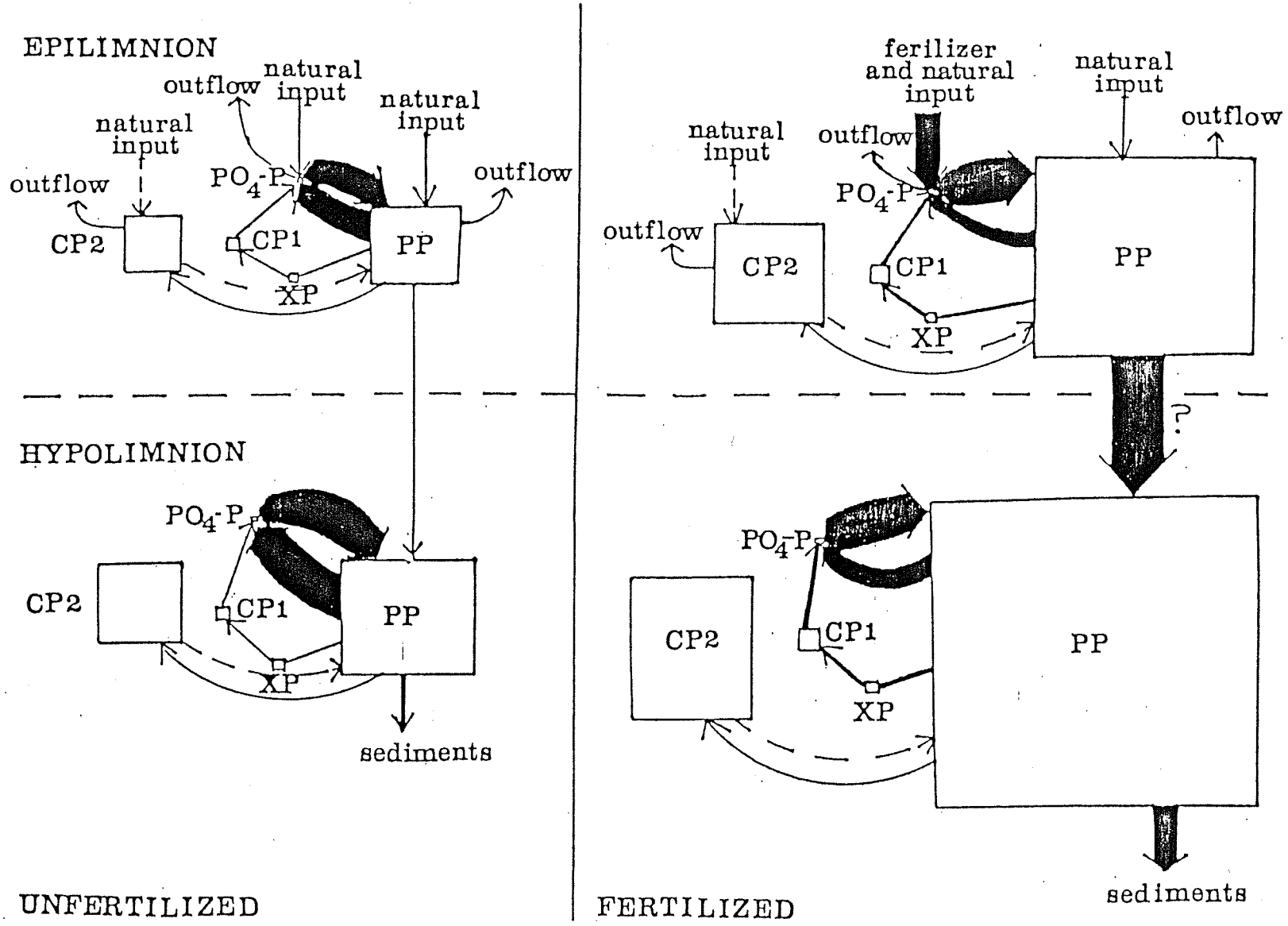
Figure 32. A model of the movement of phosphorus through the water columns of an oligotrophic lake and a fertilized lake in the Canadian Shield.

The area of each box is roughly proportional to the concentration of phosphorus in a compartment and the area of an arrow is proportional to the flux between two compartments. This diagram is for the summer season. The diagram for winter would be similar, except that the difference in pool sizes between the two lakes would be less.

Several assumptions are incorporated into this model. Most of the TDP was assumed to be PO_4-P. (This assumption may not be true for Lake 302 S). The rate constant for the movement of phosphorus from colloid to orthophosphate was not measured. Instead, Lean's (1973) estimate for Heart Lake was used to calculate the phosphorus flux. The fraction of colloidal phosphorus which is biologically labile was assumed to be that labeled two hours after $^{32}PO_4$ addition (on average about 2%).

Sedimentation rates were measured in <u>in situ</u> epilimnium columns (see Table 2, p. 19). The rate in Lake 227 is too high to be reasonable.

The flux of orthophosphate across the thermocline is not shown because it is insignificant.


EPILIMNION
outflow
natural input
natural input
natural input
outflow
outflow
PO_4-P
CP2
CP1
PP
XP
HYPOLIMNION
PO_4-P
CP2
CP1
PP
XP
sediments
UNFERTILIZED
ferilizer and natural input
natural input
natural input
outflow
outflow
PO_4-P
CP2
CP1
PP
XP
?
PO_4-P
CP2
CP1
PP
XP
sediments
FERTILIZED

pools do not expand (or expand only temporarily) in proportion to the change in phosphorus loading. Instead, the flow-through component of the model becomes more prominent (a larger fraction of the phosphorus molecules are sedimented rather than recycled). During the winter, when there is no phosphorus input, the PP and colloidal P pools shrink, while the PO_4-P pool may expand somewhat.[1] Because the PP concentration does not change significantly during the winter, the recycling of phosphorus must greatly exceed sedimentation during this period.

In the few lakes where a nutrient other than phosphorus limits biological productivity changes in the size of the orthophosphate pool may result from changes in orthophosphate loading. When phosphorus is limiting, however, fluctuations in orthophosphate loading produce variations in biomass rather than in orthophosphate concentration. Under these circumstances, the size of the orthophosphate pool is a function of the difference between the uptake and release of PO_4-P by organisms. The more efficient PO_4-P uptake is, the smaller the orthophosphate concentration is. Departures from steady state conditions (i.e. changes in the biological community or in physical factors influencing biological activity) are responsible for variations in orthophosphate concentration. In the course of this study, an inverse relationship between the dissolved oxygen concentration and the PO_4-P concentration in the "epilimnion" during the winter was noted. This suggests that organisms without

1. In Lake 302 S the PP and TDP concentrations in the epilimnion are higher during the winter than in the summer. This occurs, however, because organisms which are concentrated in the upper hypolimnion during the summer are dispersed throughout the water column in winter. The PP concentration in the hypolimnion is lowest during the winter.

sufficient oxygen for aerobic respiration are less efficient either at taking up or at retaining PO_4-P than organisms functioning under oxic conditions. This hypothesis is supported by Shapiro (1967). Shapiro found that microorganisms growing in the presence of oxygen sometimes release significant amounts of SRP[1] when they are subjected to anoxia. If anoxia is maintained long enough (as in the hypolimnion) populations of organisms proficient at using hydrogen ion acceptors other than oxygen develop. These organisms may retain PO_4-P more efficiently during anoxia than organisms which are normally aerobes. This may explain why the highest PO_4-P concentrations measured were in "epilimnion" samples taken during the winter rather than in "hypolimnion" samples.

The model of phosphorus cycling in lakes which is presented above is much simpler than the actual phosphorus cycle. The two colloidal phosphorus compartments which are described probably contain many compounds with different rates of formation and of utilization. The PP compartment definitely should be subdivided, but was not because the nature and the number of divisions necessary are uncertain. Furthermore, many of the methods needed to investigate the flow of phosphorus through this compartment have not been developed. We know that PO_4-P is not taken up by all seston, but primarily by algae and bacteria (nannoplankton are especially important in ELA lakes (Appendix IV). Whether the same organisms return PO_4-P to solution or the PO_4-P normally is passed to consumers before it is released into the water is unknown. Mass balance calculations sometimes suggest that microorganisms excrete almost as much

1. Shapiro claims that the phosphorus is PO_4-P because carbohydrates are not released simultaneously.

PO_4-P as they take up (Rigler, 1968; and Lean, 1973). A few workers have attempted to estimate the rate of PO_4-P excretion by measuring changes in SRP concentration after adding algae to a sterile medium (i.e. Kuenzler and Ketchum, 1962). This determination, however, includes excreted organic phosphorus. Lean and Nalewajko (unpublished) collected the excretory products of ^{32}P-labeled algae grown in cultures. They used Sephadex fractionation to separate the ^{32}P-labeled excretory products. Although after a few hours the medium contained more colloidal ^{32}P and $X^{32}P$ than $^{32}PO_4$, the experiment did not indicate that PO_4-P excretion is less than XP excretion. The algae were not separated from their excretory products. Thus, PO_4-P may have been taken up as rapidly as it was released.

Zooplankton may be responsible for the regeneration of most orthophosphate. Peters (1972) has found that 90% of the phosphorus released by zooplankters is PO_4-P (he identified the $^{32}PO_4$ through anion exchange, gel filtration, and uptake kinetics). Because zooplankton feed on microorganisms which are high in phosphorus, it is not surprising that they excrete orthophosphate. Peters measured zooplankton grazing rates in Lake 227. He found that 17.6% of the small particles in the trophogenic zone were removed by zooplankton every day. From this value and relationships between phosphorus ingestion and release which he obtained in the laboratory, Peters calculated the phosphorus excretion rate by zooplankton in Lake 227. It was great enough to supply algae with nearly all the phosphorus needed in primary production. Other experimenters working with zooplankton populations in other lakes (Barlow and Bishop, 1965 and Hargrave and Geen, 1968) have obtained similar results.

SUMMARY

Because conventional methods greatly overestimate the orthophosphate concentration of lake water, published seasonal studies of PO_4-P dynamics in lakes are suspect. The objectives of the current study were to test several promising methods for measuring PO_4-P and to use the best of these methods in a seasonal study.

The amount of PO_4-P normally present in lake water proved to be below the sensitivity of the chemical methods which were tried. The radiochemical methods were more sensitive. Many of these methods, however, required isotopic equilibrium. Colloidal phosphorus was found to acquire label so slowly that meeting this requirement was impractical, if not impossible.

Rigler's bioassay (Rigler, 1966) was chosen as the best available method for measuring PO_4-P. It was used during seasonal studies of the phosphorus cycles in an oligotrophic and a fertilized lake in the Experimental Lakes Area. Although this method is very sensitive and relatively free from interference, it provides only a maximum concentration estimate.

The following properties of the phosphorus cycle were uncovered during the seasonal studies:

1. The PO_4-P concentration in both the epilimnion and hypolimnion is very low (less than 0.05 µg/l in the studies lakes) during most of the year. Added PO_4-P was quickly converted to PP, so that the PO_4-P concentrations in the fertilized lake and the oligotrophic lake were similar.

2. The PO_4-P flux into seston is substantial at all depths (about 3 µg/l/day), indicating that microorganisms in the hypolimnion are as active at taking up PO_4-P as are the algae in the epilimnion. Because the flux of PO_4-P to seston exceeds phosphorus loading, a significant fraction of PP must be returned to solution as PO_4-P.
3. The rate constant for PO_4-P uptake varies inversely with PO_4-P concentration so that the flux of PO_4-P to seston does not change greatly with season. Thus, PO_4-P dynamics are controlled by organisms throughout the year.
4. Most of the phosphorus in lakes is present as PP or colloidal phosphorus. The concentrations of both of these fractions were much greater in the fertilized lake than in the oligotrophic lake, confirming that they are produced autochthonously. The existence of a second colloidal phosphorus compartment, more slowly labeled than the one described by Lean, was suggested by long-term radiochemical incubations.

These properties are combined in a model of the phosphorus cycle in lakes (page 105).

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APPENDIX I

EVALUATION OF METHODS WHICH COULD ALTER K OR PO_4-P CONCENTRATION

Adsorption by Containers

Some limnologists have reported that orthophosphate is adsorbed by polyethylene bottles (Heron, 1962, Jagitsch and Koczy, 1963). This experiment was undertaken to determine whether adsorption is significant during the short period of transport which was involved in this study.

Methods

$^{32}PO_4$ was added to a flask of lake water. Immediately after shaking, a 5 ml aliquot was withdrawn, and its ^{32}P activity determined. A polyethylene and a glass bottle were filled with the labeled water. After 1.5 hours (longer than the time of transport between the study lakes and the laboratory), a 5 ml aliquot was withdrawn from each container and its ^{32}P activity determined.

Results

After one-and-one-half hours of incubation with $^{32}PO_4$, no detectable loss of ^{32}P to the sides of either the plastic or the glass container was noted (Table A1).

TABLE A1. ^{32}P activity in a sample Lake 239 epilimnion water before and after 1.5 hours of confinement in either a glass or plastic container. 23/10/74.

$^{32}PO_4$ was added to the lake water 10 minutes before the water was poured into the two containers.

Sample	^{32}P Activity (cpm/ml)
Initial	10736
Glass bottle	10733
Plastic bottle	10761

Errors Due to Filtering

Orthophosphate Adsorption by Membrane Filters

Filter adsorption of orthophosphate could strongly influence the ability of Sephadex fractionation, Schindler bioassays, or any other method which is applied to lake water filtrate rather than to whole lake water, to produce reliable estimates of orthophosphate concentration.

Methods

Orthophosphate adsorption by filters was measured by adding $^{32}PO_4$ to filtered lake water, refiltering the sample, and measuring the ^{32}P activity on the filter.

Filter Type

Sartorius membrane filters were used to separate filterable from filtrate phosphorus during radioisotope experiments. Glass fiber filters, however, were used to separate phosphorus fractions during chemical analyses. Methods for estimating orthophosphate concentration which required that ^{32}P specific activity be equal for all phosphorus

compartments, demanded that the two types of filters separate the same phosphorus fractions. This experiment was initiated to determine whether this requirement was met.

Methods

$^{32}PO_4$ was added to lake water. After a 20 hr incubation, 5 ml aliquots of the labeled water were pumped through Sartorius and glass fiber filters. The ^{32}P activity in the filtrate of each was determined. As an extra check on this procedure, analyses for TDP were performed for unlabeled water filtered through Sartorius and glass fiber filters.

Results

Filter Adsorption

Filter adsorption of $^{32}PO_4$ by both Sartorius and glass fiber filters was measured to be about 13% of the total $^{32}PO_4$ filtered (Table A2). Methods for estimating orthophosphate concentration which require that the sample be filtered before analysis, therefore, will underestimate orthophosphate concentration by about 13%. As long as filter adsorption is constant, Rigler's bioassay, which is concerned only with the change in ^{32}P activity in the filtrate with time, will not be influenced by filter adsorption.

Filter Type

Sartorius and glass fiber filters were found to remove a similar fraction of the ^{32}P activity from a labeled lake water sample (Table A3). The Sartorius filter, however, removed a greater amount of the unlabeled phosphorus than did the glass fiber filter. The outcome of the radiochemical and chemical approaches to this experiment differed

probably because the chemical one was influenced more heavily by the large fraction of filtrate phosphorus which is more or less inert biologically (it is not labeled easily with ^{32}P). Because Sartorius and glass fiber filters retain a similar proportion of the ^{32}P in labeled lake water, a comparison of Sartorius radiochemical with glass fiber chemical fractionations is permissible.

Counting Errors Due to Fluorescence

Humic acids are known to fluoresce after being exposed to fluorescent lights. Concern that fluorescence might interfere with Cerenkov scintillation counting, and, thus, influence the individual points used to determine K, prompted this experiment.

Methods

A lake water sample containing ^{32}P was filtered under white fluorescent light and its "^{32}P" activity determined. After leaving the sample in the dark for one-half hour, "^{32}P" activity again was measured. The sample next was held for two minutes about one foot from first a yellow light (yellow light is recommended for use in rooms housing scintillation counters) and then a white light. "^{32}P" activity was counted after each exposure.

To determine how long samples should be held in the dark before counting, 70 samples containing ^{32}P were exposed to white light, and then placed in a scintillation counter. The counter was set so that each sample was counted for one minute every 70 minutes for 12 hours. On another day, a sample, which had been in the dark for 12 hours was

TABLE A2. ^{32}P activity in a $^{32}PO_4$ solution after filtration through either a glass fiber or a Sartorius membrane filter.

Filter Type	^{32}P Activity (cpm/5 ml)	% of Total (unfiltered) ^{32}P activity in the filtrate	% of Total ^{32}P activity adsorbed by the filter
Unfiltered	8296	100	-
Glass fiber	7204	86.8	13.2
Sartorius	7228	87.1	12.9

TABLE A3. The TDP concentration of unlabeled, and the ^{32}P activity of ^{32}P-labeled*, lake water (from the epilimnion of Lake 239) after filtration through either a glass fiber or a Sartorius membrane filter. 24/10/74.

Filter		TDP (µg/l)	^{32}P Activity (cpm/5 ml)	% of Total ^{32}P activity in filtrate
Unfiltered		-	6412	100
Glass fiber		-	2433	37.9
Sartorius		-	2350	36.7
Glass fiber		9	-	-
Glass fiber		9	-	-
	Average	9		
Sartorius		7		
Sartorius		6		
	Average	6.5		

* Whole lake water was incubated with $^{32}PO_4$ for 20 hr before filtration

exposed to yellow light for two minutes, placed in the counter, and counted at 60 minute intervals for 12 hours.

Results

Humic acids or other compounds in E.L.A. lake water absorb a considerable amount of energy from white fluorescent light which later is emitted as a fluorescence. This fluorescence is detected by the scintillation counter and can alter significantly the "activity" of a sample (Table A4). In this experiment 70% of the radioactivity of samples placed in the counter immediately after exposure to white fluorescent lights was due to fluorescence (Figure A1). In the dark fluorescence decreases in an exponential manner. An asymptote is reached after about 12 hours. Yellow light does not induce a significant amount of fluorescence.

Judging from this experiment, samples which are to be counted using the Cerenkov method should be held in the dark, preferably for 5 hours or more, before counting. If the samples must be exposed momentarily to light during this period, yellow light should be used. Fluorescence is not a problem when its contribution to the "activity" of each sample is relatively constant. Errors arise when a series of samples is counted immediately after exposure to white light. In this case, the contribution of fluorescence to sample "activity" will decrease progressively between the first and final sample. Most of the radioactive samples from the present study (those collected before August, 1974) were counted immediately after exposure to white light. Fortunately, no more than 6 samples were used in a determination of K

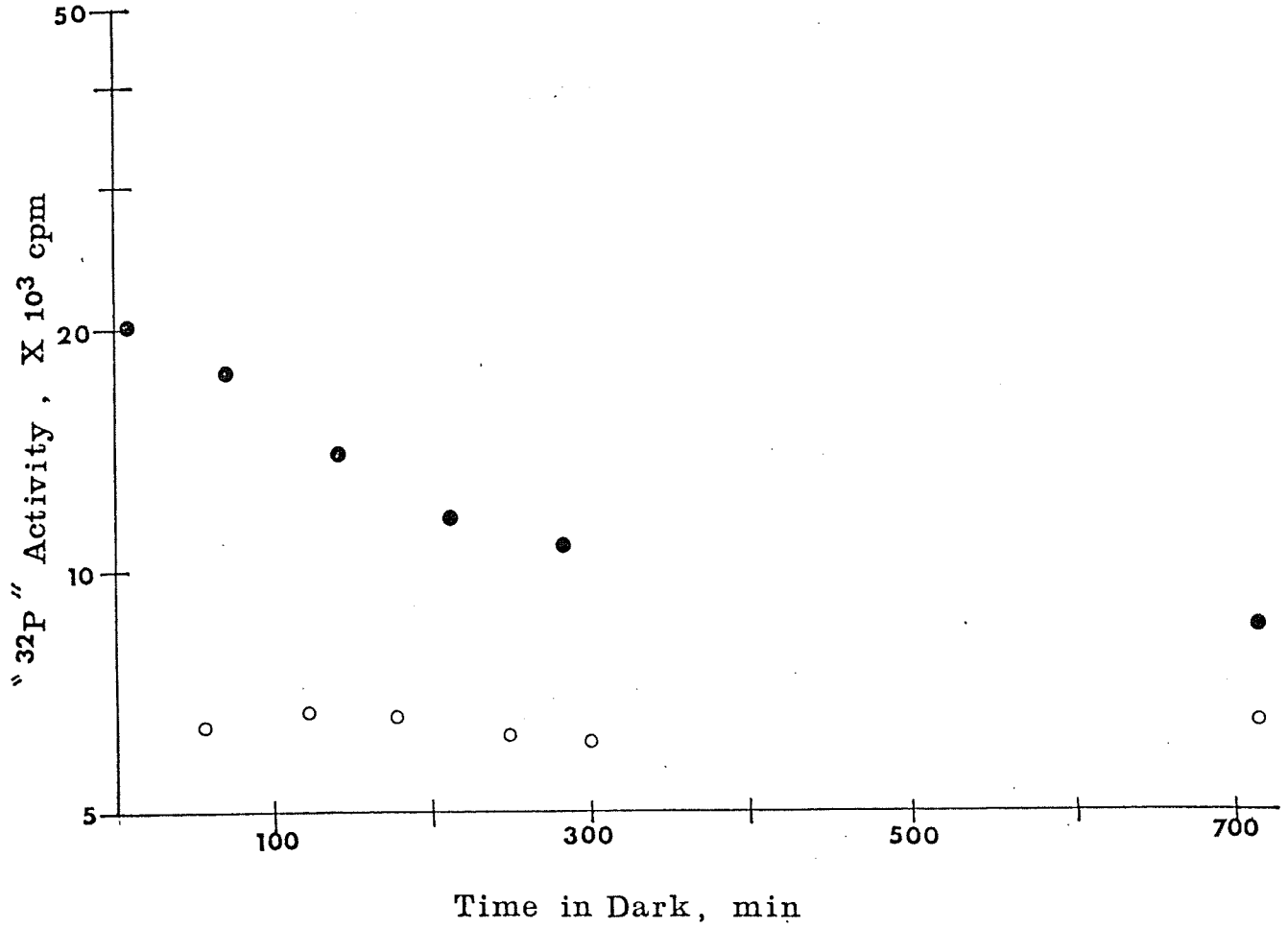
and each sample was counted only one minute. The amount of fluorescence dissipated in 6 minutes is small compared with the decrease in filtrate activity which was observed between samples.

TABLE A4. Scintillation detected by a scintillation counter after a ^{32}P-labeled sample of lake water filtrate (Lake 302S, 12/8/74) had been exposed to various light conditions.

The following conditions were imposed in the sequence given.

Condition of Sample	cpm
Immediately after filtration in a room lighted with white fluorescent lights	76992
After one-half hour in the dark	29754
After 2 minutes of exposure to yellow light (held one foot from the light source)	31369
After 2 minutes of exposure to white light (1 ft from the source)	98189

Figure A1. The radioactivity of samples held in the dark for various lengths of time after exposure to white (●) or yellow (○) fluorescent light. The experiment with white light was done on 12/8/74 with water from Lake 302S. The experiment with yellow light was done on 18/5/75 with water from Lake 239.


50
20
10
5
"32P" Activity, X 10³ cpm
100
300
500
700
Time in Dark, min

APPENDIX II

TABLE A5. r^2 values for two linear transformations of the Michaelis-Menton equation, $1/v$ vs $1/C_s$, and $1/K$ vs C_s, estimates of V_{max} derived from each equation, and a comparison of the turnover time (tt) obtained from the y-intercept of $1/K$ vs C_s with the value calculated from $^{32}PO_4$ disappearance from filtered (0.45μ) lake water.

Lake	Date	$1/v$ vs $1/C_s$		$1/K$ vs C_s			tt (min) from
		r^2	V_{max}(μg/l/min)	r^2	V_{max}(μg/l/min)	tt (min)	$^{32}PO_4$ disappearance
227	30/5/73	0.760	0.115	0.002	-2.92	14.3	6.58
0-2 m	11/6	0.991	0.0388	0.994	0.0422	14.1	14.8
	21/6	0.0011	0.0302	0.975	0.0247	-4.05	4.45
	5/7	0.987	0.107	0.987	0.0934	6.51	4.11
	25.7	0.966	0.357	0.111	3.34	3.27	1.79
	30/7	0.999	0.265	0.960	0.380	3.18	1.94
	7/8	0.997	5.20	0.987	5.52	1.35	0.408
	13/8	0.990	2.54	0.689	5.88	1.50	0.758
	16/9	0.998	-11.2	0.680	2.11	3.67	3.37
	29/9	1.00	0.872	0.904	1.10	3.13	2.65
	13/10	0.997	0.271	0.766	0.642	4.86	3.19
	27/10	0.992	0.159	0.910	0.320	6.05	3.37
	10/11	0.991	0.0131	0.954	0.0195	21.7	12.7

TABLE A5 (cont.)

Lake	Date	1/v vs 1/C_s		1/K vs C_s			tt (min) from $^{32}PO_4$ disappearance
		r^2	V_{max} (µg/l/min)	r^2	V_{max} (µg/l/min)	tt (min)	
227	18/12	0.768	0.0030	0.956	0.0022	-29.4	28.6
0-2 m	23/11/74	0.998	0.0460	0.932	0.046	261	270
	26/2	0.724	0.0197	0.943	0.0208	27.2	238
	23/4	0.989	0.0066	0.997	0.0056	984	455
	6/5	0.515	0.0083	0.833	0.0050	-57.7	17.9
	23/5/74	0.989	0.0245	0.530	0.0749	298	204
	17/6	0.997	1.29	0.572	3.00	8.49	4.44
	2/7	0.991	6.00	0.935	1.97	-1.30	4.05
	9/7	0.999	4.01	0.969	1.12	3.42	5.23
	2/8	0.856	0.0492	0.185	0.152	8.16	3.59
	16/8	0.963	0.0099	0.673	0.0379	11.1	4.99
	20/9	0.981	0.217	0.784	0.889	2.57	0.62
	31/10	1.00	0.733	0.926	1.79	4.22	3.92
	6/2/75	0.636	0.0034	0.988	0.0042	17.6	17.2
	1/4/75	0.418	0.0006	0.990	0.0009	127	93.5
227	28/5/74	0.724	0.0181	0.999	0.0164	-2.37	9.29
9 m	24/6	0.340	0.0031	0.853	0.0017	-2670	30.2

TABLE A5 (cont.)

Lake	Date	1/v vs $1/C_s$		1/K vs C_s			tt (min) from $^{32}PO_4$ disappearance
		r^2	V_{max} (µg/l/min)	r^2	V_{max} (µg/l/min)	tt (min)	
227	22/7	0.333	0.0046	0.987	0.0033	-314	86.2
9 m	22/9	0.947	0.0142	0.998	0.0208	40.6	13.4
	21/11	1.00*	0.516	1.00*	0.516	10.6	8.49
	22/1/75	0.802	0.0691	0.885	0.0691	13.2	5.77
	5/3	1.00*	0.0392	1.00*	0.0392	0.770	13.5
302 S	28/5/73	0.890	-0.0086	0.364	-0.0215	99.3	9.62
0-2 m	12/6	0.0288	0.0012	0.955	0.0009	-48.3	21.4
	3/7	0.918	0.0458	0.946	0.0381	12.5	12.4
	16/7	0.874	0.0667	0.0197	-0.239	32.6	7.43
	15/9/73	0.991	0.0590	0.757	0.0912	9.89	5.35
	30/9	1.00	0.0627	1.00	0.0573	5.94	7.42
	13/10	0.994	0.0468	0.956	0.0781	14.4	8.04
	26/10	0.993	0.0339	0.956	0.0354	9.56	11.7
	9/11	0.945	0.0059	0.602	0.0015	86.2	125
	20/12	0.967	0.0019	0.821	0.0011	113	111
	21/1/74	0.449	0.0000	0.784	0.0001	12.0	1250

* Only 2 points available

TABLE A5 (cont.)

Lake	Date	1/v vs $1/C_s$		1/K vs C_s			tt (min) from $^{32}PO_4$ disappearance
		r^2	V_{max} (µg/l/min)	r^2	V_{max} (µg/l/min)	tt (min)	
302 S	27/2	0.997	0.0050	0.0015	-0.0694	15400	11100
0-2 m	13/4	0.0397	0.0001	0.0844	-0.0008	103000	10000
	8/5	0.833	0.0080	0.945	0.0177	48.3	14.6
	22/5	1.00	0.0359	0.981	0.0327	11.3	8.67
	12/6	0.992	0.0098	0.995	0.0166	9.36	5.15
	4/7	0.999	0.1273	0.997	0.0979	3.05	4.71
	31/7	0.678	0.0057	0.187	0.0297	420	26.3
	2/9	1.00*	0.0256	1.00*	0.0256	17.5	44.3
	19/11	0.0001	0.0094	0.994	0.0077	-54.1	32.6
	18/2/75	0.109	0.0001	0.094	0.0007	41300	3330
	2/4/75	1.00*	0.0003	1.00*	0.0003	732	278
302 S	26/5	0.988	-0.1273	0.770	0.0860	42.0	12.7
9 m	21/6	0.857	0.802	0.874	0.473	-24.0	27/2
	12/7	0.956	0.0449	0.740	0.376	30.7	8.29
	26/9	1.00	0.405	1.00	0.400	4.33	3.59
	6/11	0.696	0.0120	0.997	0.0158	33.8	9.87
	21/1	1.00*	0.0730	1.00*	0.0730	16/8	23.0
	4/3	0.999	0.0291	0.996	0.0343	185	83.3

* Only 2 points available

APPENDIX III

THE INFLUENCE OF INHIBITORS AND BIOCIDES ON PO_4-P UPTAKE

Methods

One percent formaldehyde was used to terminate biological activity in a lake water sample so that inorganic reactions causing the movement of $^{32}PO_4$ to ^{32}PP could be measured. Formaldehyde, however, could induce cellular release of phosphorus. If so, the rate of non-biological reactions might be altered by the increase in filtrate phosphorus. To examine this possibility, formaldehyde (1%) was added to a sample which had been incubated with $^{32}PO_4$ for three hours. Filtrate ^{32}P activity was measured before and after the formaldehyde addition.

Penicillin (5×10^3 to 5×10^6 units/1) also was used as an inhibitor. $^{32}PO_4$ uptake was measured in treated and in control samples. Penicillin does not actually kill organisms, but prevents cell division.

To determine whether active transport is necessary for orthophosphate uptake, 10^{-6} to 10^{-5} M CCCP (carbonyl-cyanide-m-chlorophenylhydraxone) was added to lake water. CCCP inhibits both oxidative phosphorylation and photophosphorylation. As usual, $^{32}PO_4$ uptake was followed in both

the inhibited and in control samples.

Results

Formaldehyde

One percent formaldehyde virtually halted $^{32}PO_4$ uptake by seston in a lake water sample (Table A6), suggesting that biological uptake is much more significant process in phosphorus cycling than is inorganic precipitation or adsorption onto particles. However, when one percent formaldehyde was added to ^{32}P-labeled lake water, a significant return of ^{32}P from the seston to the filtrate fraction occurred (Table A7). Either excretion or cell rupture was responsible. Inorganic uptake of $^{32}PO_4$ may have been greatly obscured by extracellular release during this experiment.

TABLE A6. Rate constants for $^{32}PO_4$ uptake in a control and in a sample to which 1% formaldehyde had been added. Lake 227, epilimnion. 5/9/73.

Sample	K (1/min)
Control	0.1863
1% formaldehyde	0.0007

TABLE A7. Percent of total ^{32}P activity in the filtrate of a sample before and after addition of 1% formaldehyde. Lake 227, epilimnion. 5/9/73

Sample	% ^{32}P in filtrate
Before formaldehyde addition	6.4
After formaldehyde addition	57.7

Penicillin

Penicillin inhibited $^{32}PO_4$ uptake. The degree of inhibition increased with the amount of penicillin added (Table A8).

TABLE A8. Rate constants for $^{32}PO_4$ uptake in control samples and in samples to which from $5^4 \times 10^3$ to 5×10^6 units of penicillin per liter had been added. Lake 239, epilimnion. 20/8/73.

Penicillin Added (units/l)	K (1/min)
No addition	0.0782
5×10^3	0.0612
5×10^4	0.0257
5×10^5	0.0057
5×10^6	0.0044

CCCP

$^{32}PO_4$ uptake was inhibited strongly by 10^{-6} to 10^{-5} M CCCP (Table A9). These results indicate that the movement of ^{32}P from $^{32}PO_4$ to filterable ^{32}P was due largely to active, biological uptake. The minor role of inorganic processes was confirmed.

TABLE A9. Rate constants for $^{32}PO_4$-P uptake in controls and in samples to which 10^{-6} M or 10^{-5} M CCCP had been added. Lake 239, epilimnion. 25/7/74.

Sample	K (1/min)
"No addition"	0.0818
10% ETOH	0.0473
10^{-6} M CCCP (1% ETOH)	0.0237
10^{-5} M CCCP (10% ETOH)	0.0031

APPENDIX IV

THE INFLUENCE OF SESTON SIZE FRACTIONATION ON PO_4-P UPTAKE

Methods

To help clarify which organisms contribute most to orthophosphate uptake, 200 ml samples of lake water were pumped through 0.45μ membrane, and 10, 56, and 239μ nylon filters. $^{32}PO_4$ uptake was measured in the filtrate of each. In a later experiment, the uptake in 3μ filtrate was compared with that in whole lake water. Chlorophyll-a concentration was determined for aliquots of the 3 and 10μ filtrates.

Results

Removal of seston larger than 10μ did not influence the rate of $^{32}PO_4$ uptake significantly (Table A10). When all seston larger than 3μ were removed, however, the rate constant decreased drastically.

49% of the chlorophyll-a present in whole lake water appeared in the 10μ filtrate, but only 1% remained in 3μ filtrate. Nannoplankton, therefore, seem to be responsible for the rapid orthophosphate uptake observed in ELA lakes.

TABLE A10. Rate constants for $^{32}PO_4$-P uptake in, and the chlorophyll-a concentration of the filtrate of lake water filtered through filters of various pore sizes.

Sample	Pore Size of filter (μ)	K (1/min)	Chlorophyll-a (μg/l)
Lake 239, epilimnion 27/6/73	239	0.108	-
	56	0.129	-
	10	0.109	-
	0.45	0.0019	23.0
Lake 227, epilimnion 7/8/73	whole lake water	0.375	23.0
	10	-	11.3
	3	0.0161	2.21

APPENDIX V

TABLE A11. The rate constants for PO_4-P uptake by seston (K), PO_4-P concentrations, and estimates of PO_4-P flux into seston for "epilimnion" samples collected between May, 1973 and April, 1975.

Rate constants were obtained by measuring the disappearance of $^{32}PO_4$ from the filtrate fraction of lake water. Rigler bioassays were used to estimate PO_4-P concentration. PO_4-P flux is the product of the first two parameters.

Lake	Date	K (1/min)	PO_4-P Concentration (µg/l)	PO_4-P Flux (µg/l/min)
227	30/5/73	0.152	0.023	0.0031
	11/6	0.0675	0.053	0.0036
	21/6	0.225	0.0086	0.0019
	5/7	0.243	0.0070	0.0017
	25/7	0.557	0.0066	0.0037
	30/7	0.514	0.0094	0.0047
	7/8	2.45	0.00082	0.0020
	13/8	1.32	0.00059	0.0015
	16/9	0.297	0.011	0.0032
	29/9	0.378	0.018	0.0068
	13/10	0.313	0.011	0.0034
	27/10	0.297	0.015	0.0045
	10/11	0.0790	0.018	0.0014

TABLE A11 (cont.)

Lake	Date	K (1/min)	PO_4-P Concentration (µg/l)	PO_4-P Flux (µg/l/min)
	18/12	0.0353	0.067	0.0024
	23/11/74	0.00373	0.94	0.0035
	26/2	0.00472	0.23	0.0011
	23/4	0.00222	0.52	0.0011
	6/5	0.0558	0.039	0.0022
	23/5	0.0049	0.36	0.0018
	17/6	0.225	0.0092	0.0021
	2/7	0.247	0.053	0.013
	9/7	0.191	0.020	0.0038
	2/8	0.278	0.011	0.0031
	16/8	0.200	0.010	0.0020
	20/9	1.62	0.0097	0.0016
	31/10	0.256	0.036	0.0089
	6/2/75	0.0580	0.038	0.0022
	1/4	0.0107	0.044	0.0047
302 S	28/5/73	0.104	0.014	0.0014
	12/6	0.0468	0.024	0.0011
	3/7	0.0805	0.025	0.0020
	16/7	0.135	0.016	0.0023

TABLE A11 (cont.)

Lake	Date	K (1/min)	PO_4-P Concentration (µg/1)	PO_4-P Flux (µg/1/min)
	15/9	0.187	0.012	0.0021
	30/9	0.135	0.015	0.0020
	13/10	0.124	0.013	0.0016
	26/10	0.0856	0.023	0.0020
	9/11	0.00811	0.13	0.00011
	20/12	0.00948	0.11	0.00010
	21/1/74	0.000783	0.065	0.00005
	27/2	0.0000916	2.3	0.00021
	13/4	0.000100	0.66	0.00007
	8/5	0.0687	0.025	0.0017
	22/5	0.115	0.014	0.0016
	12/6	0.194	0.0058	0.0011
	4/7	0.212	0.016	0.0034
	31/7	0.0279	0.038	0.0011
	2/9	0.0226	0.079	0.0018
	19/11	0.0307	0.070	0.0022
	18/2/75	0.000279	0.22	0.00006
	2/4	0.0036	0.023	0.0001

TABLE A12. The rate constants for PO_4-P uptake by seston (K), PO_4-P concentrations, and estimates of PO_4-P flux into seston, for "hypolimnion" samples (9 m) over a one-year period.

Rate constants were obtained by measuring the disappearance of $^{32}PO_4$ from the filtrate fraction. Rigler bioassays were used to estimate orthophosphate concentration. Orthophosphate flux is the product of the first two parameters.

Lake	Date	K (1/min)	PO_4-P concentration (µg/l)	PO_4-P flux (µg/l/min)
227	28/5/74	0.108	0.023	0.0025
	24/6	0.0331	0.091	0.0030
	22/7	0.0116	0.18	0.0021
	27/9	0.0749	0.024	0.0018
	21/11	0.112	0.031	0.0035
	22/1/75[1]	0.173	0.0072	0.0013
	5/3	0.0740	0.015	0.0011
302S	26/5/74	0.0790	0.015	0.0012
	21/6	0.0367	0.084	0.0031
	12/7	0.121	0.065	0.0078

1. Collected at 9.5 m.

TABLE A12 (cont.)

Lake	Date	K (1/min)	PO_4-P concentration (µg/l)	PO_4-P flux (µg/l/min)
302S	26/9	0.279	0.0074	0.0021
	6/11	0.101	0.014	0.0014
	21/1/75[2]	0.0435	0.087	0.0038
	4/3	0.0120	0.13	0.0015

1. Collected at 9.5 m.

APPENDIX VI

TABLE A13. ATP concentrations in the epilimnions and hypolimnions of Lakes 227 and 302S.

Replicates and the mean concentrations are shown.

Lake	Stratum	Date	ATP Concentrations (µg/l)	Mean ATP Concentration of the replicate (µg/l)
227	epilimnion	17/6/74	0.65* 0.21*	0.43
		2/7	0.38* 0.23*	0.31
		2/8	0.91 0.81	0.86
		16/8	0.57 0.63	0.60
		20/9	0.71 0.81	0.76
		31/10	0.25 0.50	0.37
		6/2/75	0.18 0.21	0.19
		1/4	0.40 0.46	0.42

TABLE A13 (cont.)

Lake	Stratum	Date	ATP Concentration (µg/l)	Mean ATP Concentration of the replicate (µg/l)
302 S	epilimnion	12/6/74	0.34* 0.36* 0.24*	0.31
		4/7	0.036 0.039	0.037
		31/7	0.11 0.087	0.098
		19/11	0.21	0.21
		18/2/75	0.020 0.051	0.038
		2/4/75	0.39 0.46	0.42
227	hypolimnion	24/6/74	0.59* 0.85*	0.72
		22/7	1.3 0.41	0.86
		20/9	0.72 0.61	0.66
		27/9	0.59 0.52	0.55

TABLE A13 (cont.)

Lake	Stratum	Date	ATP Concentration (µg/l)	Mean ATP Concentration of the replicate (µg/l)
		7/11	0.14 0.044	0.094
		21/11	0.084 0.21	0.14
		22/1/75	0.44 2.3	1.4
		5/3	0.63 0.37	0.50
		1/4	0.27 0.40	0.33
302 S	hypolimnion	21/6/74	0.17* 0.52*	0.34
		12/7	0.072 0.096	0.084
		26/9	0.22 0.23	0.22
		6/11	0.12 0.072	0.097
		21/1/75	0.25 0.31	0.27

TABLE A13 (cont.)

Lake	Stratum	Date	ATP Concentrations (µg/1)	Mean ATP Concentration of the replicate (µg/1)
		4/3	0.27 0.17	0.21
		2/4	0.49 0.51	0.50

* Only 10 ml of water were filtered

APPENDIX VII

TABLE A14. The concentrations of TDP, PP, ATP, chlorophyll a, and dissolved oxygen (DO) in the "epilimnions"of Lakes 227 and 302S between May, 1973 and April, 1975. Temperatures also are shown. The chlorophyll a and DO concentrations given are averages of values obtained at two to three discrete depths. Some of the PP, TDP and temperature values (those marked with a *) were estimated in the same manner. All other data were obtained from the analysis of integrated epilimnion samples. ATP concentrations are the average value of two to three replicates.

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
227	28/5/73	7*	27*	-	38.1	10.85	16*
	8/6	14	31	-	-	-	-
	11/6	7	35	-	69.7	11.04	17
	21/6	8	58	-	-	-	17
	27/6	10	50	-	86.6	9.90	19
	5/7	10	83	-	-	-	20
	9/7	9	58	-	123.3	9.92	22
	10/7	11	48	-	-	-	-
	11/7	6	54	-	-	-	-

TABLE A14 (cont.)

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
227	23/7	9*	69*	-	176	9.29	22*
	25/7	14	59	-	-	-	21.5
	30.7	16	33	-	-	-	19
	6/8	24	63	-	65.3	7.38	21
	7/8	12	50	-	-	-	24
	8/8	13	42	-	-	-	-
	17/8	10	50	-	-	-	-
	20/8	14*	45*	-	28.3	6.12	21*
	3/9	16*	47*	-	37.7	8.18	20.5*
	17/9	17*	27*	-	42.0	10.73	13*
	29/9	-	-	-	-	-	12
	1/10	17*	45	-	54.4	11.24	13*
	13/10	-	-	-	-	-	10
	15/10	13*	32*	-	48.6	9.31	9*
	27/10	-	-	-	-	-	7
	29/10	14*	-	-	27.1	7.31	7*
	6/11	9*	16*	-	21.9	8.76	2*
	10/11	-	-	-	-	-	2
	12/12	13*	18*	-	16.4	9.04	2
	18/12	-	-	-	-	-	2

TABLE A14 (cont.)

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
227	22/1/74	8*	14*	-	8.0	0.42	4*
	23/1	-	-	-	-	-	1
	26/2	7*	24*	-	9.1	0.10	1.5
	2/4	6	22	-	6.5	0	1
	23/4	-	-	-	-	-	2
	6/5	9*	15*	-	12.5	6.03	4
	21/5	7*	8*	-	6.5	9.11	11*
	23/5	-	-	-	-	-	12
	4/6	6*	15*	-	22.7	10.77	15*
	18/6	8	33	0.31	56.5	11.08	17
	2/7	11*	44*	0.31	62.8	10.23	22
	9/7	8	45	0.86	-	-	24
	16/7	10*	32*	-	28.7	7.40	23*
	30/7	12*	16*	-	15.1	9.19	19*
	2/8	15	54	0.86	-	-	18
	13/8	11*	31*	-	44.4	10.35	19.5*
	16/8	9	47	0.60	-	-	20
	27/8	8*	28*	-	28.8	9.32	16*
	10/9	11*	28*	-	42.2	9.85	14*
	20/9	-	-	0.76	-	-	13

TABLE A14 (cont.)

Lake	Date	TDP (µg/1)	PP (µg/1)	ATP (µg/1)	Chlorophyll a (µg/1)	DO (mg/1)	T (°C)
227	24/9	9*	33*	-	37.1	10.83	11*
	8/10	9*	31*	-	40.1	7.33	6*
	22/10	8*	25*	-	41.2	7.25	5*
	31/10	-	-	0.37	-	-	7
	5/11	8*	26*	-	26.8	8.61	4*
	21/11	9*	22*	-	29.3	10.66	-
	11/12	9*	16*	-	27.0	10.67	-
	22/1/75	8*	11*	-	9.7	9.97	-
	6/2	-	-	0.20	-	-	1.5
	4/3	5*	8*	-	7.1	7.12	-
	1/4	5*	8*	-	3.8	5.86	-
302S	21/5/73	3*	4*	-	4.4	10.44	14*
	28/5	-	-	-	-	-	15
	4/6	6*	7*		4.5	9.21	17*
	12/6	3	6	-	-	-	16
	18/6	3*	7*	-	3.3	8.90	18*
	2/7	4*	4*	-	2.7	8.63	20.5*
	3/7	4	8	-	-	-	19
	16/7	3	3	-	3.0	8.37	20

TABLE A14 (cont.)

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
302S	17/7	3	5	-	-	-	-
	18/7	1	1	-	-	-	-
	19/7	2	6	-	-	-	-
	23/7	5	2	-	-	-	-
	30/7	3*	4*	-	4.2	8.79	19*
	13/8	5*	-	-	2.8	8.44	21*
	27/8	4*	3*	-	2.2	8.23	22*
	10/9	3*	5*	-	5.9	8.28	19*
	15/9	-	-	-	-	-	15
	24/9	24*	18*	-	4.8	8.78	13*
	30/9	-	-	-	-	-	12
	8/10	4*	5*	-	5.4	9.32	13*
	13/10	-	-	-	-	-	10
	22/10	3*	6*	-	6.5	9.90	9*
	26/10	-	-	-	-	-	7
	5/11	4*	10*	-	10.9	10.31	5*
	9/11	-	-	-	-	-	2
	19/12	4*	11*	-	9.0	11.37	2*
	20/12	-	-	-	-	-	2 I
302S	21/1/74	-	-	-	-	-	1

TABLE A14 (cont.)

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
302S	23/1	5*	10*	-	2.8	9.90	3*
	26/2	6*	6*	-	1.2	6.90	3*
	27/2	-	-	-	-	-	1.5
	2/4	4*	9*	-	1.0	4.99	2*
	13/4	-	-	-	-	-	1.5
	1/5	4*	7*	-	1.3	4.78	4*
	8/5	3	7	-	-	-	5
	22/5	4	8	-	-	-	12.5
	5/6	4*	5*	-	2.8	9.50	18*
	12/6	8	6	0.31	-	-	17
	3/7	3*	5*	-	5.3	8.20	21.5*
	4/7	3	7	0.037	-	-	21
	31/7	3	7	0.10	2.9	8.40	18
	26/8	3*	5*	-	10.0	8.87	17.5
	2/9	-	-	-	-	-	15
	25/9	3*	5*	-	6.1	9.14	11.5*
	23/10	4*	5*	-	5.8	10.29	6*
	4/11	4*	8*	-	10.8	10.86	5*
	19/11	6*	6*	0.12	6.1	12.65	1.5
	9/12	5*	5*	-	7.9	12.68	-

TABLE A14(cont.)

Lake	Date	TDP (µg/1)	PP (µg/1)	ATP (µg/1)	Chlorophyll a (µg/1)	DO (mg/1)	T (°C)
302S	21/1/75	5*	6*	-	2.0	11.50	-
	18/2	-	-	0.038	-	-	1.0
	4/3	5*	4*	-	1.7	11.97	-
	2/4	4*	3*	0.42	2.2	10.57	-

TABLE A15. The concentrations of TDP, PP, ATP, chlorophyll a, and dissolved oxygen (DO) in the hypolimnions (9 m) of Lakes 227 and 302S between May, 1974 and April, 1975. Temperature also is given. ATP concentrations are the average value of two to three replicates.

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
227	16/5/74	14	81	-	-	-	5
	21/5	17	98	-	110	0	4
	28/5	17	102	-	-	-	5
	4/6	16	104	-	114	0	4
	18/6	15	93	-	117	0	4
	24/6	14	90	0.72	-	-	9[1]
	2/7	18	98	-	111	0	4.5
	16/7	22	68	-	89.6	0	4.5
	22/7	15	85	0.86	-	-	6.5[1]
	30/7	19	95	-	98.6	0	4.5
	13/8	17	146	-	115	0	4.5
	27/8	21	148	-	109.5	0	4.5
	10/9	14	117	-	123	0	4.5
	20/9	-	-	0.67	-	-	-
	24/9	32	94	-	150	0	4.5

TABLE A15(cont.)

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
227	27/9	17	117	0.55	-	-	6
	8/10	15	117	-	120	0	5
	22/10	7	25	-	40.9	6.68	5
	5/11	6	26	-	26.3	8.05	4
	7/11	-	-	0.094	-	-	-
	21/11	8		-	26.8	6.80	3.5
	11/12	10	79	-	24.1	0	-
	22/1/75[2]	14	64	1.39	20.6	0	4
	4/3	11	57	-	29.3	0	-
	5/3	-	-	0.50	-	-	5
	1/4	14	58	0.33	46.0	0	-
302 S	26/5	3	9	-	-	-	5
	5/6	5	11	-	11.9	2.50	5.5
	21/6	5	29	0.34	-	-	6.5
	3/7	6	45	-	48.2	0.22	7

TABLE A15 (cont.)

Lake	Date	TDP (µg/l)	PP (µg/l)	ATP (µg/l)	Chlorophyll a (µg/l)	DO (mg/l)	T (°C)
302 S	12/7	7	16	0.084	-	-	7
	31/7	8	48	-	114	0	7
	26/8	9	46	-	94.9	0	7.5
	25/9	6	31	-	90.2	2.89	8
	26/9	7	33	0.23	-	-	10.5
	23/10	4	6	-	6.4	9.95	6
	4/11	5	7	-	9.6	10.91	5
	6/11	-	-	0.10	-	-	4.5
	19/11	6	9	-	7.2	8.79	-
	9/12	5	14	-	4.4	2.80	-
	21/1/75[2]	11	30	0.28	9.9	0.0	4
	4/3	6	35	0.22	21.5	1.95	4
	2/4	6	36	0.50	17.3	0	-

1. These temperatures were measured in bottles. The bottles were filled before the syringes, but temperature was measured after the syringes were filled. Obviously some warming occurred.
2. Collected at 9.5 m.