THE METABOLISM OF NONPARTICULATE PHOSPHORUS IN AN ACID BOG LAKE

by

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DISCLAIMER

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INTRODUCTION

In North Gate Lake, an acid bog lake located on the northern Michigan-Wisconsin border, U.S.A., the algal nutrient inorganic phosphate (FRP) is not detectable by chemical means. Organic phosphorus (FUP) represents 100% of the detectable filterable phosphorus. The availability and cycling of this organic fraction is of considerable interest in regard to the primary productivity of this system. To clarify these relationships, I studied the cycling of nonparticulate forms of phosphorus (see Appendix) found in the epilimnion of this lake.

I. The Link Between Primary Productivity and Phosphorus

Waters of highly stained bog lakes of the north temperate zone typically show a very low level of primary productivity (Ruttner 1963; Hutchinson 1957). At the same time among naturally and artifically enriched eutrophic lakes, a high phosphorus content is ordinarily associated with high productivity and a large biomass of plants and animals (Edmondson 1969). Phosphorus concentration has been linked with various indices of eutrophication of lakes in Asia, Europe and North America (Peters 1975). When introduced into a lake, inorganic phosphorus is rapidly taken out of solution and is transferred to the entire biota (Coffin et al. 1949). However, certain Michigan bog lakes do not follow this pattern since they contain high concentrations of phosphorus and have extremely low levels of primary production.

Low primary production may be caused by (1) a lack of dissolved electrolytes (cations) which are selectively removed by the surrounding Sphagnum mat and exchanged for hydrogen ions (H_3O^+); this process also reduces the pH of the water (Clymo 1964; 1967); (2) the reduction in

light penetration into the water by colloidal organic matter (COM) (Hasler et al. 1951; Stross and Hasler 1960); and (3) the control COM may exert on the availability of essential nutrients (Koenings 1976; Koenings and Hooper 1976). Other factors stressed by Waters (1957) include the importance of a low pH and the lack of alkaline earth metals which are needed to maintain a supply of bound CO_2. However, in geologically similar lakes on the Canadian Shield, phosphorus is believed to be the most important factor in lake eutrophication (Schindler et al. 1971).

Since bog lakes do not fit the model of the phosphorus cycles of eutrophic and oligotrophic lakes presented by Hutchinson (1957), both the supply and form of phosphorus in the lake water as well as its availability to phytoplankton must be considered as possible limitations to productivity. In bog lakes the input of phosphorus from ground water or surface drainage is drastically changed by the seepage of water through the biochemically active Sphagnum mat. Also, bog lakes lack a true littoral zone which in other lake types acts as an active site of phosphate regeneration and resupply when assisted by wind-driven currents. Finally, the protection then afforded to such small lakes by the surrounding vegetation (i.e., black spruce forest), the low surface area to volume ratio, and the ability of the stained waters to quickly absorb sunlight all tend to reduce water circulation and thereby restrict the resupply of mineralized phosphate from the hypolimnion. Bog lakes of this type may develop a permanent chemocline and monimolimnion (Hooper 1969).

North Gate Lake has a surface area of 0.4 ha (Figure 1). It is located near the Michigan-Wisconsin border and is in close

geographical proximity to the lakes studied in the pioneering limnological research of C. Juday and E.A. Birge (Juday and Birge 1931). It is within the same watershed as Peter and Paul lakes studied by Hasler et al. (1951). The total phosphorus content (maximum of 80-90 $\mu g\ l^{-1}$ as PO_4-P) in the epilimnetic waters of this unproductive bog lake is greater than the average phosphorus content of 479 neighboring Wisconsin and Michigan lakes (23 $\mu g\ l^{-1}$ P-P) (Juday and Birge 1931). This phosphorus concentration places it among lakes of the world classified as eutrophic according to the classification scheme of Vollenweider (1968).

The organic phosphorus fraction (FUP) represents nearly 55% of the total phosphorus in North Gate Lake and 100% of the filterable phosphorus. Thus, the production of this fraction and its use by the biota is pivotal to the understanding of the impact of phosphorus availability upon the primary production of bog lakes. Little is known of the availability of FUP to photosynthetic organisms and of its rate of turnover (recycling) in natural lake water. Data on the physiological availability of the FUP pool in these lakes is of importance to the understanding of phosphorus dynamics not only in this lake but also in other freshwater and marine systems (Vollenweider 1968).

II. Nutrient Cycling

Phosphorus concentrations in natural bodies of water are often linked to the productivity of the phytoplankton (Rigler 1964). Atkins (1923) and Harvey (1940) asserted that phosphorus supply was the limiting factor in the growth of algae or phytoplankton. Dissolved

inorganic phosphate (DIP) was long assumed to be the only form of phosphorus assimilated by the plankton. Its importance to growth was soon recognized because phosphorus is concentrated from the water by plankton to a greater extent ($1 \times 10^7 - 1 \times 10^8$) than any element required for growth (Vallentyne 1974; Armstrong 1965). It has also been noted that on many occasions the amount of inorganic phosphate did not decrease with an increase in the phytoplankton population and on some occasions actually increased (Juday and Birge 1931; Rigler 1964). Through fertilization experiments it has been found that virtually all of the nutrients added to the epilimnion disappear within a few days (Phillips 1964). Waters (1957) and Koenings and Hooper (1973), however, noted that the phosphorus content of the epilimnion increased upon addition of lime. In addition, it has been shown that the stores of soluble phosphate measured in the open water trophogenic zones of lakes and oceans are inadequate to provide the phosphate requirements of the phytoplankton for any extended period of time (Hutchinson 1941; Juday et al. 1928; Redfield et al. 1937). These lake studies were refined by the radiophosphate (^{32}P) experiments of Rigler (1956), Hayes et al. (1952) and Coffin et al. (1949) which demonstrated the natural flux of phosphorus within the epilimnion.

Thus, from several sources of evidence it became apparent that the phosphate content of the open water is not a static entity but is in constant contact with zones of nutrient resupply and removal. Such a dynamic model of the phosphorus cycle focused attention upon mechanisms responsible for the regeneration of the phosphate phosphorus pool within the trophogenic zones of lakes and oceans.

Mechanisms of resupply or removal of inorganic phosphate have previously been identified as being (1) the exchange with the littoral zone of lakes (Hutchinson and Bowen 1950; Hayes et al. 1952; Rigler 1956; Chamberlain 1968; Confer 1972), (2) regeneration from the hypolimnion and its sediments (Mortimer 1941; 1942; Harrison et al. 1972; Fitzgerald 1970), (3) bacterial regeneration (Watt and Hayes 1963; Grill and Richards 1964; Hayes and Phillips 1958), (4) algal autolysis (Harvey 1963; Golterman 1973), and (5) zooplankton fecal material (Ketchum 1962; Corner 1973; Rigler 1964; Pomeroy et al. 1963).

These studies indicate the phosphate exists in a state of dynamic equilibrium between the particulate and dissolved states. This was shown by Rigler (1956) who found a turnover time (the time it takes for the phosphorus in the dissolved phase to replace itself) of 5 min for a bog lake and that the range of equivalent turnover times in the summer plankton of a variety of lakes was from less than 1 min to 7.5 min. In marine waters Watt and Hayes (1963) found a turnover time of 1.5 days for dissolved phosphate. Recently, Sabetich (1975) summarized the turnover times of several compartments of marine and freshwater systems. From this summary it is apparent that phosphate-phosphorus is not only concentrated, but is also recycled rapidly through the water and its biota.

III. Filterable Unreactive Phosphorus (FUP)

It became apparent during the analysis of phosphorus in lakes and oceans that a sizable pool of filterable phosphorus exists which requires acid digestion before it (FUP) reacts as inorganic phosphate. This fraction was designated soluble unreactive phosphorus (SUP) by

Strickland and Parsons (1972) but is often designated as dissolved organic phosphorus (DOP). Seasonal and diel peaks were observed in FUP in fresh and marine waters (Overbeck 1962; Soeder et al. 1971; Watt and Hayes 1963; Harris 1957; Harvey 1963; Reichardt 1971; Minear 1972) which indicated recycling (i.e., utilization and regeneration). Hutchinson and Bowen (1950) found that the phytoplankton quickly took up $DI^{32}P$ (inorganic) rapidly and excreted the tracer in a less assimilatable (organic) form. They considered the radiophosphorus to be in equilibrium with sestonic phosphorus, the less assimilatable (organic) form and ionic phosphate.

In unpolluted marine systems (Johannes 1964; Redfield et al. 1937; Watt and Hayes 1963; Harvey 1963; Armstrong 1965) and in freshwater systems (Juday and Birge 1931; Hutchinson 1941; Rigler 1964; Tucker 1957; Overbeck 1962), the FUP fraction often dominates the steady state distribution of the total phosphorus. In the trophogenic waters, FUP almost always exceeds filterable reactive phosphorus (FRP) (FRP is used as a measure of inorganic phosphate) and can exceed the phosphorus found in particulate form (Tucker 1957; Redfield et al. 1937; Juday and Birge 1931). Particulate phosphorus and FUP often decrease with increasing depth in the tropholytic zone as FRP increases (Hutchinson 1957; Redfield et al. 1937). Thus, FUP production was very early attributed to the phytoplankton (Redfield et al. 1937; Harvey 1963; Holm-Hansen 1966; Blanchard 1975). A description of the typical relationship between FRP, FUP, and particulate phosphorus (Part. P) in the sea is provided by Holm-Hansen et al. (1966). It was found that both Part. P and FUP were highest in the epilimnion and decreased in importance with depth. FRP, however, showed the

reverse trend, i.e., increasing in importance with depth and with a minimum concentration in the epilimnion. Of considerable interest is the fact that these authors found that dissolved organic carbon and particulate carbon followed the same trend as did their phosphorus counterparts. However, Rigler (1964) showed that the absolute amounts of FUP in natural waters varied with the procedures used to separate the particulate organic fraction from the FUP fraction but that FUP always exceeded inorganic phosphorus. The observations of Kuenzler and Ketchum (1962) and Rigler (1968) indicate that the chemical procedure used to estimate inorganic phosphate overestimates the abundance of this fraction 10 to 100 times. Thus, for example, instead of an observed ratio of filterable organic to inorganic phosphorus of 4.6:1, it may be closer to 500:1 (Rigler 1966).

Stable chemical analyses have suggested the importance of phytoplankton in the production of FUP and the potential importance of this fraction in the phosphorus cycle. Major emphasis has been placed on algal decomposition by bacteria and/or autolysis in the process of production of FUP (Grill and Richards 1964; Watt and Hayes 1963; Hayes and Phillips 1958; Phillips 1964; Golterman 1973). However, Johannes (1964) showed that neither dead nor living bacteria constitute significant sources of FUP in the sea and found that animals release FUP and FRP at lower rates when dead than when alive. He also found that when marine bacteria assimilate FUP, they regenerate very little FRP, but diatoms on the other hand can excrete FUP before they reach a stationary phase of growth, i.e., still in a completely viable state. Johannes attributed the difference in his results

compared to those of other workers to the methods others used in killing algae (chloroform, heat, ultraviolet light).

Studies on the rate of mineralization of algae in the dark (with bacteria present) (Grill and Richards 1964; Goldberg et al. 1951) suggest that significant decomposition does not take place until 2-3 weeks after light is excluded. A critical look at the data presented indicates that silica levels reached pre-darkness levels on day 13. During this time silica was taken out of solution, indicating growth of the diatom culture. In addition, FRP levels dropped during this period while Part. P levels increased. It wasn't until day 14 that all the data (in particular an increase in FRP) indicated that the diatoms were undergoing decomposition. Goldberg et al. (1951) suggested that these results might be explained by the observation that the diatom frustule does not open until the decay process is nearly complete. At any rate, during the experimental period (414 days), FUP levels were never more than 50% of the levels at the start of the experiment when the diatom cultures were in the light. Thus, to say that FUP pools are mainly the result of autolysis and especially bacterial decomposition (Watt and Hayes 1963; Rigler 1956) does not fit all observations. Further evidence for this conclusion comes from the work of Golterman (1975) who observed that after a few hours of autolysis, 50%, and after a few days, 70-80% of the particulate phosphorus returned into solution as mineralized phosphate. In addition, Watt and Hayes (1963) cite the work of Matsue who found that up to 98% of the organic cell phosphorus of Skeletonema is released to the water as orthophosphate within 23 hours.

Johannes (1964) observed secretion of FUP by algal cultures in a nonstationary growth phase. This was also reported by Kuenzler and Ketchum 1962; Kuenzler 1967; Antia et al. 1963; Strickland and Solorizano 1966. Ketchum and Corwin (1965) point out that the filterable organic phosphorus appearing in their study may have come from excretion by phytoplankton. Strickland and Solorizano (1966) found that appreciable quantities of hydrolyzable ester material were excreted after algae had used up all the inorganic phosphate in the medium. Kuenzler (1970) noted that a significant amount of FUP was excreted in cultures of several species of healthy marine algae during and after phosphate depletion. Aaronson (1971) reported the production of FUP from viable cells of a cultured phytoflagellate. Thus, there is abundant evidence that a variety of algae can produce FUP without autolysis or bacterial decomposition. Autolysis and bacterial regeneration are important in nutrient regeneration and perhaps also in FUP production, however, these processes cannot now be considered the sole factors responsible for the production of FUP.

IV. Enzymes Acting on FUP

Dissolved or membrane-bound enzymes in contact with the open water can degrade FUP into an organic moeity and inorganic phosphate. These phosphomonoesterases (PMase) have been detected in both the marine and freshwater systems. Estimates of the stability of enzymes are: 80% retained activity after 20 days (Berman 1970); a half-life of 7 hours (Reichardt 1971); half-life of 3 days at 18° C (Reichardt et al. 1967). Extracellular PMases have been shown to exist in sea water (Strickland and Solorizano 1966; Yentsch et al. 1972) and in freshwater (Berman 1970; Berman et al. 1972; Reichardt

1971; Reichardt et al. 1967). Specific sources of this enzyme are Euglena gracilus (Price 1962), the benthic gamarid amphopod Lembos intermedius (Johannes 1964), the crustacean Daphnia magna (Rigler 1961), the bacterium E. coli (Garen and Levinthal 1960), several species of phytoplankton (Kuenzler 1970; Kuenzler and Perras 1965; Aaronson 1971; Fitzgerald and Nelson 1966), and the ciliate protozoan Tetrahymena geleii (Elliott and Hunter 1951). Hooper and Elliott (1953) showed that this benthic ciliate can release inorganic phosphate from organic phosphorus compounds. Thus, cellular enzymes produced by aquatic organisms may be ecologically important as a source of new inorganic phosphate for growth of algae once the phosphate in the water is low or exhausted.

V. Organophosphorus Utilization

The ability of the phytoplankton or algae to use the phosphorus of organic phosphorus compounds has been established. Chu (1946) clearly demonstrated the ability of three diatoms in axenic culture to use the phosphate in phytic acid as a source of phosphorus for growth. Harvey (1953) found that a culture of Nitzechia closterium could use 10-12 µg of the phosphorus from phytic acid and 8.5 µg of inorganic phosphate during a 3-hr incubation period. He also found utilization of glycerophosphorus by this species but noted that this ability may have been due to the presence of extracellular hydrolytic enzymes present in phosphorus-deficient cells. Phosphorus-replete cells did not use this compound. Fogg and Miller (1958), Provasoli (1958), and Kuenzler (1970) concluded that several phosphate esters could be utilized as readily as inorganic phosphate by several species

of phytoplankton with or without bacteria present. Compounds utilized include glycerophosphate, phytic acid, yeast nucleic acid, adenosene monophosphate, adenylic acid, cytidylic acid, hexose-1, 6-diphosphate, riboflavin monophosphate, glucose-6-phosphate (Johannes 1964; Kuenzler 1965), deoxyribonucleic acid, phosphoserine and phosphoryl choline (Perry 1976). Anacystis nidulans used glycerol-2-phosphate, pyrophosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1, 6-diphosphate and 3-phosphoglyceric acid. In addition, Aphanizomenon sp. used the phosphorus of phytin (Reichardt 1971). Thus, esterified forms of phosphorus of a variety of compounds and arising from degradation by a variety of hydrolytic enzymes can be used by algae as a phosphorus source.

In culture experiments organic phosphorus ($FU^{32}P$) of recent metabolic origin has been demonstrated to be partially available to the phytoplankton. Utilization by algae of the $FU^{32}P$ produced by algae in stationary phase varied from 4 to 97% depending on the species tested (Kuenzler 1970). Johannes (1964), however, found only 40% reutilization by algae and Watt and Hayes (1963) found that in a 7 day period bacteria could reutilize only 23% of the $FU^{32}P$ concentrated from sea water. Yet Johannes (1964) also reported that bacteria could use up to 92% of the $FU^{32}P$ produced by a benthic amphipod.

Kuenzler (1965; 1970) showed that the ability of various algae to use the phosphorus in organic linkages was related to the ability of the various species of plankton to produce an active phosphatase. The algal species tested varied in this ability and thus reassimilated different amounts of FUP from culture experiments. However, the

presence of external PMase was necessary since cleavage of inorganic phosphate from the organic moeity takes place in the media with subsequent absorption of the phosphate with the carbon skeleton remaining in the media.

It is of interest to note that the production of PMase often increases when phytoplankton, bacteria, and fungi reach the lag phase of growth in culture and when phosphate becomes deficient. Upon addition of phosphate to the culture, enzyme activity falls to the initial value (Kuenzler 1970; Kuenzler and Perras 1965; Price 1962; Torriani 1960; Blum 1965). Fitzgerald and Nelson (1966) found that when green algae, blue-green algae, and diatoms are grown with surplus phosphate and with low levels of phosphate, that they store orthophosphate in proportion to that present in the medium, but the PMase activity is inversely related to the amount of stored phosphate. This was later confirmed by Rhee (1973) who further found that surplus phosphate was quantitatively equivalent to the pool of stored polyphosphate which in turn was inversely related to the activity of an alkaline PMase. Thus, the enzyme was produced when the cells became DIP deficient, and the presence of excess DIP repressed the de novo synthesis of this enzyme. The ecological significance of this phenomenon is that some algae, bacteria, and fungi, when faced with nutrient-deficient conditions, produce a hydrolytic enzyme that is external to the cell membrane. Quantitatively, this production may constitute up to 6% of total cell protein. This enzyme enables the cell to obtain needed phosphate from FUP compounds in the media (Kuenzler 1970). The ability to form the external enzyme is not universal among algae (Kuenzler 1970) so this phenomenon may offer

a competitive advantage to the species capable of producing a PMase.

VI. Filterable Organic Phosphorus

Identification of organic compounds making up FUP has been limited, however, compounds have been identified from both laboratory algal cultures and from naturally existing FUP. FUP isolated from algal cultures has been identified by thin layer chromatography and consisted of orthophosphate (DIP), and two sugar phosphates; three remaining spots were not identified (Krause and Stegman 1968). Phillips (1964) with paper chromatography tentatively identified six compounds which included: three nucleotides or polynucleotides and three phosphorylated carbohydrates. Kuenzler (1967; 1970) found seven to nine substances with paper chromatography. Strickland and Solorizano (1966) report that considerable dissolved ester-hydrolyzable material was produced by Dunaliella tertiolecta in culture experiments. These are the same class of low molecular weight substances that have been shown to be utilized by algae as a source of phosphorus through extracellular hydrolytic enzymes. In addition to these low molecular weight compounds, Aaronson (1971) presented evidence supporting the existence of a dissolved nucleic acid-like material in algal culture media.

Herbes et al. (1975) indicates the presence of phytic acid in natural lake water. This appeared at highest concentrations during the spring and declined through the early summer. The seasonal pattern suggests that this form of FUP may be of allochthonous origin. Minear (1972) identified nucleic acid as a form of FUP and estimated that it

contained 40-60% of the high molecular weight material. The high molecular weight fraction made up only 5-14% of natural lake FUP. So of natural lake FUP, nucleic acid equalled 1.28-2.03% of total phosphorus or a maximum of from 3.1 to 6.5% of natural lake water FUP. These results must be evaluated by taking into account the concentration procedure used. The procedures gave erratic results, perhaps were not specific for recovery of FUP. There may have been further loss by "extensive" hydrolysis of FUP in Minear's Sephadex columns. The latter point seems to be especially important to consider in light of Lean's work (1973) with phosphorus kinetics derived solely from Sephadex studies. Koenings and Hooper (1973) identified nucleotides and polyphosphates in a small acid bog lake. However, the amount of FUP accounted for was usually less than 50% of the FUP found.

Further characterization of natural FUP has been done with bacterial enzymes. The addition of bacterial PMase to a sample of water containing natural FUP results in a slow and incomplete degradation of FUP at least as compared to PMase action on phosphate esters such as glucose-6-phosphate. Kuenzler et al. (1963) found that only 17% of natural "bound" FUP was released and Berman (1969; 1970) found that only 40-60% of the total initial phosphorus present in unfiltered lake water was released as DIP within 4.5 days. These results included the enzyme-catalyzed release of DIP from organic phosphorus compounds in the dead plankton, not just natural FUP compounds. The experiments of Berman should be regarded with caution in light of the experiments of Jones (1963) that demonstrated that the addition of chloroform to unfiltered lake water resulted in the release of DIP from particulate organic material. The rate of release

in Berman's experiments was only 2.6-8% of the rate with p-nitrophenyl phosphate (PNPP) as a substrate. Jones (1972) found that release of DIP from naturally occurring phosphorus compounds was 15-100 times slower than that for PNPP. Rigler (1961) found that Ottawa River water PMase could hydrolyze glycerophosphate, but that naturally occurring organic phosphorus compounds were unaffected. Strickland and Solorizano (1966) failed to find appreciable FUP in open sea water that was enzyme hydrolyzable, but found a small quantity in near-shore ocean water. Herbes et al. (1975) found no FUP hydrolyzable by PMase in two lakes, and found that only 9% of natural lake FUP was utilized by the biota of the lake in seven days. Perry (1976) concluded that the pool of FUP in the North Pacific was not turning over, and Rigler (1964) found no evidence for a seasonal cycle of FUP in several north temperate lakes. Kuenzler et al. (1963) reported that a bulk of the "bound" phosphorus in sea water was not directly assimilatable by a diatom or rapidly hydrolyzed extracellularly. Berman and Moses (1972) found free dissolved phosphatases in filtered samples of fish-pond water, however, neither these enzymes nor added phosphatases released significant amounts of DIP from the FUP.

From the evidence just presented, it can be concluded that most of the natural FUP in lake waters is not hydrolyzed by PMases. Compounds identified as part of the FU^{32}P isolated from algal culture experiments can be hydrolyzed by PMases, and since these same compounds can be used as sources of phosphorus by algae, most naturally occurring FUP pools may not be present as the same compounds tentatively identified as algal excretion products in culture experiments. However, this conclusion may be premature since it is not known if natural FUP

compounds are in an enzyme-hydrolyzable form. This is important since organic phosphorus compounds complexed with metal ions are not susceptible to enzymatic degradation.

VII. Abiotic Organic Phosphate Formation

Abiotic formation of filterable (colloidal) organic phosphorus is thought to occur in natural lake waters (Golterman 1973). This can be measured either as FRP or FUP depending upon the form of phosphorus held on a humic-iron complex. Chamberlain and Shapiro (1973) suggest that such complexes may be responsible for the discrepancies found between estimates of DIP and FRP. Lehmusluoto and Ryhanen (1972) report Sephadex separation of two fractions of humic material and that the higher weight material contains iron and phosphorus. The formation of a colloidal complex between iron and organic matter and phosphate was demonstrated in natural lake water by Koenings and Hooper (1976). This finding is of significance since recent studies on the form of FUP in natural lake waters have found a low molecular weight fraction and a high molecular weight fraction (Herbes et al. 1975; Minear 1972; Lean 1973). A COM-metal-phosphate complex would go far to explain differences between both the chemical analysis and algal utilization of DIP and FRP. Colloidal complex formation could also account for the slow enzyme hydrolysis rate of natural FUP compared to the rate of hydrolysis of exogenous phosphate esters, e.g., PNPP, recently added to filtered lake water. It may also account for the existence of FUP esters in natural lake waters that are hydrolyzable by high concentrations of bacterial PMase yet are not attacked by low levels of natural dissolved enzymes, i.e., changes

caused by assay conditions. Thus, specific phosphorus esters may be protected from decomposition by virtue of metal-organic acid complexation, e.g., phytic acid is known to form complexes like phosphate and these salts are resistant to phytase (Hesse 1973). In addition, Scharpf (1973) reports that organic phosphates, like inorganic phosphates, have the ability to form complexes, chelates, and insoluble salts with many metal ions. Thus, recently produced $FU^{32}P$ from algal cultures may be more utilizable by algae than natural lake water $FU^{31}P$ since the latter may exist entirely in a complexed form.

Of particular importance to this study is that bog lakes contain very high levels of colloidal organic acids which are associated with high levels of dissolved iron (Shapiro 1966; Hutchinson 1957; Juday et al. 1938; Koenings and Hooper 1976) and possess higher levels of dissolved organic phosphorus (Juday and Birge 1931). Organic phosphorus in peats and humus is believed to be highly resistant to microbial degradation (Hesse 1973). This may be due to complex formation, but little is known about the matter.

SECTION I

The Chemical and Biological Limnology of North Gate Lake

Formation and Characteristics of Bog Lakes

A bog lake ecosystem is intermediate between typical terrestrial and lake systems in many respects. Bog lakes are surrounded by vegetation which causes the partial filling in of the old lake basin, so that the lake sediments both profundal and littoral are always found below the peat layers of the bog.

During the process of filling, the lake which is normally supplied with mineral nutrients from precipitation and surrounding soils is slowly cut off from the latter supply by selective filling with organic sediments. This is especially rapid nearer the lake edges, where conditions of higher productivity prevail. Continued autochthonous deposition of organic matter impedes the flow of water, and isolates the central parts of the open lake area from the supply of mineral nutrients. Deposition of organic matter continues but there is little mineralization. This leads to a depletion of mineral nutrients and a resulting decrease in productivity. However, the concentration of organic matter is high because the rate of decomposition is only about 1/3 that of other ecosystems (Malmer 1975).

Isolation of the drainage into the bog allows only a surficial flow of water. Although this flow may be rapid (>0.04 cm sec^{-1}), it is limited to the upper 25 cm through the horizon of living Sphagnum (Boelter 1970). The water level and especially the supply of mineral nutrients is dependent directly or indirectly on precipitation since nutrients (multivalent cations) are stripped from the surface flow of water by cation exchange reactions from living Sphagnum plants (Clymo

1967). This reaction along with the fact that a great many metallic cations are fixed on the humus colloids (Malmer 1963; Koenings and Hooper 1976), reduces the sum of cations below that of the sum of anions. The low pH (4-5) reduces the formation of bicarbonate ions, thus the only significant anions are often chloride and sulfate. These reactions account for the low conductivities observed in such lake systems.

Fertilization experiments of the bog mat indicate that plant-available phosphorus limits productivity. Thus the internal recycling of the minerals between green parts and storage organs may be important for the maintenance of plant growth (Malmer 1975). The bog mat shows a paradox of a very low productivity coupled with a high total amount of nutrients. This suggests a shortage of plant-available nutrients which must arise because of a slow remineralization process.

The phorphorus of the Sphagnum beds surrounding the open lake water is concentrated in the surface layers. This concentration zone is seen for only one other element, iron. Studies by Stewart and Robertson (1970) on the depth distribution of elements in peat support these observations. Thus, iron and phosphorus concentrations are highest in the surface layer of Sphagnum and this is the only strata likely to be involved in the lakeward migration of mat interstitial water. Such flow or bulk movement arises from rainfall. Thus, the Sphagnum mat is the principal source (besides direct rainfall on the open water) of phosphorus for the lake.

Chemical Cycles and Biological Communities

Methods

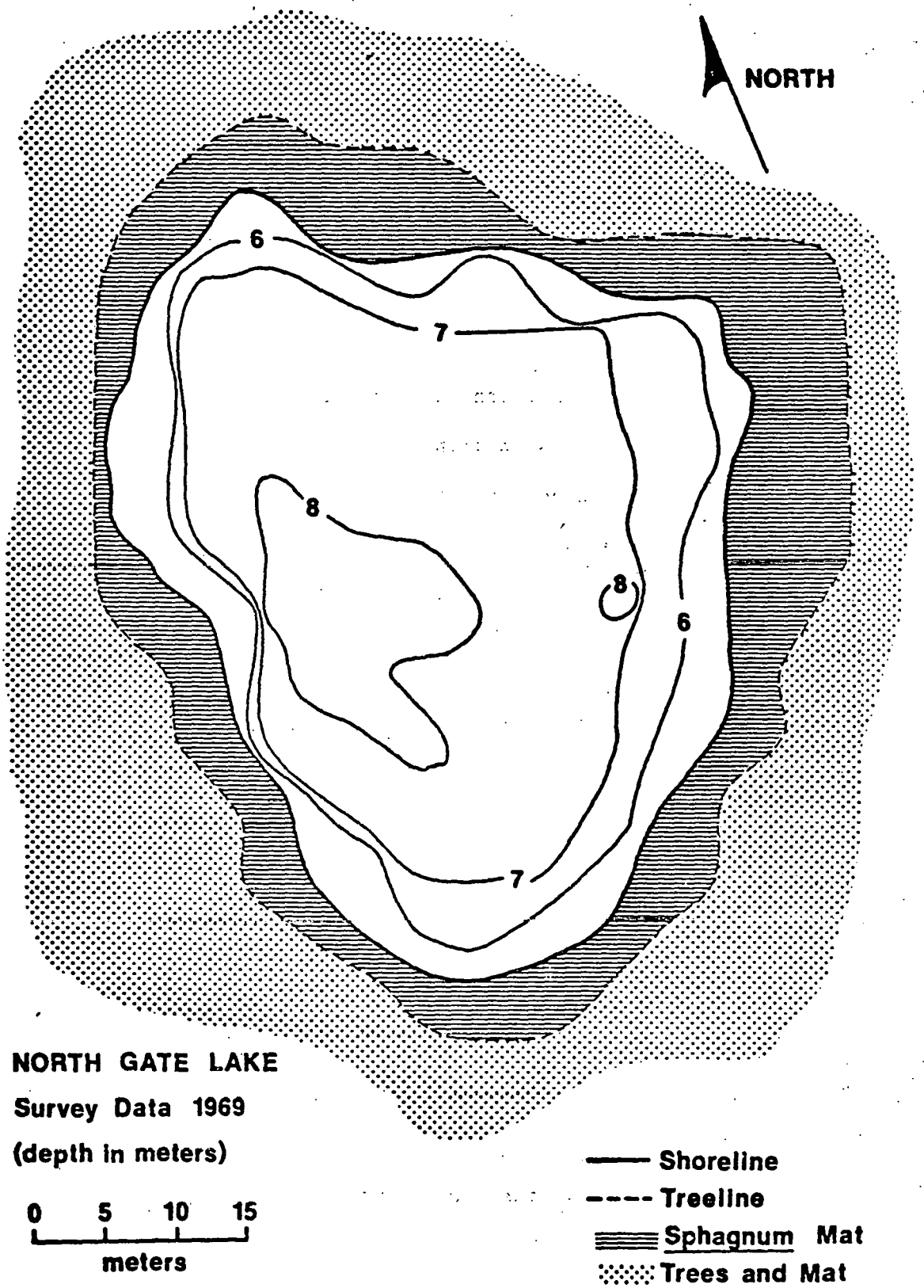
Study Area

North Gate Lake is a small, highly stained (tea color) meromictic lake located within sector 37 of T45N, R42W, Gogebic County in the Upper Peninsula of Michigan (Figure 1). The lake is one of numerous bog lakes on a 2,225 ha reserve owned by The University of Notre Dame. The open water is surrounded by a Sphagnum-dominated mat which extends into a black spruce (Picea mariana) forest. Additional mat vegetation consists of Carex, Chamaedaphne, Vaccinium, Eriophorum, and Drosera. The lake has a surface area of approximately 0.4 ha, dimensions of approximately 50 m long by 40 m wide and a maximum depth of 8 m. North Gate Lake lacks a true littoral zone. The bottom falls to 6 m at the edge of the Sphanum mat. Two-thirds of the lake bottom is enclosed by the 7 m contour and only a small area exceeds 8 m in depth (Figure 1). From physical and chemical properties, the lake can be split into four zones. From 0 m to 1.0-1.5 m represents the euphotic zone with a Secchi disk depth of approximately 1 m. The thermocline or metalimnion is at 2 m. Below this to approximately 6.5 m is the hypolimnion with 6.5 m being the beginning of the permanent zone of nonmixing or monimolimnion which extends to 8 m.

Stable Chemistry

Routine chemical analyses were taken during the summer of 1974 to determine background limnological characteristics of the open water and surrounding Sphagnum mat. In 1973 a study lasting 30 days was

Figure 1. Map of North Gate Lake and surrounding vegetation.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Trees and Mat

Figure 1.

conducted comparing the open water at 3 m from the edge of the mat with the open water at the center of the lake. Samples of conductivity, pH, alkalinity, dissolved oxygen, hydrogen sulphide were taken every day at 6 depths (1 m intervals). Those data showed that the chemistries at the two stations were very similar and more importantly that changes occurring at one station were mirrored by the changes at the second station. Thus, the center station was chosen for data collection in 1974-1975 and results from this one station will be considered to be representative of the open lake water as a whole.

Mat interstitial water and the mat-open water interface were sampled at ten sites through the use of lysimeters installed in 1973. Samples from the lysimeters can be considered to be from "undisturbed" sites since they had a full year to equilibrate with the surrounding interstitial waters before use. These lysimeters were constructed entirely of plastic and glass.

Physical-chemical determinations were made every four days at the same time each day for four months. Those tests included total, filterable (0.45 μ membrane filtered) total, filterable reactive phosphorus, dissolved oxygen and hydrogen sulfide. All tests were performed according to Strickland and Parsons (1972).

Alkalinity values were obtained according to the low level method (APHA 1975) using a pH meter for end point determination. A Coleman pH meter was used with glass electrodes for both the alkalinity and pH determinations. Conductivity measurements were made with a Wheatstone bridge and results were corrected to 18°C. Temperature was determined with a resistance thermometer. Samples for dissolved oxygen were analyzed at site. Phosphorus, H_2S, organic carbon, pH, alkalinity, and

conductivity were analyzed within 20 minutes after collection at the Notre Dame laboratory or after storage at 4°C for 2-3 hours. The depths sampled for pH, alkalinity, temperature, dissolved oxygen, hydrogen sulphide were usually every meter. Phosphorus, organic carbon, and conductivity were sampled at representative depths: surface (epilimnion), 2 m (thermocline), 5 m (hypolimnion), and 7.5 m (monimolimnion). Samples were analyzed in duplicate with standards and blanks run where appropriate.

Results and Discussion

Biological Communities

Before discussing the cycle of phosphorus in North Gate Lake, a general description of the community of organisms at the various strata must be considered. Biological populations are responsible to a great extent for the cycling of elements in natural waters and through biochemical reactions determine or at least influence the chemical composition of the aquatic environment.

Communities of organisms differ in the various strata primarily because of physical factors. Photosynthesis is limited to the euphotic zone or the Secchi disk depth (Figure 2). Within this zone (0.0 m - 1.0 m) numerical distribution of phytoplankton parallels the particulate ^{14}C activity. Thus, this zone contains populations of active, autotrophic phytoplankton. Below this consistently oxygenated euphotic zone, very little ^{14}C is found in the particulate phase in either light or dark incubated samples.

The phytoplankton in soft water meromictic lakes is often so poor that the bacterial production of organic carbon by chemosynthetic

Figure 2. The depth dependent distribution of biological communities and physical factors in North Gate Lake.

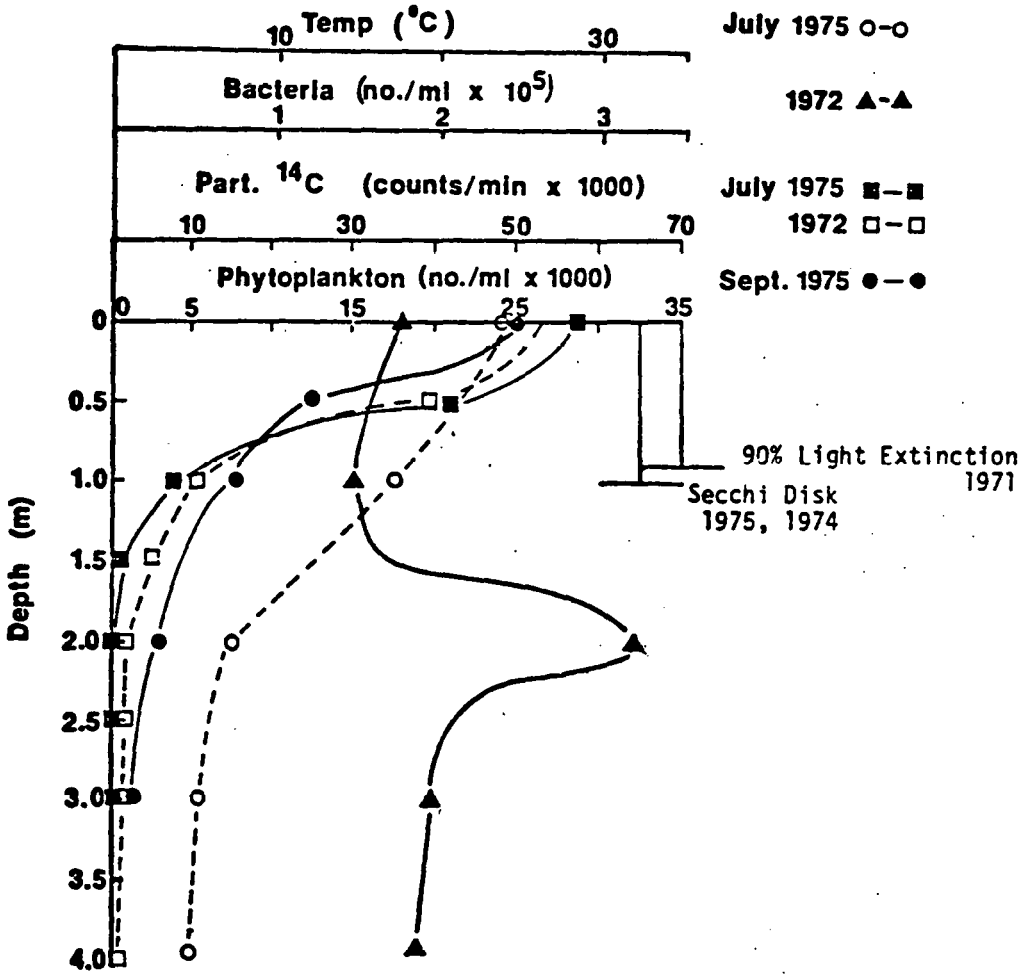

Temp (°C)
10
30
July 1975 o-o
Bacteria (no./ml x 10^5)
1
2
3
1972 ▲-▲
Part. 14C (counts/min x 1000)
July 1975 ■-■
10
30
50
70
1972 □-□
Phytoplankton (no./ml x 1000)
Sept. 1975 ●-●
0
5
15
25
35
Depth (m)
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
90% Light Extinction
1971
Secchi Disk
1975, 1974

Figure 2.

reactions may equal that of the photosynthetically fixed carbon (Sorokin 1970). Montroy (1974) described the distribution of bacteria in North Gate lake and found a maximum of bacteria at 2 m. This same lens was qualitatively followed at this depth from 1973 to 1975. Since the bacterial community occurs at the junction of anaerobic and aerobic zones, both reduced (energy-rich) substrates and dissolved oxygen are available for chemosynthetic metabolism. However, since little dark $^{14}CO_2$ incorporation was observed in North Gate Lake, chemosynthetic reactions appear to be minimal. The bacterial lens showed minimal <3% assimilation of ^{14}C instead of 30-40% characteristic of chemosynthetic reactions (Sorokin 1972). Thus, the bacteria appear to be heterotrophs which utilize as substrates senescent phytoplankton which sink from the euphotic zone and concentrate at the top of the thermocline. This probably accounts for the large amount of detritus found in the 2 m strata of the lake (Vogel 1976).

A general picture of the biota of North Gate Lake indicates an active population of phytoplankton at 0.0 m, 0.5 m, and marginally functional at 1.0 m. A residual population of photosynthetically inactive plankton exists at 1.5 m. Below this zone populations of bacteria increase concentrating in a lens at the top of the thermocline that extends from 2.0 m to 2.5 m. Thus, 0 m, 0.5 m, and 1.0 m appear to be dominated by autotrophic phytoplankton, and 1.5 m, 2.0 m and 2.5 m by heterotrophic bacteria.

Chemical Cycles

Dissolved oxygen

The euphotic zone (surface to 1.0 m) remained consistently

aerobic throughout the summer; however, the 1 m stratum periodically fell below 1 mg l^{-1} of dissolved oxygen (Figure 3). The surface layer (6 cm) averaged 4-6 mg l^{-1} yet was only 50-70% saturated. At 1 m oxygen fell to <1-4 mg l^{-1} (6-40% saturation) but was renewed aperiodically by physical mixing with the surface waters during cold windy weather. During those mixing periods, oxygen concentrations at the surface fell as it received the lower oxygenated water from 1 m. After mixing, the oxygen levels increased at the surface, and fell at 1 m. This cycle occurred several times during the summer which enabled 1 m to remain aerobic.

Thus, the oxygen demand of the organic matter stain reduced oxygen in the epilimnion to $\geq$ 30% undersaturation. Since oxygen concentration was always inversely related to temperature, it is apparent that physical factors were primarily responsible for regeneration of oxygen levels. Biotic renewal of oxygen through photosynthesis was of minor importance.

Hydrogen sulphide

Strata below 1.0 m were always anaerobic and contained hydrogen sulfide (H_2S). Levels of H_2S increase to over 2.0 mg l^{-1} at 2.0 m at the end of the summer (Figure 3). H_2S increased with depth reaching a maximum of 2.7 mg l^{-1} in the monimolimnion. Since the pH of the hypolimnion is approximately 5.5, most of the H_2S was in the form of the toxic undissociated acid. However, the concentration of H_2S in the hypolimnion was not sufficient to precipitate ferrous iron as FeS. In the monimolimnion H_2S values (1 mg l^{-1}) were compatible with a ferrous iron concentration of only 485 µg l^{-1} at pH 6.14 (Stumm and Lee 1960).

Figure 3. Depth profiles of temperature (a), oxygen (b), and hydrogen sulphide (c) in North Gate Lake.

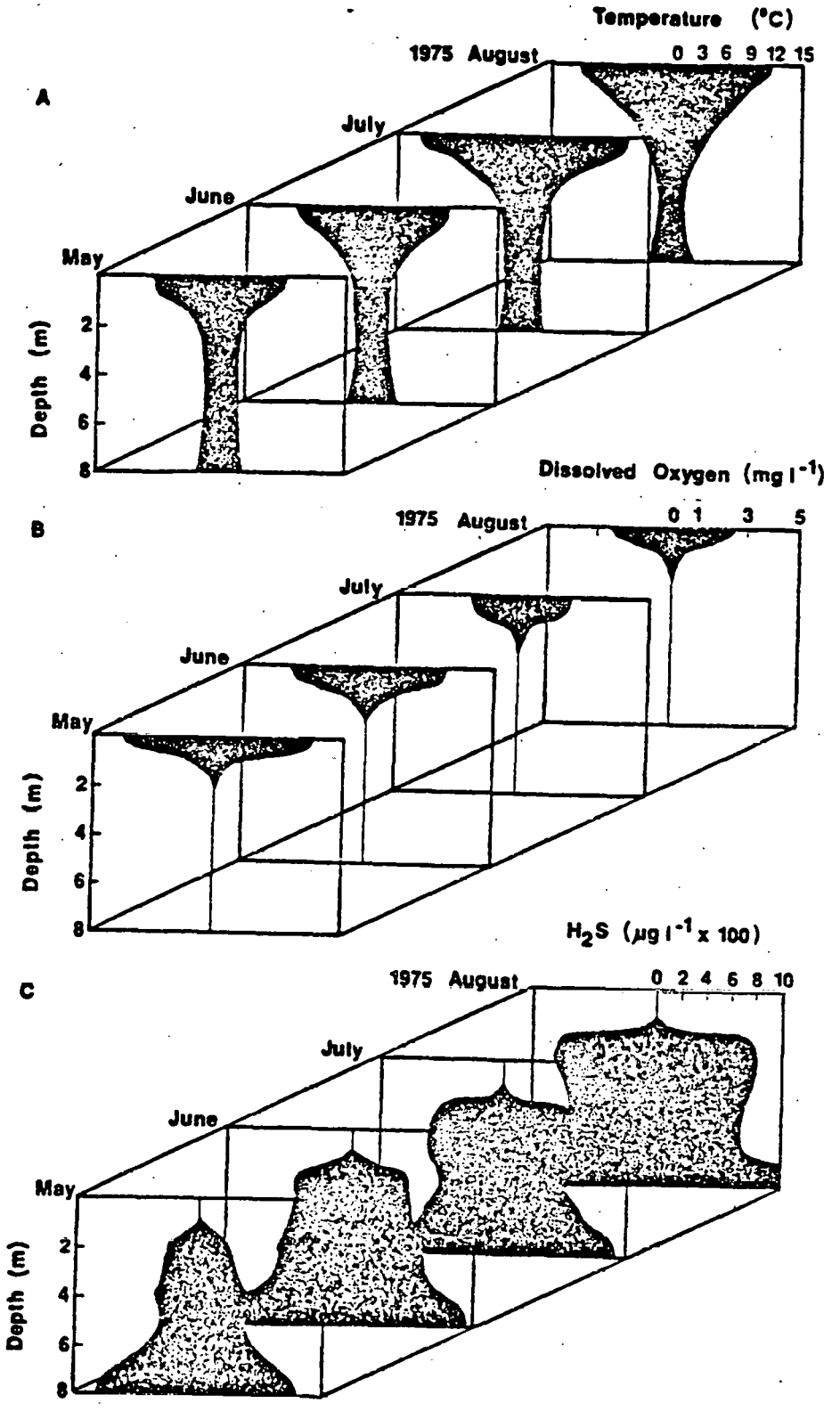

Temperature (°C)
A
1975 August
0 3 6 9 12 15
July
June
May
Depth (m)
2
4
6
8
Dissolved Oxygen (mg l-1)
B
1975 August
0 1 3 5
July
June
May
Depth (m)
2
4
6
8
H2S (µg l-1 x 100)
C
1975 August
0 2 4 6 8 10
July
June
May
Depth (m)
2
4
6
8

Figure 3.

However, levels of ferrous iron reached 1,055 μg l^{-1}. Similar nonequilibrium concentrations of ferrous iron were found by Juday and Birge (1933) in Lake Sabietch, Wisconsin.

Temperature

Temperature profiles departed from typical eutrophic lake profiles (Figure 3). During May temperature decreases linearly through the euphotic zone from 15°C at the surface to 4.70°C at 2 m. It had increased to 22.3°C at the surface, to 11.75°C at 2 m, and 6.90°C at 3 m by August. Thus, the strongly defined thermocline deepens during the summer heating period from 2 m to 3 m. The density differences responisble for the thermocline is imparted by temperature. The brown organic matter strongly absorbs solar radiation so that the lake warms rapidly. Thus, thermal stratification is quickly established after ice-out, especially if there is sunny calm weather. Little wind-generated mixing occurs because of the protection extended by the surrounding black spruce forest. Thus, the lake rarely mixes below a depth of 2 m.

The temperature reached a minimum value of 4.6°C at 4-5 m. Below this depth the water again warms to 4.9-5.0°C. This is probably due to heat accrued from the surrounding basin by heat exchange reactions involving water flowing through the Sphagnum mat down to the chemocline.

pH and alkalinity

The pH of the epilimnion ranged from 4.1-5.1 and average 4.5 (Figure 4). These low pH values reduced the amount of titratable alkalinity to less than 1 mg l^{-1} (as $CaCO_3$). Obviously, the only

Figure 4. Depth profiles of conductivity (a), alkalinity (b), and pH (c) in North Gate Lake.

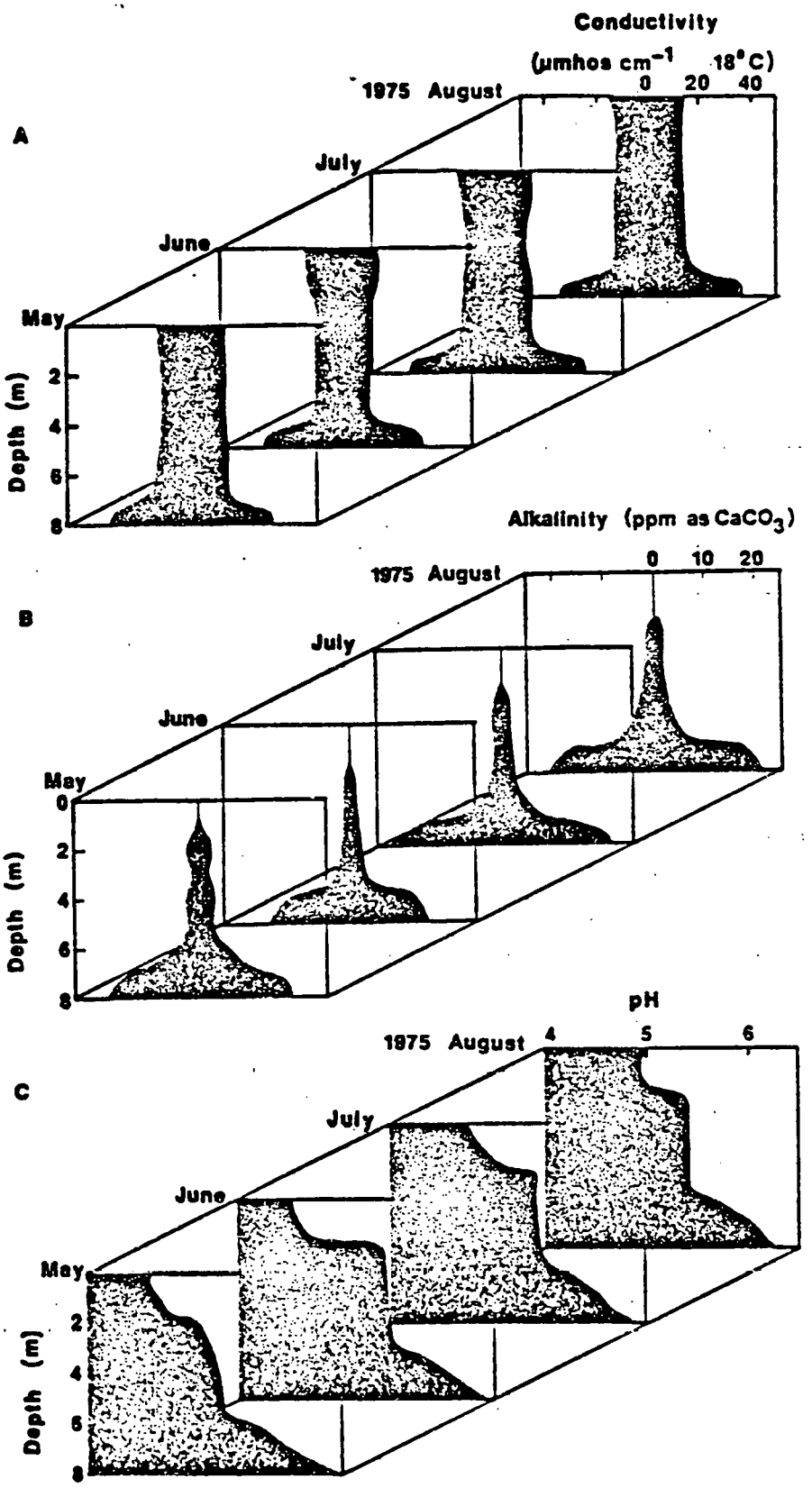

Conductivity
(μmhos cm⁻¹ 18°C)
A
1975 August
July
June
May
0 20 40
Depth (m)
2
4
6
8
Alkalinity (ppm as CaCO3)
B
1975 August
July
June
May
0 10 20
Depth (m)
0
2
4
6
8
pH
C
1975 August
July
June
May
4 5 6
Depth (m)
2
4
6
8

Figure 4.

available carbon in the trophogenic zone is free CO_2. This presents methodological problems in measuring the productivity because the addition of radioactive carbon ($H^{14}CO_3^-$) in presumptive tracer amounts may in fact add a significant amount of bound CO_2 to the system thereby disturbing the equilibrium ratios of carbon species and abridging one of the basic tenets of tracer methodology (Nygarrd 1968). At 2 m the pH rises to around 5.3 and the amount of bound CO_2 rises to 3.4 mg l^{-1} (as $CaCO_3$). Thus, the bicarbonate ion concentration rises under anaerobiosis. At 6.5-7.0 m the bicarbonate ion again increases to a maximum of 30-40 mg l^{-1} (as $CaCO_3$). Again there is an accompanying rise in pH to 6.14, suggesting that the formation of bicarbonate ion is responsible for the increase in pH.

Conductivity

Another aspect of considerable interest in bog lakes is the lack of electrolytes. The conductivity at 18°C of the lake averaged 25 µmhos cm^{-1}. Those values compare to 1-2 µmhos cm^{-1} for distilled water and 300 µmhos cm^{-1} for moderately hard water lakes and streams. This lack of nutrients is caused by the cation stripping action of the Sphagnum mat, and the complexing ability of the bog acids of the open water (Ruttner 1963; Clymo 1967; Malmer 1963; Koenings and Hooper 1976). In anaerobic strata at 2 m and 5 m the conductivity remains fairly constant, but in the monimolimnion it nearly triples, reaching a high of 71 µmhos cm^{-1} while averaging 62-67 µmhos cm^{-1} (Figure 4). This spectacular increase is, of course, due to the accumulation of salts at the bottom of this meromictic lake. This is consistent with the results of Koenings and Hooper (1976) who found high concentrations

of ionic (dialyzable) ferrous iron and phosphate phosphorus in the monimolimnion. These ions would contribute to the observed higher conductivity.

The euphotic-aphotic zone interface

The 2 m zone demarcates changes in several chemical factors in North Gate Lake. Alkalinity values rose from an undetectable value to above 3-4 mg l^{-1} (as $CaCO_3$), pH rose from 4.66 to 5.32, while the dissolved oxygen fell to zero. At 2 m there were also major changes in the amount and oxidation state of iron. The sharp changes in the chemical environment beginning at 2 m and below compared to the upper strata arose from the onset of anaerobic conditions.

Koenings (1976) and Koenings and Hooper (1976) demonstrated the ability of the organic matter of the lake to coordinate ferric iron and minimize the formation of ferric hydroxide [$Fe(OH)_3$] and thereby make this form of iron a minor fraction of the total iron in the aerobic zone. Thus, precipitation and reduction reactions involving $Fe(OH)_3$ are of minimal importance. Further evidence was offered by these authors that organically complexed ferric and ferrous iron dominate the iron species in aerobic zones, free ferrous iron increases with depth and with increasing time of anaerobiosis.

The chemical reactions of organic iron in combination with the CO_2 produced by bacterial metabolism in the lake may be responsible for the changes observed in the water chemistry of the anaerobic strata according to the following reactions. Organic matter coordinates ferric iron in aerobic strata which is then partially reduced by the COM to ferrous iron.

$$Fe^{+3} + COM_{(red.)} \rightarrow Fe^{+3} - COM_{(red.)} + Fe^{+2} - COM_{(oxid)} + CO_2 \quad (1)$$

Such a reaction produces an organic-Fe complex as a result of the initial binding of COM to ferric iron and accounts for both ferric and ferrous organic iron being present in the euphotic zone of North Gate Lake. The CO_2 produced contributes to the decreased pH of the surface waters.

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H_3O^+ + HCO_3^- \quad (2)$$

In anaerobic strata the now stable ferrous iron slowly goes into solution. This reaction may be inhibited in low-pH waters.

$$2COM\text{-}Fe^{+2} + 2CO_2 + 2H_2O \rightarrow Fe(HCO_3^-)_2 + 2COM - H_2 \quad (3)$$

This forms a solution of ferrous bicarbonate which accounts for the slight rise in alkalinity in the anaerobic strata and the increase in free ferrous iron (Koenings and Hooper 1976). The bicarbonate ion hydrolyzes readily to form the hydroxyl ion which increases the pH.

$$HCO_3^- + H_2O \rightarrow H_2CO_3 + OH^- \quad (4)$$

Thus, the abiotic reactions involving ferrous iron and COM together with biotic production of CO_2 may be responsible for the unusual situation of a rise in pH with depth in the anaerobic strata. The more typical reaction in stratified lakes is a lowering of pH due to an increase in respired CO_2.

The hypolimnetic-monimolimentic interface

The chemocline at 6.5-7.0 m is not apparently formed by either ectogenic (catastrophic) or biogenic (biological) processes but may be included in an expanded definition of Hutchinson's (1957) ectogenic

meromixis. The latter would include lakes whose monimolimnion is formed by dense water entering the surface of the lake and flowing to the bottom by density currents (Hooper and Koenings 1975). The chemical constituents of the surface layer of the Sphagnum mat are similar to those found in the monimolimnetic strata especially in regard to iron and phosphate. In addition, the warmer temperatures of this layer would have their origin in the 5.2°C temperature of the lower mat. Flow down the sides of the vertical mat would equilibrate with this temperature and warm the bottom water, and the high salt contributed by this flow maintains a positive density gradient. Kjensmo (1962) described two meromictic lakes in Norway that are similar to North Gate Lake in that they have extensive bogs in their drainage areas, and the lake water is in direct contact with Sphagnum. These lakes exhibited much higher concentrations of iron and organic matter in the monimolimnion compared to the mixolimnion than in North Gate. Thus, North Gate's meromixis may be of more recent origin. Nonetheless, a key chemical feature is the same, i.e., at the depths these acid bog lakes become anaerobic there is an increase in pH, alkalinity and ferrous iron. Kjensmo (1962, 1968) attributes the formation of the monimolimnion to an enrichment of ferrous bicarbonates. It is suggested that a sublacustrine inflow of iron-laden waters was important, especially in lakes in direct contact with bog vegetation (Sphagnum) which supplies organic detritus that is effective in reducing iron. Thus, the mechanism of chemocline formation described by Hooper and Koenings (1975) may be of considerable importance and general application to acid bog lakes in the United States and Europe.

Colloidal Organic Matter (COM): Molecular Size and Metal Coordination

Many chemical features of acid bog lakes, i.e., low pH, alkalinity, conductivity (electrolytes), low light penetration, high surface temperature and low productivity may indirectly or directly be attributed to the presence of large concentrations of colloidal organic acids (COM). By absorption of solar energy COM is responsible for the vertical thermal density gradient that restricts mixing to a very shallow strata and prevents the transfer of mineralized nutrients from the hypolimnion to the euphotic zone. The higher temperatures also increase microbial activity which in turn maintain the oxygen concentration considerably below saturation. Bog acids may compete with the phytoplankton for the specific wavelengths of light that initiate photosynthesis. However, the major impact of these acids on the lake is their ability to reduce the pH and the conductivity of the water by cation exchange reactions (e.g., $2R\text{-}COOH + Ca^{++} \rightarrow 2R\text{-}COOCa + 2H_3O^{+}$), which results in the removal of cations necessary for plankton growth. Since these acids may exert considerable influence on the chemistry and biology of the lake, a more detailed study was done of their functional role in elemental cycling.

Methods

Gel chromatography

Sephadex gel (C-100) chromatography was used to measure the molecular size of the COM. Lake water was filtered (0.45 μ) and 500 ml of the sample was then concentrated on a Virtris freeze dryer. The resulting powder was weighed and stored under refrigeration (4°C) until

applied to the column. This powder was readily soluble in buffer (pH 7.2 0.2 M tris and 0.02 M NaCl).

The G-100 column (1.8 x 49 cm) was calibrated with Bovine serum albumin (BSA) fraction V (Miles Laboratories), Lysozyme (egg white, Nutritional Biochemicals), and α-chymotrypsinogen-A (Sigma Chemical Company). Columns were allowed to flow with buffer for two days to ensure reproducible fractionation. Standards (3-4 mg protein per 2 ml buffer) and samples were added below column buffer level with a pasteur pipette. Fractions of 3 ml volume (51 drops) were collected on a LKB fraction collector at elution rates of $\leq$ 0.5 ml min^{-1}.

A Beckman DB Spectrophotometer was used to detect the eluted compounds at the following specific wavelengths: FMN (410 nm), blue dextran (630 nm), proteins (280 nm), Rhodamine B (530 nm), and bog acids (340 nm). Total and filterable organic carbon were analyzed by a modification of the method of Maciolek (1962) with a glucose standard curve prepared according to Strickland and Parsons (1972). Dialyzable organic carbon was obtained by in situ dialysis for 5 days.

In situ dialysis

In situ dialysis experiments were performed using dialysis tubing of regenerated cellulose. Tubing was hydrated for 24 hours, washed with 10% HCl, rinsed with distilled water and filled with either anaerobic or aerobic distilled water for use at the appropriate depths. Five days was determined adequate for quantitative dialysis (Koenings 1976). Samples dialyzed for up to three weeks showed no increase in dialyzable material beyond that at 5 days. The dialysis membrane used had a molecular cutoff in regard to proteins of 12,000 and a pore size

of 48 Å.

Chemical analysis for ferrous iron and ferric iron, reactive and nonreactive phosphorus, and organic carbon were carried out on the dialyzable, filterable and unfiltered fractions. When used in combination with 0.45 μ membrane filtration, the dialysis procedure separated particulate (>0.45 μ), colloidal (<0.45 μ, >48 Å), and truly dissolved (<48 Å) materials.

Results and Discussion

Molecular Size

Gel chromatography gave an average molecular weight of 7,000 for the bog acids (in regard to proteins) (Figure 5). Elution positions (i.e., Ve/Vo and Rsa) of known weight marker proteins derived from this and other studies (Andrews 1964; Determann and Michell 1966) are comparable, indicating a correct calibration of the sephadex column (Table 1). Calibration is important since it defines the linear region of a sigmoid curve which is the relationship between the log of molecular size and elution volume (Figure 5).

The elution profiles for COM for two separate determinations (Figure 6) indicate a broad range of material, but give identical and reproducible peak positions. The range of molecular weight is from 3,800 to 12,000 with a peak at 7,000. However, previous studies on colored lake acids have shown that COM possesses aromatic polyhydroxyl, phenolic and polycarboxyl groups (Christman 1970; Christman and Minear 1971; Schnitzer 1971). The partial sorption of aromatic groups to the Sephadex gel matrix causes delayed elution (Pharmacia 1972) which combined with the use of globular proteins (which migrate faster through

Table 1. The molecular weights and volume ratios (V_e/V_o) of standard proteins determined in this study (*) compared to similar molecules used in other studies (+ and ++) for G-100 gels. V_e = elution position of molecule, V_o = void volume, and R_{sa} = elution volume of BSA/elution volume of molecule.

Table 1.

Molecule	Molecular Weight	V_e*	Determann+ V_e/V_o	V_e/V_o	Andrews++ R_{sa}	R_{sa}*	Determinations
1) Blue Dextran	2,000,000	48	1.00	1.00	----	----	3
2) Bovine Serum Albumin (BSA)	67,500	63	1.32	1.31	1.00	1.00	3
3) Chymotrypsinogen A	23,650	87	----	1.81	0.70	0.72	2
4) Lysozyme	13,930	102	----	2.12	----	0.62	1
Cytochrome C	13,000	--	2.14	----	----	----	
Ribonuclease	13,700	--	----	----	0.61	----	
5) FMN	514	149	----	----	----	0.42	1
Rhodamine B	442	150	----	----	----	0.42	2
Sucrose	342	--	----	----	0.44		

*Determined for this study

+From Determann and Michel (1966)

++From Andrews (1964)

Figure 5. Elution positions of marker proteins of known molecular weight (Bovine serum albumin, α-chymotrypsinogen-A, and Lysozyme) compared to that of the bog acids from North Gate Lake.

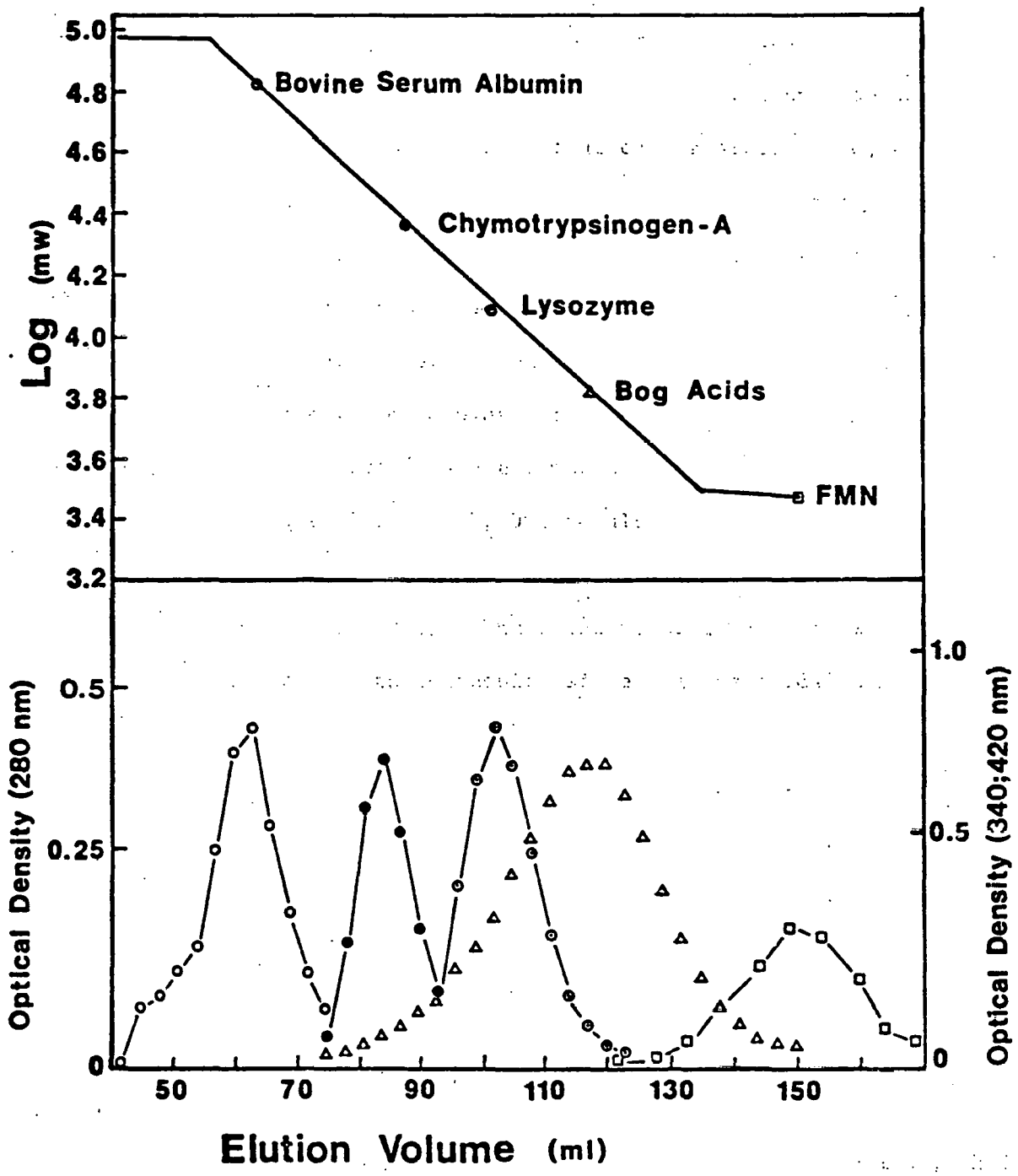

Log (mw)
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
Bovine Serum Albumin
Chymotrypsinogen-A
Lysozyme
Bog Acids
FMN
Optical Density (280 nm)
0.5
0.25
0
1.0
0.5
0
Optical Density (340;420 nm)
50
70
90
110
130
150
Elution Volume (ml)

Figure 5.

the gel due to their relatively compact structures) (Reiland 1968) suggests that the values reported here represent a minimum molecular weight for COM.

In addition, the assumption of separation solely by molecular size is not always met because separations achieved by Sephadex columns are dependent to a great extent on the size, shape, and functional groups present on the material fractionated (Christman and Minear 1971). Thus, until the validity of the Sephadex data is confirmed by other methods of molecular size analysis, the data cannot be taken as conclusive. However, gel filtration has been employed extensively to estimate the molecular weight of the colored organic acids in lake water. Shapiro (1967) estimated that most of the yellow substances had a molecular of $\leq$10,000, results substantiated by Gjessing (1967), Gjessing and Lee (1967), Ghassemi and Christman (1969) and Christman and Minear (1971). Thus, the results obtained in this study are in agreement with those results since the bog acids' molecular weight averaged 7,000.

However, previous in situ lake dialysis studies of Griffing (1969) and Koenings and Hooper (1976) have shown that these same colored organic acids are excluded from dialysis membranes (exclusion limit >12,000 in regard to proteins). I do not believe this difference is due to the concentration procedure but is probably a combination of the minimum molecular weights estimated for these acids and the different in situ molecular size of COM.

The organic matter present in lakes (Juday and Birge 1931; Christman and Minear 1971; Maciolek 1962), in soil (Schnitzer 1971) and bogs (Kovalev and Generalova 1967) has been found to be 50% carbon. Thus,

Figure 6. Reproducibility of bog acid molecular size determinations on a Sephadex G-100 column.

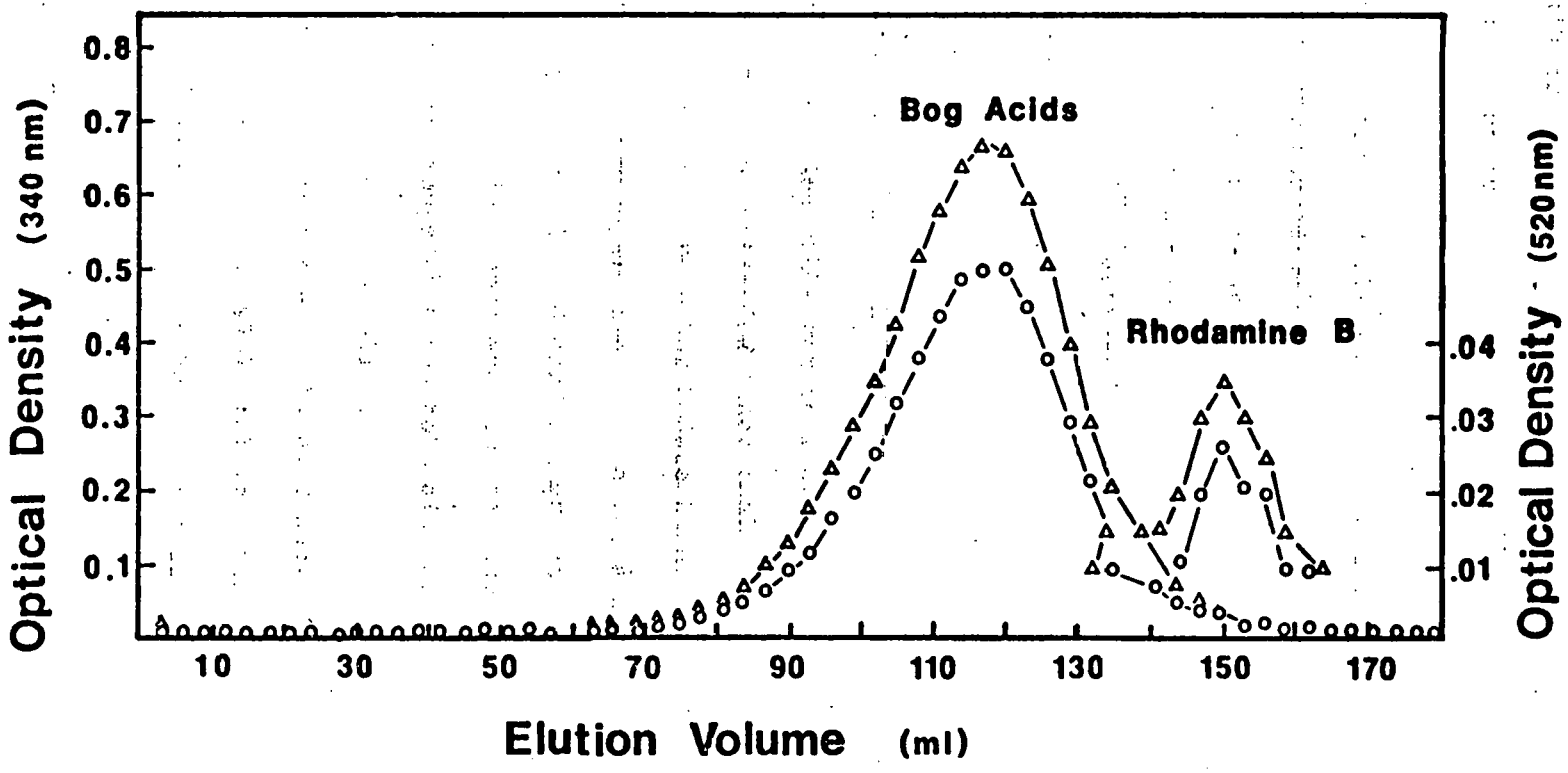

Optical Density (340 nm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Bog Acids
Rhodamine B
.04
.03
.02
.01
Optical Density - (520nm)
10
30
50
70
90
110
130
150
170
Elution Volume (ml)

Figure 6.

the amount of the organic matter in North Gate Lake can be readily estimated from the content of organic carbon. The organic carbon of the open lake ranges from 34.2 mg l^{-1} to 18.6 mg l^{-1}. Of this from 82% to 90% is 0.45 μ membrane filterable. Of the total carbon 21.2% to 6.3% is dialyzable, depending on the depth sampled (Figure 7). COM accounts for 61% to 81% of the organic material or a concentration of 4.0×10^{-6} moles l^{-1} to 6.52×10^{-6}. This is in the upper range of the values ($2\text{-}6 \times 10^{-6}$ moles l^{-1}) estimated by Christman and Minear (1971) to exist in natural waters.

Metal coordination

Clymo (1963; 1964; 1967) demonstrated the ability of detrital Sphagnum to act as a cation exchanger to long chain polymers of unesterified polyuronic acids containing functional carboxyl groups. A fraction of the organic matter in North Gate Lake is of colloidal (filterable but not dialyzable) size (Figure 7), and could contain functional phenolic, hydroxyl, and carboxyl groups. This class of organic matter is of crucial importance to bog lake chemistry since it may contain functional groups that can both bind metal ions and serve as effective electron donors (Martell 1971).

Lake sediment organic matter (Schindler and Alberts 1975), humic acids from peat (Szilagyi 1971; 1973), fulvic acids from soil (Schnitzer 1969; Levesque and Schnitzer 1967), bog water acids (Clymo 1963; 1967; Malmer 1963), and lake water (Shapiro 1957; Christman and Minear 1971) are organic compounds that may have different properties (e.g., solubility, molecular weights, color, etc.), but they all have at least two properties in common. They all contain functional carboxyl, phenolic

and hydroxyl groups which not only complex metal ions, but enable the organic matter to enter into redox reactions with the metal ion (Martell 1971). Thus, the COM of North Gate Lake which enters into coordination reactions with iron may have the ability by virtue of these functional groups to reduce ferric iron.

The ability of COM to reduce the concentration of free metals is supported by several investigators. Stumm and Morgan (1970) suggested that many hydrophilic colloids form highly colored complexes with ferric iron. Ong et al. (1970) considered bog waters as dilute suspensions of negatively charged hydrophilic colloids, and Clymo (1963; 1964; 1967) and Spearing (1972) have attributed the cation exchange ability of Sphagnum towards Ca^{++} and Fe^{+++} to be due to unesterified polyuronic acids. Clymo (1964) found organic acids containing functional carboxyl groups in the open water of the bog, and suggested that a long-term breakdown of plant constituents was a source of these free acids.

Szilagyi (1971; 1973) found that large molecular humic acids from peat sorb metal ions by a cation exchange process similar to cation exchange resins containing carboxyl groups. More importantly, it was found that the redox potential of a solution of humic acids was such that if coupled to the ferric-ferrous iron redox couple, it could bring about reduction of ferric iron. The reduction capacity of these organic acids was smaller than their absorption capacity. As a result, after the addition of inorganic ferric iron to a solution of peat humic acid, both ferric and ferrous iron were found sorbed to the organic acids.

Similar mixed ferric and ferrous iron colloidal organic complexes

were found in North Gate Lake and may arise by similar reduction and complexing reactions of bog COM. Koenings and Hooper (1976) found that the colloidal bog acids in North Gate Lake complexed ferric iron and that in the aerobic surface waters, ferrous iron made up 45% of the total iron (Figure 7). Since the lake water contained both colloidal ferrous and ferric iron, COM appears to be able to either (1) complex both oxidized and reduced iron or (2) to complex ferric iron and subsequently reduce a fraction of the iron. Both mechanisms would result in mixed ferric and ferrous colloidal organic iron. Since the ferrous iron in the anaerobic strata shows little complexing ability with COM (Figure 7) the latter mechanism seems more likely.

Interactions of iron and organic matter similar to those found in North Gate Lake have been reported in other systems (Tompsett 1940; Kovalev and Generalova 1967). Tompsett (1940) reported that in plasma and serum, ferric iron is in part reduced to the ferrous state with a consequent change from a nonultrafilterable (an undissociated colloidal complex) to an ultrafilterable state. The dialysis of ferric iron, but not of ferrous iron, is inhibited by phosphotides and phosphoproteins, and the reduction of ferric iron takes place only in an acidified solution (which stabilizes the reduced ferrous iron so it is detectable). Kovalev and Generalova (1967) working with iron and organic matter from peat soils, found that the interaction of ferrous and ferric iron with organic matter was affected by pH, and the iron-organic matter ratio. Iron and organic matter became more soluble as the pH was reduced from 9 to 5 and the ratio of iron to organic carbon approached 1:1. At pH's of 4-5, Fe^{+++} and $Fe(OH)^{++}$ predominated and reacted with the negative valances of the humic radicals. At a pH >6, the formation of

Figure 7. Distributions of phosphorus, iron, and organic matter by size and with depth in North Gate Lake.

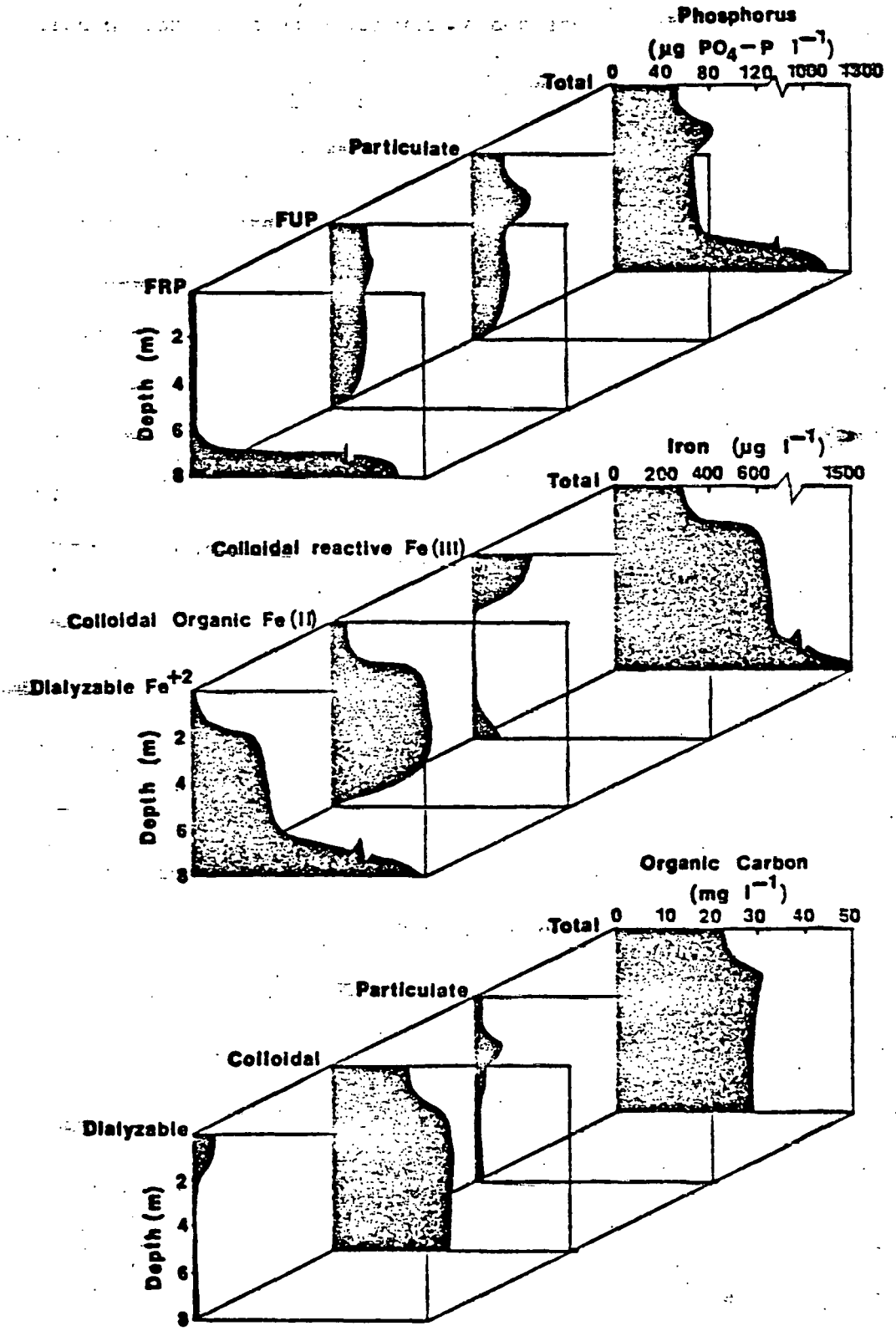

Phosphorus
(µg PO4−P l−1)
Total
0 40 80 120 1000 1300
Particulate
FUP
FRP
Depth (m)
2
4
6
8
Iron (µg l−1)
Total
0 200 400 600 1500
Colloidal reactive Fe(III)
Colloidal Organic Fe(II)
Dialyzable Fe+2
Depth (m)
2
4
6
8
Organic Carbon
(mg l−1)
Total
0 10 20 30 40 50
Particulate
Colloidal
Dialyzable
Depth (m)
2
4
6
8

Figure 7.

iron hydroxide takes place with a decrease in organic iron.

Relationship of COM-Iron to Phosphorus

If phosphate sorbs to an iron-COM colloid in the trophogenic zone of North Gate Lake, and still reacts chemically as DIP, but is in fact not free phosphate, the absorbtion of such colloidal bound phosphate (FRP) by algae might be slower than the absorbtion of recently added $DI^{32}P$. Thus, the assumption that the uptake of $DI^{32}P$ is equivalent to $FR^{31}P$ needed investigation if absolute uptake rates were to be derived from both sets of data. This is especially needed when the uptake of $DI^{32}P$ is determined in short-term experiments since the $DI^{32}P$ may not have sufficient time to equilibrate with the lake colloids.

Methods

Ion Exchange Chromatography

Dowex 1-8x, 100-50 mesh anion exchange resin in combination with either formic acid-ammonium formate or acetic acid-sodium acetate gradient elution systems was used to separate and identify orthophosphate from other phosphorus compounds. The gradient elution system consisted of 0.0 M to 3.0 M acid + 3.0 M salt (2:1 v/v). The resin was changed from its chloride form to the desired form by washing the column with 1 N NaOH and neutralizing with the appropriate 1 N acid. Columns were 0.9 in x 7 cm, and were eluted at 1.0 ml min^{-1} with 15-ml fractions collected on an automatic fraction collector. Reactive and total phosphorus measurements were made in duplicate on each fraction after Strickland and Parsons (1972).

This gradient elution system made it possible to separate

phosphate from other forms of phosphorus. By comparison of the known position of orthophosphate to the elution position of FRP, the chemical equivalence of FRP to orthophosphate was determined.

Hydrophobic chromatography

To determine the anion exchange capacity of the COM-iron complex, I used Amberlite XAD-2 polymeric adsorbent (20-50 mesh) in columns of 0.9 cm x 6 cm.

This hydrophobic adsorbent is a synthetic insoluble crosslinked polystyrene polymer with macroreticular physical porosity, high surface area and it possesses a unique nonionic structure which differentiates it from anion exchangers. XAD-2 selectively removes organic substances (e.g., COM) from water by adsorption of these materials through the hydrophobic portion of the molecule to the surface of the polymeric resin. The hydrophilic section of the molecule remains oriented in the aqueous phase.

I used this resin to determine the adsorption capacity of filtered lake water for phosphate. Filtered lake water (100 ml) was passed through the resin and the amount of FRP and organic material removed was measured. To test whether or not the adsorbed phosphate is available to be measured as FRP, I eluted the column with 0.12 N HCl. The reasoning was that if adsorbed phosphate is released by HCl, it would be available to the highly acidic "mixed reagent" used in the FRP analysis. I also ran standards (K_2HPO_4) through the column before and after the lake water to determine if exogenous phosphate was adsorbed by the resin and/or by the adsorbed COM-iron colloid. The eluted sample was analyzed for phosphate and compared to affluent phosphate

(after volume correction) to determine the extent of phosphate release and/or uptake.

Amberlite XAD-2 resin has (1) a macroreticular porosity, and (2) a nonionic structure. This enables the nonpolar (hydrophobic) portion of organic molecules to be preferentially adsorbed onto the surface of the adsorbent (Grieser and Pietrzyk 1973). The more polar section of the molecule remained oriented in the aqueous phase (Rohm and Haas 1974). Because of its structure the XAD-2 resin sorbs various classes of organic compounds; however, ionic solutes pass quantitatively through the resin (Rohm and Haas 1974). Because of these properties Montgomery and Echevarria (1975) effectively differentiated between ionic and organically complexed copper and zinc.

Ong and Bisque (1968) showed that iron-organic acid associations can be considered hydrophobic colloids, whereas the free organic acid can be considered as a hydrophilic colloid. That is, the organic molecule changes from a hydrophilic to a hydrophobic state upon complexation with a metal cation. So, organic iron would be removed from solution by the resin while inorganic iron and other ions (e.g. phosphate) would not. Thus, by the XAD-2 procedure, I could determine (1) the presence of a COM-iron-phosphate complex by the binding of the colloid to the resin, (2) the formation of colloidal reactive phosphate by the absorption of phosphate to the resin bound complex, and (3) the ability of dilute acid to release the phosphate from the bound complex.

Results and Discussion

COM, iron and phosphate

Water soluble organic matter (fulvic acids) extracted from soils by Levesque and Schnitzer (1967) and Schnitzer (1969) formed a complex with phosphate and iron. These complexes were found to be filterable if the molar ratio of Fe^{+3} to organic matter was 1:1 (Schnitzer 1971). In North Gate Lake this ratio equalled 1.06:1, which would result in the iron remaining entirely in the colloidal state. This is in agreement with what was observed in an in situ radiochemical study in this lake (Koenings 1976). The molar ratios of iron to COM increased with depth to 3:1 indicating that more ferrous iron could be complexed compared to ferric iron and still remain colloidal.

Direct phosphate sorption to COM does not take place (Szilagyi 1973; Levesque and Schnitzer 1967; Schnitzer 1969; Clymo 1963; Koenings and Hooper 1976). Thus, fixation of phosphate to organic colloids requires a metal bridge.

Colloidal and noncolloidal phosphate

Material reacting as phosphate in North Gate Lake was studied using anion exchange chromatography with a gradient elution. This procedure was found to be useful to separate and characterize the molybdate reactive substances (e.g., FRP). Molybdate reactive material found in both dialyzed and undialyzed samples eluted off the resin in the same peak position as known orthophosphate standards (Figure 8). This was taken as evidence that the reactive material in the lake water was orthophosphate.

Using this ion exchange procedure (and in situ dialysis), Koenings

Figure 8. Overlap in peak positions between molybdate reactive material found in North Gate Lake and orthophosphate standards using anion exchange chromatography.

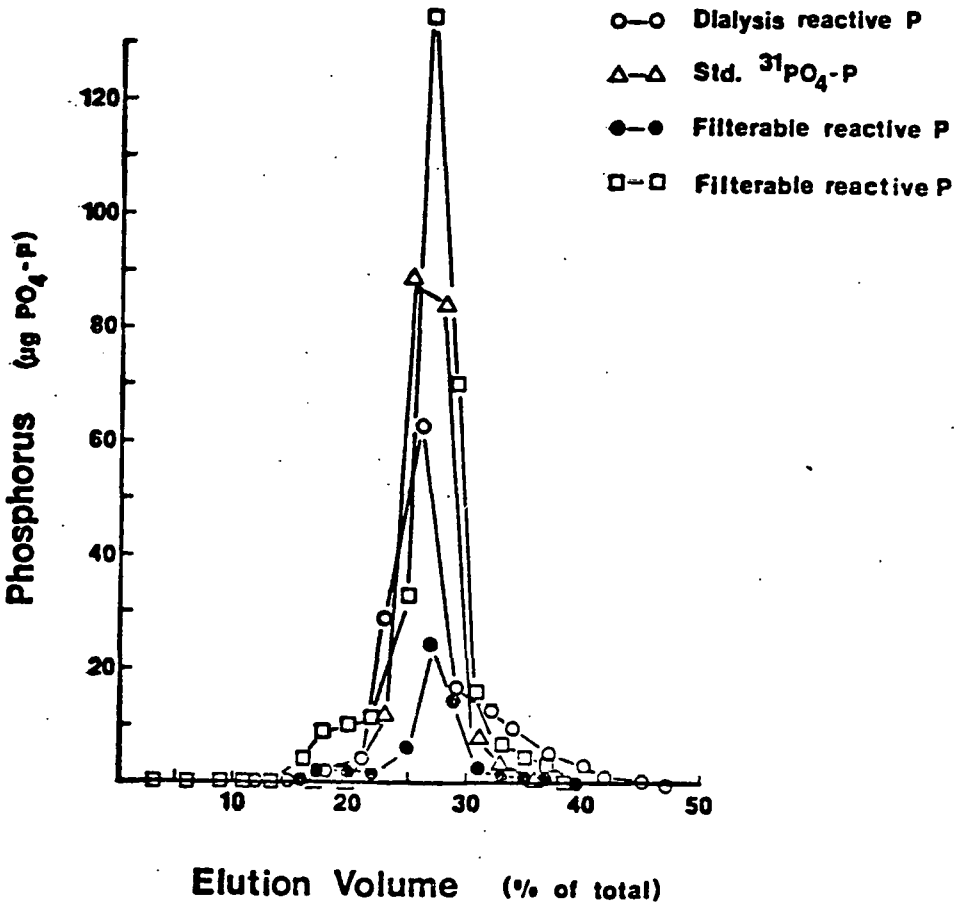

Dialysis reactive P
Std. $^{31}PO_4$-P
Filterable reactive P
Filterable reactive P
Phosphorus (µg PO_4-P)
120
100
80
60
40
20
10
20
30
40
50
Elution Volume (% of total)

Figure 8.

and Hooper (1976) presented evidence in support of the abiotic formation of colloidal phosphate. This phosphate reacted as FRP but not as free phosphate when COM was present (i.e., undialyzed water). Equivalent results in support of colloidal phosphate formation were obtained for both procedures; however, neither was completely free of errors. For example, a portion of the nonrecovery of phosphate from the ion-exchange studies (which was used as evidence for the removal of phosphate from true solution by a noneluting COM complex) could have been caused by phosphate capture by the gel (a pseudo pore) resin. Such problems are avoided by the use of a macroreticular (true pore resin) adsorbent which could selectively adsorb the COM (and its bound ions) through the hydrophobic (organic) portion of the colloidal complex.

The XAD-2 resin showed little adsorptive activity towards phosphate (Table 2), since consistently less than 1% of the standard phosphate solutions (six determinations) was removed by the resin. However, 45% of the COM (as measured by a decrease in adsorbance at 420 nm) was removed. Along with this fraction 134 $\mu g\ l^{-1}$ of FRP or 16.5% of the FRP added was retained by the resin. A distilled water wash failed to remove the bound phosphate, however, by washing with 0.12 N HCl, 111.0 $\mu g\ l^{-1}$ of FRP was recovered; this equalled nearly 83% of the previously retained FRP. The organic acids remained on the resin during both the distilled water and dilute acid washes.

In a second set of experiments, COM was added first to a new column. Again a portion (33.0 $\mu g\ l^{-1}$) of FRP and 34% of the COM was removed from 7.5 m lake water. This represented 25% of the FRP added. Then a standard phosphate (200 $mg\ l^{-1}$) solution was added. Instead of

Table 2. Recoveries of standard phosphate (Std. PO_4-P) solutions and FRP (aerobic lake water) from XAD-2 resin.

Table 2.

Reaction Order	Experiment #1			Experiment #2			Reaction Order	
	Affluent	Effluent	% Recovery	Affluent	Effluent	% Recovery		
1 Std. PO_4-P*	196.0	194.0	98.9	185.0	123.0	66.0	Color (lake)+	1
2 Color (lake)+	208.0	115.0	55.0	1349.0	1016.0	75.0	FRP (lake)*	2
3 FRP (lake)*	812.0	678.0	83.5	201.9	178.0	88.0	Std. PO_4-P*	3
Total PO_4-P	1008.0	872.0	87.0	1550.0	1194.0	77.0	Total PO_4-P	
COM-PO_4-P	136.0			356.0			COM-PO_4-P	
Dist. water	0.0	0.0	0.0	0.0	0.0	0.0	Dist. water	
HCl (0.12 N)	0.0	111.0	81.6	0.0	305.0	85.7	HCl (0.12 N)	

*$\mu g\ l^{-1}$ PO_4-P

+Klett absorbance (420 nm)

a 1% nonrecovery, the column removed 23 μg l^{-1} of phosphate or 12% of the added DIP. When eluted with HCl, after the distilled water wash, 305.0 μg l^{-1} of phosphate was recovered. Thus, the COM-iron complex reduced the recovery of FRP as free phosphate, and also complexed with secondarily added DIP forming colloidal reactive phosphate.

In a further experiment a series of lake water samples, using anaerobic water and water aerated for various amounts of time, was passed through the XAD-2 resin. The anaerobic sample retained 1.45% of added FRP (8.79 μg l^{-1}), while water aerated for 8 hours retained 4.55% of added FRP (27.75 μg l^{-1}) and finally sample water aerated for 48 hours retained 6.78% of the FRP (40.41 μg l^{-1}). This again is consistent with the formation of COM-bound phosphate through the bridging action of ferric iron since Koenings and Hooper (1976) demonstrated that ferrous iron, COM and phosphate did not form a complex, but that such a complex did develop upon aeration of previously anaerobic water.

Thus, North Gate Lake water does have the potential to reduce the in situ concentration of free phosphate, while leaving the phosphate reactive as FRP. In addition, any phosphate added to the water in the dissolved state will become colloidal over time. This could be of paramount importance in any observed differences in the cycling rates of $FR^{31}P$ and $DI^{32}P$ in North Gate Lake.

SECTION II

Stable Phosphorus and Enzymatic Activity in the Open Water of North Gate Lake

Methods

Phosphorus samples were collected with a modified Hale sampler using acid cleaned 300 ml BOD bottles. Samples were darkened, taken to the laboratory and analyzed within 10-20 minutes after collection. Unfiltered samples used for total phosphorus analysis were digested with perchloric acid, neutralized, and then diluted to volume with distilled water (Strickland and Parsons 1972). Samples were then tested for filterable reactive phosphorus (i.e., FRP) using a Klett-Summerson colorimeter equipped with a 600 nm filter and a light path of 4 cm. Standards and blank determinations were done with each set of samples. Standard curves for total and filterable reactive determinations differed slightly so calculations of each were made from a standard curve prepared and analyzed exactly as were the lake samples.

Lake water filtered with a 0.45 μ filter was tested for reactive phosphorus before and after digestion. These analyses give values for FRP and total filterable phosphorus. Phosphorus fractions were then calculated as follows:

Total Phosphorus (unfiltered sample) - Total Phosphorus (filtered sample) = Particulate Phosphorus (Part-P)

Total Phosphorus (filtered) - FRP = Filterable Unreactive Phosphorus (FUP)

FRP = Filterable Reactive Phosphorus

If dialysis membranes were used to further characterize filterable phosphorus, then colloidal phosphorus could be separated from dissolved phosphorus. Compounds passing through dialysis membranes were termed

dissolved; those not dialysable but filterable are hereafter designated colloidal.

Further problems associated with phosphorus analysis were found in this study. In anaerobic water H_2S was formed. It was found that the H_2S not only reduced the 12-molybdophosphoric acid complex in the absence of other reducing agents, but also formed a dark brown colloidal precipitate of molybdenum sulphide. This absorbed maximally at 420 nm but the tail of the absorption curve overlapped the 660 nm wavelength used for phosphate determination and increased the estimation of orthophosphate from 0.0 $\mu g\ l^{-1}$ to 12-15 $\mu g\ PO_4\text{-}P\ l^{-1}$. This complex was also extracted into n-hexanol, and isopropanol-benzene (Yanagita 1964). Also, the high amount of colored colloidal organic material decreased in absorbance at 660 nm in the presence of the acid "mixed" reagent." Thus, using a color blank of lake water diluted with distilled water (the same volume as "mixed reagent") would underestimate the concentration of orthophosphate. This was solved by adding citric acid (1 M) to the ascorbic acid reagent. The citric acid solution complexed the molybdate and reduced the pH of the solution as did the "mixed reagent", and gave an accurate estimate of sample color.

All glassware and polyethylene bottles were cleaned with dichromate cleaning solution (potassium dichromate plus concentrated sulphuric acid) and washed three times with distilled water. Various membrane filters have been reported to release significant amounts of detergent phosphorus during filtration (Burton 1973; Rigler 1964; Minear 1972), thus all filters (Gelman Instrument Company) were soaked or washed with 1% HCl before use and then rinsed with distilled water. Filters tested after this procedure resulted neither in an increase nor decrease

in phosphate from standard solutions prepared with filtered and refiltered bog water.

Results and Discussion

Total Phosphorous

The total phosphorus of the surface strata averaged 56 μg l^{-1} (Table 3). Epilimnetic phosphorus exhibited little change in the spring-summer period studied. This is unusual in that a majority of lakes typically undergo complete mixing during the spring and fall overturn periods. During lake overturn sestonic phosphorus which has settled to the hypolimnion and become mineralized is again brought to the surface. This results in two (spring and fall) periods of higher concentrations of epilimnetic phosphate at times when there is a uniform vertical concentration in the entire lake basin. This typical seasonal trend was not observed in North Gate Lake and was not reported in the acid bog lakes studied by Hayes and Anthony (1958).

North Gate Lake did not show a distinct spring (5 May - 6 June) maximum, and maintained a depth specific phosphorus stratification because the lake did not mix beyond a depth of 5 m (Table 3, Figure 7). The chemocline at 6.5-7.0 m remained intact, thus retaining a high concentration of noncirculating phosphorus in the monimolimnion.

Variations in total phosphorus in the epilimnion appear to be linked to weather conditions. Fluctuations in total P in the surface waters followed periods of intense storms, i.e., rapidly falling temperatures, high levels of precipitation, and high winds. These conditions produced a net phosphorus flux into the lake from the pool of phosphorus in the surface interstitial water of the surrounding

Table 3. The distribution of inorganic phosphorus (FRP), filterable unreactive phosphorus (FUP), particulate, and total phosphorus in North Gate Lake.

Table 3.

Stable Phosphorus in North Gate Lake
May - August 1974[1]

Depth (m)	FRP	FUP	Particulate	Total
0 (Surface)	0.0	30.7	25.4	56.1
2 (Thermocline)	0.0	33.2	46.5	79.7
5 (Hypolimnion)	0.0	33.3	32.3	65.6
7.5 (Monimolimnion)	1163.2	0.0	3.0	1166.0

[1]Values in μg PO_4-P l^{-1}; averages of over twenty duplicated determinations.

Sphagnum mat (Hooper 1972; Hooper and Koenings 1975). Phosphorus levels rose from an average of 50 µg $PO_4P\ l^{-1}$ to 54 µg PO_4-P l^{-1} after the first storm, then increased again to an average of 61 µg PO_4-P l^{-1} after the second storm (Figure 9).

This aperiodic flow of phosphorus into the lake follows thermal density gradients once bulk movement in the mat is initiated by cold heavy rains. The colder, denser water moves toward the open lake water and either enters the lake at water layers of equal density or continues to the bottom waters reinforcing the chemocline at 7.0 m (Hooper and Koenings 1975).

During the summer at 2 m total phosphorus increases to an average of 79 µg PO_4-P l^{-1}, again fluctuations in total P appear to follow periods of heavy weather, but are not nearly as well defined. Total P rose from an average of 74 µg PO_4-P l^{-1} to an average of 85 µg PO_4-P l^{-1} but finally fell to 80 µg PO_4-P l^{-1}; the 5 m stratum showed a less distinct trend rising from an average of 64 µg PO_4-P l^{-1} to 67 µg PO_4-P l^{-1}. These latter values show relatively small changes so that major inputs of phosphorus may be confined to the surface to 2 m layer.

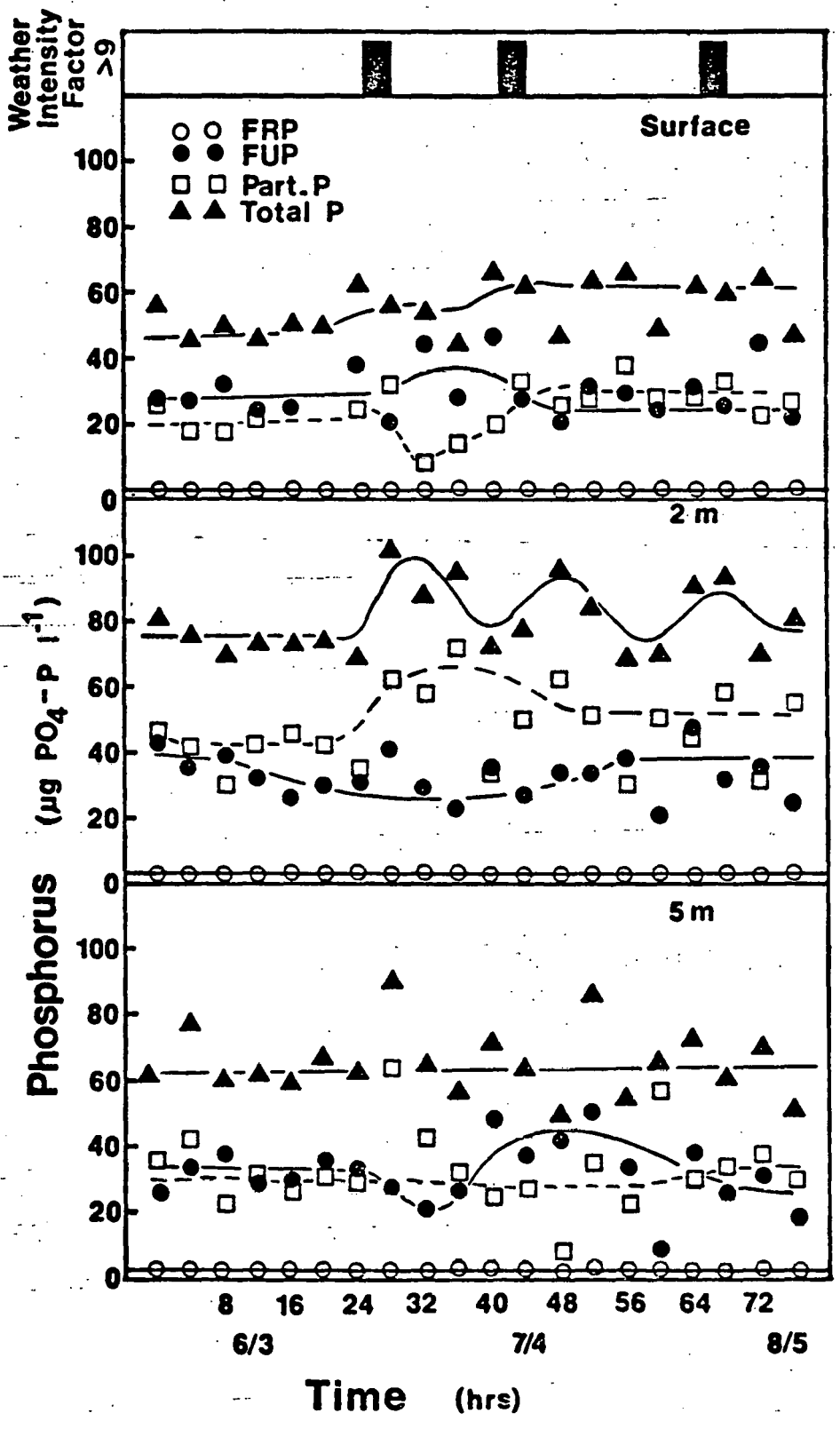
In the monimolimnion at 7.5 m total phosphorus increases dramatically to an average of 1,166 µg l^{-1}. Such a high concentration results from an accumulation of phosphorus due to several factors including the lack of wind generated mixing, accrual from the Sphagnum mat, and microbial degradation.

Filterable Reactive Phosphorus (FRP)

Filterable reactive phosphorus (all filterable inorganic phosphate, not just free phosphate) was undetectable, i.e., below 3 µg PO_4-P l^{-1},

Figure 9. The distribution of stable phosphorus in the surface, 2 m, and 5 m strata of North Gate Lake during the summer of 1974. Conditions of cold temperature, rainfall and high winds were combined to give the weather intensity factor.

Figure 9.


Weather Intensity Factor >9
FRP
FUP
Part.P
Total P
Surface
2 m
5 m
Phosphorus (µg PO4-P l-1)
0
20
40
60
80
100
8
16
24
32
40
48
56
64
72
6/3
7/4
8/5
Time (hrs)

at the surface, 2 m and 5 m (Table 3, Figure 7). Such low levels are caused by a combination of rapid biotic uptake, and the insufficient seasonal renewal of FRP from the monimolimnetic stratum. The absence of mixing causes FRP to build up in the latter layer since it is continually supplied with phosphorus from the Sphagnum mat via density currents, and from the open water via sedimenting plankton. Mineralized phosphate from both sources is trapped in this layer and thus does not resupply the epilimnion. Thus, FRP remains undectable throughout the summer period in the photic zone but increases slowly in the monimolimnion.

Filterable Unreactive Phosphorus (FUP) and Particulate Phosphorus (Part-P)

In the upper three strata (i.e., surface, 2 m, 5 m) levels of FUP and Part-P show little correlation except during the midsummer period of 6 June to 20 July (Figure 9). However, even during this period any relationship is ill defined requiring time periods of weeks for any observable change.

I found little evidence for seasonal changes in FUP in North Gate Lake. At the surface where phytoplankton dominate, FUP may increase for a short period during midsummer. Here the increase in FUP is matched roughly by a decrease in Part-P suggesting excretion and/or plankton autolysis. At 2 m where actively growing phytoplankton cells are absent, and bacteria dominate the system, the Part-P pool increases and the FUP fraction slowly decreases during the summer. The FUP fraction at 2 m decreases from an average value of 40 μg PO_4-P l^{-1} to 25 μg PO_4-P l^{-1} while Part-P increases from 42 μg PO_4-P l^{-1} to 68 μg PO_4-P l^{-1} (Figure 9). However, both the rate and beginning of the

increase in Part-P (day 24) appear to be closely linked to the increase in total P rather than to the decrease in FUP. This new phosphorus was presumably from the Sphagnum mat.

These small seasonal differences suggest that there may be a net increase in FUP during short periods which arises from autotrophic phytoplankton in the surface, and a small decrease at 2 m which arises from utilization by bacteria. However, the FUP pool on the average is quantitatively very similar at all three depths sampled (Table 3), and only differ by <2 μg PO_4-P l^{-1}. The uniformity in FUP at all three depths indicates that little release and utilization of FUP takes place, and that lake FUP consists largely of inert and noncycling compounds.

Changes in time periods of weeks were noted in the FUP pool by Strickland and Austin (1960), and by Harvey (1963). These authors concluded that in these marine systems the FUP pool was slowly turning over. They found a correlation between Part-P and FUP. However, both authors found that FRP was readily detectable and at times even higher in concentration than FUP. The plankton apparently were not phosphorus deficient with phophorus being more readily available than in North Gate Lake. Thus, the difference in phosphorus availibility may in part determine any detectable seasonal cycling of FUP.

Conclusions drawn from stable chemical analysis of FUP production and/or utilization by the plankton are questionable. However, the data seem to indicate that there is a large FUP pool (which exceeds the amount of Part-P at 2 and 5 m) which is relatively inert and is only partially used. If FUP represents a non-metabolic form of phosphorus,

it is not surprising that FUP concentrations are on the average about the same throughout the lake even though they exist with vastly different amounts and types of seston. This idea is supported by the observations of Ruttner (1963) who considers freshwater colloidal organic phosphorus a metabolic end product that is resistant to further decomposition, and Perry (1976) who observed little turnover of FUP in the Pacific Ocean.

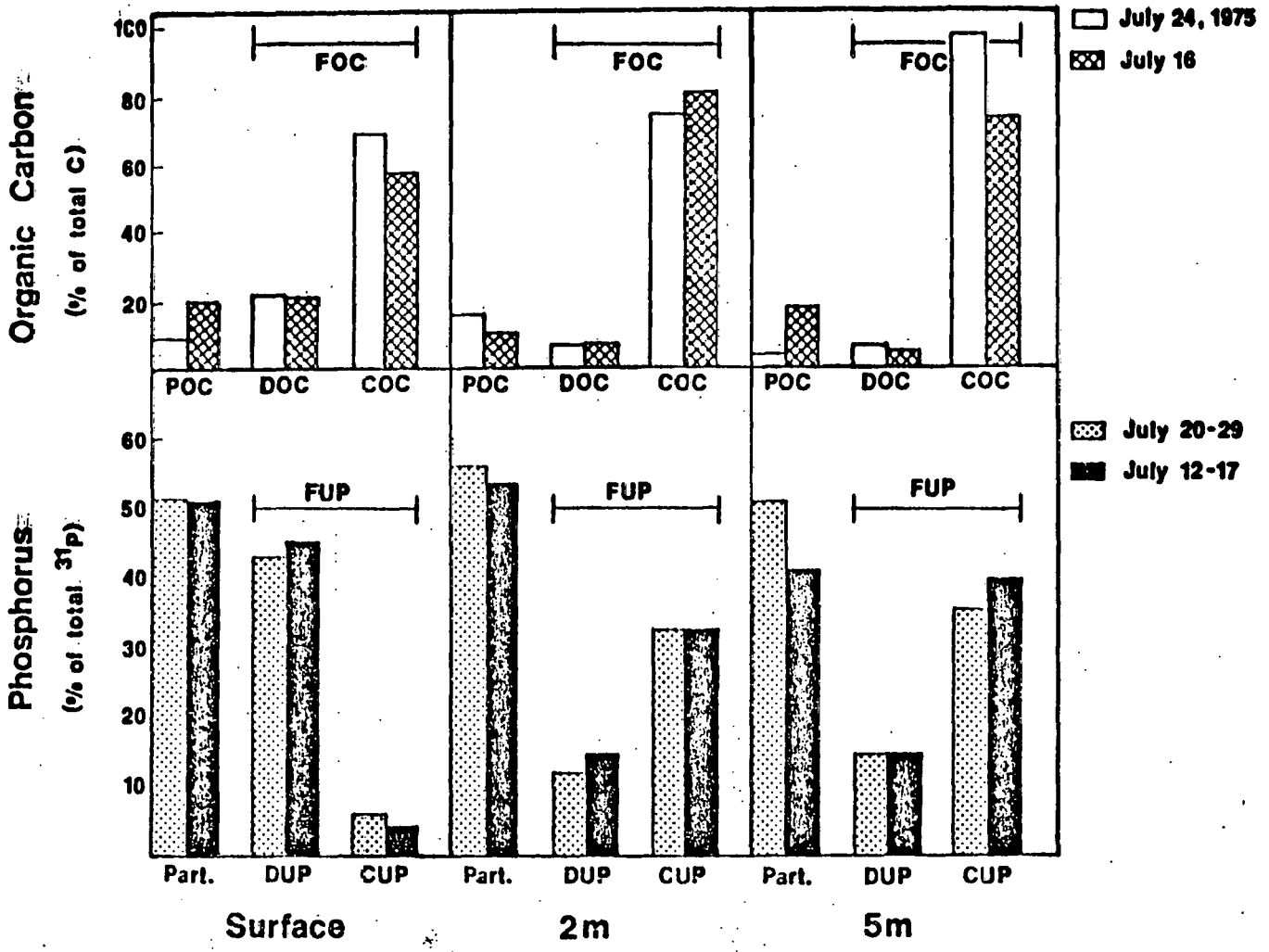
Thus, like Juday and Birge (1931) and Hayes and Anthony (1958) I concluded that FUP may be present as part of a humic colloid. If so, then chemically determined FUP may dominate metabolic FUP that is actually cycling. In North Gate Lake then, any metabolically active fraction of nonparticulate phosphorus may be masked by this large concentration of noncycling material.

Colloidal and Truly Soluble Organic Phosphorus

A more detailed analysis of FUP showed that the FUP could be split into two pools (Figure 10). The first pool consists of truly dissolved unreactive phosphorus (DUP) which was separated by two experiments involving in situ dialysis. These compounds were of low molecular weight (<12,000), and were dialyzable. This pool had a maximum concentration at the surface and decreased with depth. The opposite trend was noted for the second component, colloidal unreactive phosphorus (CUP), which showed a minimum in the surface waters and increased with depth. There exist major differences in these two fractions among the layers of the lake sampled. The surface water showed a summer maximum of FUP consisting of low molecular weight DUP which perhaps was produced by the phytoplankton. The 2 m and

Figure 10. The distribution of organic carbon and organic phosphorus by strata in North Gate Lake. Filterable organic carbon (FOC) and FUP are further split into dialyzable and colloidal pools. POC = particulate organic carbon, DOC = dialyzable organic carbon, and COC = colloidal organic carbon; DUP = dialyzable unreactive phosphorus and CUP = colloidal unreactive phosphorus.

Figure 10.


Organic Carbon
(% of total C)
100
80
60
40
20
FOC
POC
DOC
COC
July 24, 1975
July 16
Phosphorus
(% of total 31P)
60
50
40
30
20
10
FUP
Part.
DUP
CUP
July 20-29
July 12-17
Surface
2m
5m

5 m depths which were dominated by aquatic bacteria had more of a colloidal or high molecular weight fraction of FUP.

If ratios of organic phosphorus to organic carbon are calculated for the colloidal, as well as the dialyzable and particulate fractions, it becomes apparent that three pools of organic phosphorus are present in North Gate Lake (Figure 11).

Unlike phosphorus, the predominant form of dissolved organic carbon at all depths in the lake was the colloidal fraction (Figure 11). However, like phosphorus, truly dissolved organic carbon (DOC) was highest at the surface. In the euphotic zone the C:P ratio of the DOC to the DUP was almost equal to the C:P ratio of particulate organic carbon to Part-P. The low molecular weight organic phosphorus (DUP) of the euphotic zone may be of more recent metabolic origin since its C:P ratio is similar to that of the particulate fraction. Pool I is Part-P which possesses an organic C to P ratio from 147:1 to 639:1. Pool II is dialyzable phosphorus which has ratios of from 383:1 to 698:1. The overlap of the particulate pool (I) with the dialyzed pool (II) occurs for only the surface sample (Figure 11).

The C:P ratios discussed above are contrasted to those from the third pool which consists of colloidal organic phosphorus (CUP) which vary from 2,467:1 to 15,427:1. Thus, there exists in this lake a considerable portion of the FUP pool (CUP) which has been stripped of most of its phosphorus and can be classified as refractory, especially in regard to the cycling time of free orthophosphate found for North Gate Lake. The COP pool does not overlap the C:P ratios of the previous two pools. This may account for the refractory nature of the

Figure 11. Organic phosphorus:organic carbon ratios in North Gate Lake which define three separate pools of organic phosphorus: particulate (I), dialyzable (II), and colloidal (III). The only overlap occurs between the particulate and dialyzable pools of the surface samples. ([1]results of two separate experiments; [2]summer averages)

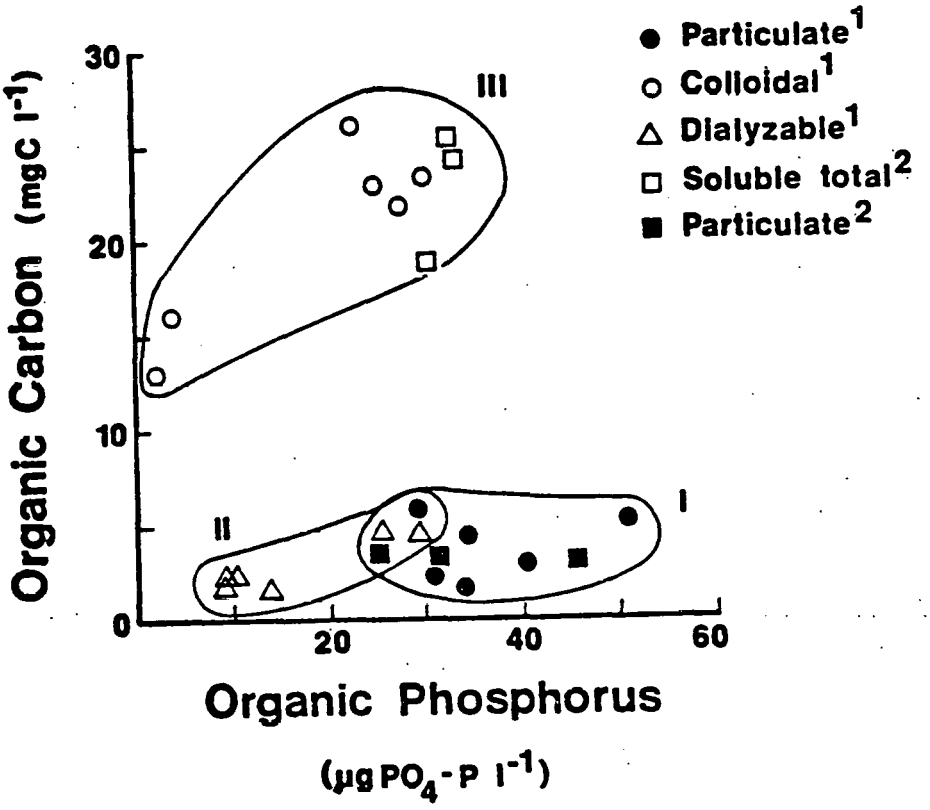

Particulate[1]
Colloidal[1]
Dialyzable[1]
Soluble total[2]
Particulate[2]
Organic Carbon (mgC l-1)
30
20
10
0
III
II
I
20
40
60
Organic Phosphorus
(µg PO4-P l-1)

Figure 11.

entire FUP pool noticed for the summer period. It is the COP fraction which may overwhelm or mask the cycle of autochthonous FUP. This colloidal organic material may be the same material which caused Hayes and Anthony (1958) and Juday and Birge (1931) to conclude that there was not a relationship between phosphorus pools and productivity in the acid bog lakes they studied. These authors attributed the lack of such a coupling to the "humus" materials imparted to the water by the surrounding Sphagnum vegetation. Bog lakes may possess higher levels of total phosphorus than their productivity would predict because a major fraction of the total phosphorus is FUP, not a portion of the available phosphorus pool. This is an important point since the total P level is considered to be an indicator of the potential productivity of a body of water (Vollenweider 1968; Dillon and Rigler 1974; Jones and Bachmann 1976). Obviously, refractory FUP components of total phosphorus should not be used in productivity forecasts. If the total phosphorus is considered and North Gate Lake classified as to potential productivity, it would fit into Vollenweider's (1968) scheme as a productive mesoeutrophic lake, but if the FUP is subtracted, this lake would be classified as oligotrophic (unproductive). Radiochemical experiments described below give further support to these conclusions.

The Enzymatic Identification of Lake FUP

Methods

Enzymes were used to characterize the naturally existing FUP at three depths in North Gate Lake. These were: The surface (top of the epilimnion), 2 m (bottom of the epilimnion), and 5 m (hypolimnion).

These enzymes function as follows:

I. Deoxyribonucleate oligonucleotide hydrolase (Deoxyribonuclease DNase I). Enzyme code number (E.C.) 3.1.4.5 from beef pancreas. (Sigma Chemical Co.)

This enzyme reacts with DNA to form deoxyribonucleotides at pH 7.0 according to the general reaction:

$$DNA + (n-1)H_2O \rightarrow \text{deoxy-5'-ribonucleotides}$$

This enzyme requires the metal activator Mg^{++} and is an endonuclease that does not require a free 3'-hydroxyl end to initiate degradation as is required by the exonuclease phosphodiesterase E.C. 3.1.4.1.

II. Meso-inositol-hexaphosphate phosphohydrolase (Phytase) E.C. 3.1.3.8 from wheat. (Sigma Chemical Co.)

This enzyme also requires Mg^{++} for activation and reacts to degrade monophosphate esters plus pyrophosphate as well as phytic acid (inositol hexaphosphate) to inorganic phosphate according to the general reaction:

$$\text{meso-inositol hexaphosphate} + 6H_2O \rightarrow \text{meso-inositol} + 6H_3PO_4$$

III. Orthophosphoric monoester phosphohydrolase (alkaline phosphatase; PMase) E.C. 3.1.3.1 from hog intestinal mucosa. (Sigma Chemical Co.)

This enzyme requires Mg^{++} for activation and reacts to hydrolyze monoesters of phosphoric acid (sugar phosphates, deoxy- and ribonucleotides and pyrophosphate) to inorganic phosphate, in an alkaline (pH 7-9) medium by the reaction:

$$\text{orthophosphoric monoester} + H_2O \rightarrow \text{an alcohol} + H_3PO_4$$

This enzyme hydrolyzes the same enzymes as Phytase, except it does not

hydrolyze forms of phytic acid.

IV. Orthophosphoric monoester phosphohydrolase (acid phosphatase; PMase) E.C. 3.1.3.2 from wheat germ. (Sigma Chemical Co.)

This enzyme requires an acid pH (4-5) for maximum activity, but does not require a metal activator. Otherwise, its reactions are the same as for alkaline phosphatase.

Thus, orthophosphate (P_i) appearing in lake water samples after hydrolysis of acid and alkaline phosphatase will be from sugar phosphates, deoxyribonucleotides, ribonucleotides, and pyrophosphate. Orthophosphate appearing in samples incubated with phytase should be equal to the above compounds plus those from forms of phytic acid. Finally, orthophosphate recovered from samples incubated with DNase plus alkaline PMase would equal phosphate from DNA plus that liberated by the alkaline PMase.

This reaction proceeds as follows:

$$\text{DNA} + (n-1)H_2O \xrightarrow{+\text{DNase I}} n \text{ oligodeoxy-5'-ribonucleotides}$$

$$\xrightarrow{+\text{PMase}} \text{an alcohol} + \text{inorganic phosphate}$$

Thus, classes of compounds could be separated as follows:

(1) P_i from DNase + alkaline PMase - P_i from alkaline PMase = DNA phosphate

(2) P_i from Phytase - P_i from alkaline and acid PMase = phytic acid phosphate

(3) P_i from alkaline PMase or from acid PMase = sugar,

nucleotide and pyrophosphate phosphorus

Enzyme assays were carried out using 45.0 ml of filtered lake water and 1.0-0.5 mg of enzyme preparation per sample. Incubation with enzymes did not interfere with P_i estimation by the Strickland and Parsons procedure. However, phytase was contaminated with a high level of P_i which had to be removed with an anion exchange resin Amberlite IRA-904 (Cl^- form) before it could be used. Buffers used were 0.1 M phthalate (pH 5.0) and citric acid (pH 5.0). The phthalate did not interfere with the phosphorus estimation but the organic acid binding of molybdate by citric acid prevented its reaction with phosphate.

The buffer used at pH 8.0 and 7.0 was 0.1 M Tris (hydroxmethyl-amino methane). This buffer did not interfere with phosphate analysis and was not contaminated with orthophosphate. Because freezing [recommended by Strickland and Parsons (1972) and Burton (1973)] may affect orthophosphate concentration, refrigeration as well as freezing was used for the enzyme studies. Lake water was collected from the surface, 2 m, and 5 m with a plastic (1 liter) Kenmerer sampler. At each depth 3-6 samples were combined to give a single sample which was filtered (0.45 μ), stored in 1-liter polyethylene bottles and then was either frozen or refrigerated.

All samples were analyzed in duplicate or triplicate, along with blanks consisting of lake water to which the buffer and chloroform were added. The blank was determined by adding the enzyme to the sample, followed immediately by the "mixed reagent," and then analyzed as given above for FRP. This rapid analysis resulted in minimal hydrolysis of organic phosphorus esters. Internal standards of phytic

acid nucleotide (AMP) and sugar phosphates (glucose-6-phosphate) were run with the appropriate samples. Complete hydrolysis of standards had always taken place when enzyme-lake samples were analyzed, a time period between 8 and 24 hrs. Hydrolysis of sugar phosphates took up to 4 hrs. so the time was doubled for lake samples to further ensure complete hydrolysis.

A standard curve was prepared using glucose-6-phosphate and alkaline phosphate. This insured that complete hydrolysis occurred over all phosphate ester concentrations.

A general assay was as follows:

To 45 ml of 0.45 μ filtered sample, add:

(1) 1.0 ml of enzyme solution prepared by dissolving 0.5 to 1.0 mg of enzyme preparation per ml of buffer.

(2) 4.0 ml of buffer (some buffers required 0.001 M Mg^{++}) and 0.1 ml of $CHCl_3$. (Note: the buffer insured a pH compatible with the maximum activity of the enzyme, as does the metal activator where appropriate and the chloroform reduces bacterial contamination.)

(3) Incubate at 25°C in the dark for 8-24 hrs.

(4) Add 5 ml of mixed reagent and read at 660 nm after 10 min.

Levels of phosphate cleaved from natural lake FUP were calculated from the organic phosphorus standard curve after subtraction of all appropriate blanks from optical density readings of the sample solutions. All samples, blanks, and enzyme activity standards were run in duplicate

or triplicate.

Results and Discussion

Samples stored up to 3 months by either refrigeration or freezing did not differ significantly so the results of five duplicated tests were averaged to give the fraction of FUP hydrolyzed by alkaline PMase (Table 4). The amount of phosphate released from FUP decreased from 7.76 µg PO_4-P l^{-1} at the surface, to 3.64 µg PO_4-P l^{-1} at 5 m. The amount of FUP hydrolyzed by PMase was different, depending upon the enzyme used. For example, acid PMase hydrolyzed only 27%, 50% and 71% of the FUP compared to alkaline PMase. Since acid PMase is the only form of active PMase found in North Gate Lake, the difference in hydrolysis could be due either to different specificities of the enzymes, or to the different pH's of the incubation media.

Phytase acted on a fraction of FUP different from PMase only at 5 m where 57% of the FUP present was degraded to inorganic phosphate.

A greater fraction of the FUP pool was hydrolyzed by the combination of DNase I and alkaline PMase compared to PMase alone. This fraction was relatively large at all depths sampled.

The classes of compounds hydrolyzed by these enzymes does overlap; however each enzyme is specific for a class of organic-P compounds. Thus, the difference in DIP found between the PMase and phytase assays represents a form of phytic acid (Table 5). The classes of FUP found in North Gate Lake by this procedure changed with depth (Table 5). For example, at the surface, more PMase hydrolyzable compounds were found, while phytic acid was only found at 5 m, and DNA fragments were relatively constant. Since phytic acid does not make up a significant

Table 4. The amount of phosphate released from the FUP of North Gate Lake by alkaline phosphomonoesterase (Alk. PMase), acid phosphomonoesterase (Acid PMase), phytase, and a combination of deoxyribonuclease and alk. PMase (DNase + Alk. PMase).

Table 4.

Enzymatic Degradation of Natural Lake Water FUP[++]

Depth	Alk. PMase	Acid PMase	Phytase	DNase + Alk. PMase	FUP
0 m	7.76±1.37*	3.88±5.49*	7.76±1.12+	14.07±0.97+	23.4±1.94
2 m	5.50±1.02*	3.88±0.00*	2.91±0.00+	11.64±2.24+	24.8±0.00
5 m	3.64±1.22*	0.97±1.37*	12.61±4.48+	10.67±0.00+	22.0±1.95

++values in μg PO_4-P l^{-1}.

*average of 3 samples ±1 standard deviation.

+average of 4 samples ±1 standard deviation.

Table 5. The amount of enzyme hydrolyzable phosphorus in compounds composing FUP.

Table 5.

Organic Phosphorus Compounds in North Gate Lake[+]

Depth	Sugar Phosphates + Nucleotides + Polyphosphate	Phytic Acid	DNA Fragments	Total Found	Lake FUP	% Recovery
0 m	7.76	0.00	6.31	14.07	23.4	60.1
2 m	5.50	0.00	6.14	11.64	24.8	47.0
5 m	3.64	8.97	7.03	19.64	22.0	89.0

[+]Fractions in μg PO_4-P l^{-1}

portion of stable FUP in the euphotic zone, it may be rapidly degraded and used by the seston as a source of phosphorus. In contrast, DNA fragments seem to be relatively inert since their concentration remains constant with depth at 6-7 μg PO_4-P l^{-1}. PMase hydrolyzable material seems to be greater in the euphotic zone, decreasing with depth. It is this fraction that may be the form of low molecular weight DUP (Figure 10) found in the euphotic zone in North Gate Lake.

From 47% to 89% of the FUP in North Gate Lake was hydrolyzed by the three classes of enzymes used. The unhydrolyzed fraction may be RNA or organic-P compounds complexed to COM and thus resistant to enzymatic action. However, it must be stressed that these tests were made only during the summer period, and results might be expected to change seasonally.

Lake Enzymes

Methods

I assayed the water and suspended solids of both the lake water and the interstitial water of the Sphagnum mat for enzyme activity. Two hydrolytic enzymes were assayed. These were: acid phosphomonoesterase (acid PMase) and alkaline phosphomonoesterase (alkaline PMase). The substrate used for the phosphomonoesterases was para-nitrophenyl phosphate (PNPP) purchased from Sigma Chemical Company. When hydrolyzed PNPP yields para-nitrophenol which turns a brilliant yellow at a pH of 9.0. Appearance of para-nitrophenol color at 410 nm was used as an indication of enzyme activity.

Buffers used were 0.1 M glycine-NaOH pH 8.4 and 10.0, and 0.1 M

citric acid-Na citrate pH 4.5. Assay procedure was as follows:

(1) 0.4 ml of PNPP (0.1 g of PNPP in 50 ml of distilled water). In those experiments requiring a metal activator, 2.5 g of $MgSO_4$ was added to the PNPP solution.

(2) 1.0 ml buffer plus 0.05 ml chloroform ($CHCl_3$).

(3) Incubate in the dark at 25°C for 12-24 hrs.

(4) Add 2 ml of pH 10 glycine-NaOH buffer and read absorbance at 410 nm.

Color blanks were treated as above except 0.4 ml distilled water replaced the PNPP solution. PNPP blanks were also run, these consisted of PNPP in 10 ml of distilled water with buffer and $CHCl_3$.

Assays were run with no metal activators since I wanted to determine activities as close to in situ conditions as possible. However, to determine if I could activate or increase enzyme activity, I added Mg^{++} ($MgSO_4$) to a PMase experiment. The unit of enzyme activity reported is the release of 1 µg PO_4-P per hour per liter.

Results and Discussion

The Levels of Enzyme Activity

Alkaline phosphatase activity was not found in any layer of North Gate Lake whereas there was an appreciable pool of acid phosphatase at all levels (Figure 12). This would be expected to occur in a lake where the pH averages 4-5 much of the year.

The assay for the enzyme was linear for both the soluble (filtered) and total (unfiltered) activity (Figure 13). The filterable

Figure 12. Acid phosphomonoesterase (PMase) activity in North Gate Lake.

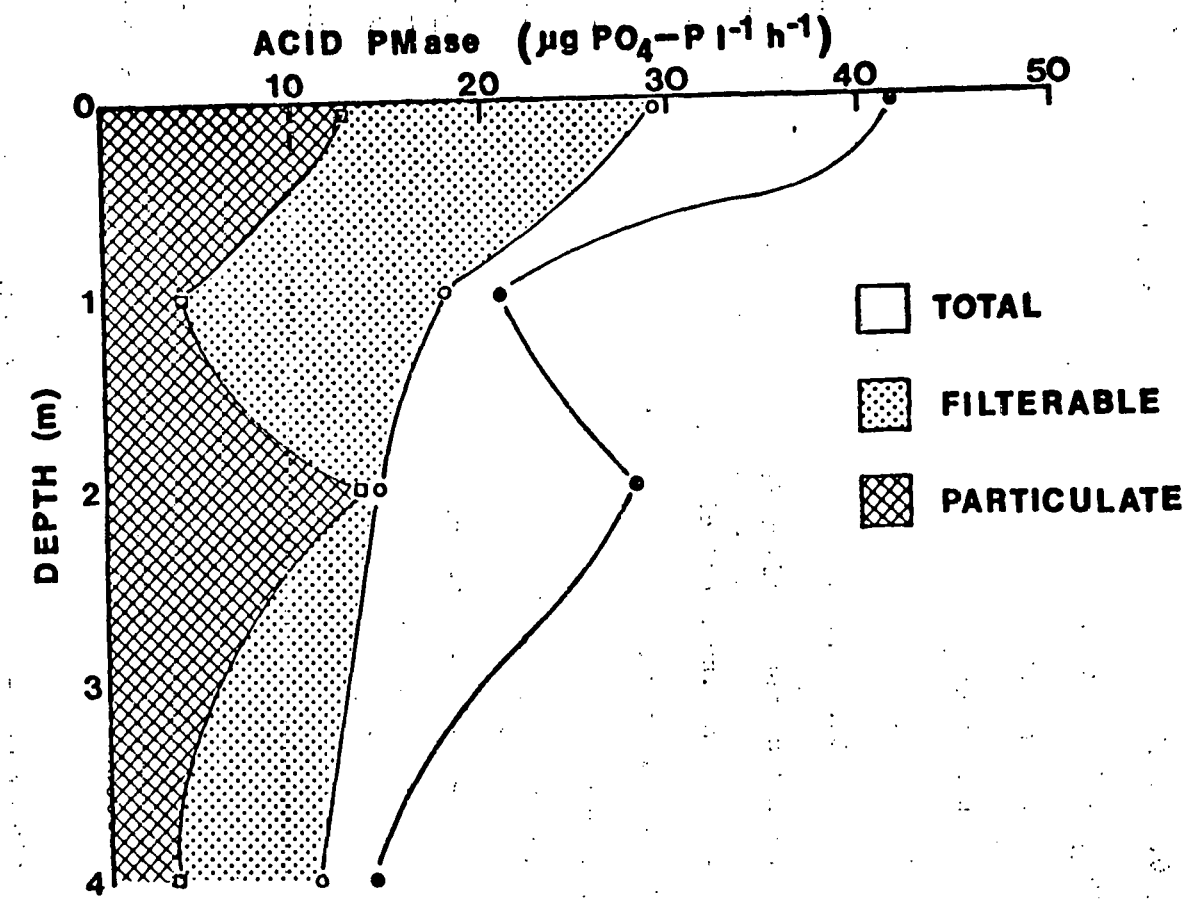

ACID PMase (µg PO4–P l-1 h-1)
10
20
30
40
50
0
1
2
3
4
DEPTH (m)
TOTAL
FILTERABLE
PARTICULATE

Figure 12.

activity represented >68% of the total activity throughout the time period of the assay. K_m values (the concentration at which 1/2 the maximum activity of the enzyme occurs) for the unfiltered water PMase ranged from 140 µM PNPP to 23 µM PNPP (Figure 13). This falls within the lower range of values found by Berman (1970) of from 13.0 µM PNPP to 670 µM PNPP and of Reichardt et al. (1967) who found a K_m of 1.09 µM for the dissolved enzyme and from 54 to 500 µM PNPP for the algal enzyme. In comparison the K_m for the alkaline PMase of the bacterium E. coli is 15-12 µM PNPP (Garen and Levinthal 1960).

Acid PMase activity was not equally distributed with depth (Figure 12). Enzyme activity was generally higher in the surface strata with 69% being in the filterable fraction. At one meter enzyme activity dropped by 50%, with filterable activity increasing to 80% of total enzyme activity. In the thermocline at 2 m, enzyme activity increased but the amount of filterable enzyme dropped to 50% of total activity. In the hypolimnion enzyme activity reached a minimum. However, like 1 m over 75% of the activity was filterable. Thus, where the bacteria and the phytoplankton are concentrated (i.e. 2 m and surface), there is a peak in total enzyme activity, and in particulate activity. In contrast, at 1 m and 4 m, the enzyme activity reaches a minimum, and the particulate fraction is reduced to only 20-25% of the total.

Since bog lakes are very low in cations, I tested whether the addition of a metal co-factor or activator would increase PMase activity. Initially, there was little effect of the metal (Mg^{++}) on enzyme activity (Figure 13). However, over time the enzyme activity actually decreased. Therefore, a metal activator which is necessary for the

Figure 13. The determination of (1) acid PMase activity (A) using p-nitrophenol (PNP) in the presence and absence of an added metal cofactor (Mg^{++}) and (2) the K_m value (B) for PMase in the surface strata. Error bars in (A) equal $\pm$ 1 std. deviation.

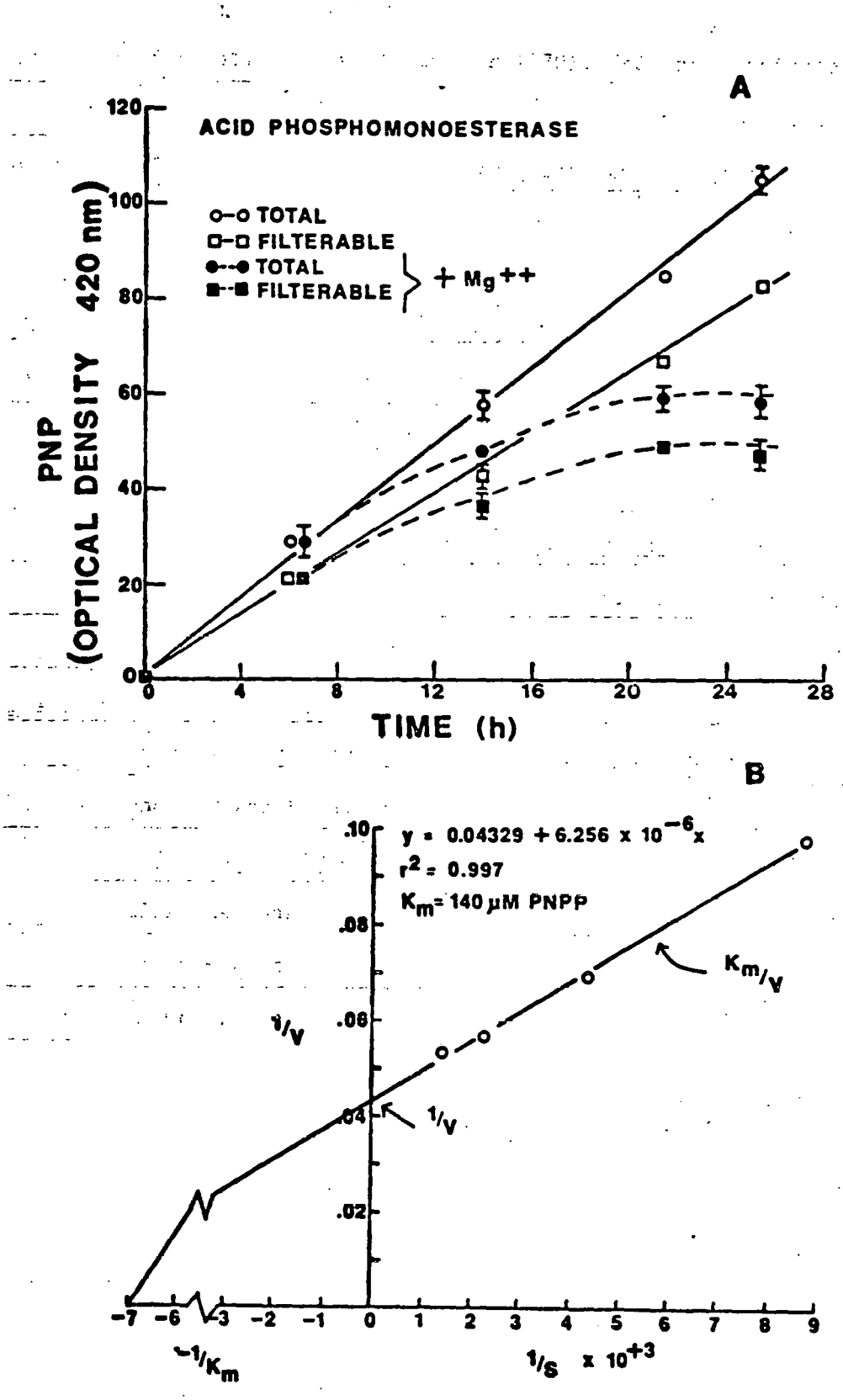

A
ACID PHOSPHOMONOESTERASE
o–o TOTAL
□–□ FILTERABLE
●–● TOTAL
■–■ FILTERABLE
+ Mg++
PNP (OPTICAL DENSITY 420 nm)
TIME (h)
B
y = 0.04329 + 6.256 x 10⁻⁶x
r² = 0.997
Km= 140 µM PNPP
Km/V
1/V
1/V
-1/Km
1/S x 10+3

Figure 13.

activity of alkaline PMase (Kuenzler 1970), does not activate this acid PMase. The lack of the need of a metal cofactor, and the activity of the enzyme at an acid pH closely matches this enzyme to the chemical environment of North Gate Lake.

The acid PMase activity in North Gate Lake is also unusual in that 68% (39 to 100%) of the activity was in solution, i.e. not associated with the particulate fraction. In Lake Kinneret Berman et al. (1972) found that soluble enzyme alkaline phosphatase averaged only 12.9% (range of 5-20%). The reason for this difference may be twofold. First, Healey (1973) found that the amount of noncellcular alkaline phosphatase increased as an algal cell becomes phosphorus deficient. Thus, the plankton in Lake Kinneret may be phosphorus sufficient. Second, acid phosphatase was found by Aaronson (1971) to be predominantly in the supernatant of his algal cultures, while alkaline phosphatase was found attached to the cell.

Acid PMase may differ then in its physiological properties compared to alkaline PMase. Therefore, acid PMase size distributions may also differ from that of alkaline PMase especially in a phosphorus deficient system.

SECTION III

The Kinetics of Radiophosphorus Flux in North Gate Lake and Its Relation to Stable Phosphorus Flow

The Radiochemical Phosphorus Cycle

Methods

Radiochemistry

Radioisotopes ^{32}P ($H_3{}^{32}PO_4$ in HCl), ^{59}Fe ($^{59}FeCl_3$ in HCl), and ^{14}C ($NaH^{14}CO_3$) were purchased from New England Nuclear Corporation and were diluted to workable specific activities before use in lake experiments. A Nuclear Chicago low beta background planchette (47 mm) counter was used. This instrument had a 50% efficiency with a ^{14}C source. Samples were counted the length of time required to obtain at least 10 times the background count. All samples were corrected for isotopic decay and background. Planchettes were monitored before use for background contamination and were used only if background values were equal to or less than 2 counts/minute.

The Determination of the Uptake Kinetics for $DI^{32}P$, $FU^{32}P$, and $FR^{32}P$

In experiments designed to measure the uptake of $DI^{32}P$, the production of $FU^{32}P$ and the utilization of $FR^{32}P$ and $FU^{32}P$ in situ, I used 4-liter bag of polyethylene 0.5 mm thick. The large size of the enclosures minimized the surface-to-volume effects (i.e., adsorption and bacterial growth). Samples were incubated only for short periods of time so as to avoid changes in planktonic flora. The plastic used is nontoxic, inert, and highly permeable to O_2 and CO_2. Bags were

filled with four individual 1-liter Kemmerer subsamples from the appropriate depth. This minimized variations resulting from plankton patchiness. Samples were taken while drifting randomly over the surface of the lake. Bags were covered during filling so as to minimize any effects of sunlight on the phytoplankton. The bags are good for one to six days; after this time fouling is evident (Porter 1972).

To make possible in situ sampling of bags placed at several depths, I installed a device which allowed surface sampling. This consisted of a plastic tube (3.18 mm ID) placed through a rubber stopper which was inserted into the bag. The other end of the tube was attached to tygon tubing (3.18 mm ID) which extended to the surface. At the surface, connection was made to a glass tube placed through a rubber stopper sized to seal with either a 250 ml or 125 ml polyethylene sampling bottle. During sampling, a polybottle was placed on the stopper, collapsed by hand, and then released. This suction filled the bottle within 30 seconds. When more than one depth was sampled, bottles were started as described, and then placed below the water line in a bucket attached to the raft. This allowed the samples to fill by siphon.

The rubber stopper was attached to the bag with several loops of string which fit into a groove on the side of the stopper. This and the fact that the large end of the tapered stopper was placed inside the bag, insured that the sampling device did not pull out of the bag during sampling. The rubber stopper also contained a second rigid plastic tube fitted with a septum. This allowed injection of isotope or antibiotic to the contents of the bag with a syringe ensuring exact measurement of starting times for the time-dependent $DI^{32}P$ uptake

Figure 14. Radiophosphorus separation scheme used to differentiate between Part-^{32}P, $FR^{32}P$ and $FU^{32}P$. Filtrate ^{32}P is split into $FR^{32}P$ and $FU^{32}P$ by a 30-second extraction of an $FR^{32}P$-molybdate complex in n-hexanol, a water immisable compound.

Total ^{32}P (125 ml sample)

↳ 40 ml (two 10 ml subsamples = total ^{32}P dried on monitored planchetts)

↓ 85 ml

0.45 μ filtered (+5 ml distilled H_2O wash, optional)

↓

Filterable total ^{32}P

↳ 35 ml (two 10 ml subsamples = filterable total ^{32}P dried on monitored planchetts)

↓ 50 ml

Extraction procedure for $FR^{32}P$

↳ organic extract = $FR^{32}P$

↓

Aqueous extract

$FU^{32}P$ (two 10 ml subsamples = $FU^{32}P$ dried on monitored planchetts)

Then:

Total ^{32}P - filterable $^{32}P^*$ = $\underline{\text{Particulate } ^{32}P}$

Filterable $^{32}P^*$ - $FU^{32}P^*$ = $\underline{FR^{32}P}$

$FU^{32}P$ = $\underline{FU^{32}P^*}$

*Corrected for dilution by reagents and corrected for isotopic decay.

Figure 14.

experiments. Also, the corners of the bags made excellent mixing pockets when inverted into the interior of the labelled bag.

Immediately after sampling the bags, subsamples were taken from the polybottles and filtered through 0.45 μ membranes that had previously been washed with dilute HCl and distilled water. The filters were then quickly washed with 5 ml of distilled water. All the filtration was done in a boat on the lake immediately after sampling. Multiple experiments at several depths were run at the same time to reduce the effect of any natural physiological uptake rhythms on the uptake and excretion experiments.

After filtering, those samples that were analyzed for $FU^{32}P$ production rates were taken immediately to the dock, the filtered samples were subsampled and then tested for $DI^{32}P$ and $FU^{32}P$ according to the modified method of Shapiro (1973). Samples taken just for $DI^{32}P$ utilization were not extracted, thus the filtrate included both $FU^{32}P$ as well as $DI^{32}P$. The sampling scheme resulted in three fractions: total ^{32}P, total filterable ^{32}P, and filterable unreactive ^{32}P (FUP). From these data, particulate ^{32}P (Part-P), filterable reactive ^{32}P ($FR^{32}P$), and filterable unreactive ^{32}P ($FU^{32}P$) were calculated (Figure 14).

The exact methodology used for the separation of $FR^{32}P$ from $FU^{32}P$ is:

(1) Filter lake water (0.45 μ membrane).

(2) Place in a 125-ml separatory funnel, in sequence, 50 ml of sample, 15 ml of n-hexanol, 5 ml of a 1% aqueous solution of ammonium molybdate (previously made to pH 8.0 with NH_4-OH).

(3) Add 2.0 ml of 4.0 N HCl, stopper and immediately shake. After 1 min, let stand for 2 min, then drain first 25 ml into an acid-washed polybottle.

(4) Take two 10-ml subsamples of the aqueous extract, dry at 70-80°C on previously monitored planchettes.

(5) The remaining solution, i.e., (a) unfiltered solution for total ^{32}P and (b) filtered but nonextracted solution for filterable total ^{32}P, are treated as in procedure (4).

This procedure was tested to determine the contact times required between acidified molybdate and the sample water containing $^{32}P_i$. Times of 1, 2, 4, and 6 min gave no differences in the amount extracted. Drying of samples did not alter the $^{32}P_i$ content as all $^{32}P_i$ added was recovered after drying. However, it was found that upon drying, the added extracts of both n-butanol and n-hexanol were incompletely recovered and that variable results were obtained. This analysis was discontinued in subsequent lake samples.

It was also determined that the order of addition of samples was important; e.g., if HCl is added before molybdate, twice as much $DI^{32}P$ remains unextracted. I also tested the method for extraction of $DI^{32}P$ at various concentrations to ensure the independence of the method from $DI^{32}P$ concentration (Table 6). Since extraction of $DI^{32}P$ was near 97% except for the lowest activity tested which equalled close to 95%, I corrected all $FU^{32}P$ values for 5% nonextracted $FR^{32}P$. All samples were duplicated.

The extraction of the 12-molybdo-phosphoric acid into organic solvents has been reviewed by Olsen (1967). In seawater Protor and

Table 6. Efficiency of DI^{32}P extraction from lake water.

Table 6.

µCi Activity Added	Total Counts Added	Total Counts Not Extracted*	Percent Nonrecovery
1.0	1,364	70	5.15
5.0	6,820	218	3.20
10.0	13,641	369	2.70
25.0	34,104	1,016	2.98
40.0	54,566	1,592	2.92
50.0	68,258	1,659	2.43
			$\bar{x}$ = 3.23

*Corrected for dilution by the extraction reagents.

Hood (1954) recommended the use of isobutanol and Stephens (1963) suggested its use for either fresh or marine waters. Shapiro (1973) reported a quick method which limits the time of contact of the acidified molybdate reagent with lake water. Lindberg and Ernstein (1956) recommend the use of organic extraction by equal volumes of benzene and isobutanol. This limited contact time to less than 15 seconds, and these authors reported that the great advantage of this method is that it is practically independent of concentration over the entire range of orthophosphate used. Golterman and Wurtz (1961) and Heron (1961) used n-hexanol which is much less soluble than the other organic alcohols used for extraction. For this reason, I used n-hexanol in experiments reported here. Hexanol extraction also reduces the acid-catalyzed hydrolysis of the linkage by which the phosphate is bound to organic radicals. The 10-min reduction of the 12-molybdophosphoric acid complex in acid called "free phosphate" analysis includes, besides orthophosphate, acetyl phosphate, ribose-1-phosphate, carbamyl phosphate, and partially creatine and arginine phosphates. Weil-Malberke and Green (1951) found that the effect of molybdate on the removal of phosphate from organic phosphate esters (molybdenolysis) was decreased by extracting the molybdophosphoric acid complex into isobutanol. Thus, extraction with organic solutions seems justified to reduce acid hydrolysis of labile phosphate esters.

The Calculation of ^{32}P Kinetics

Phosphate is removed from solution either exponentially or linearly depending on the temperature, biomass, and nutrient level of the sample. There are several assumptions made when radio labelled

tracers are used to measure the flux rates of their stable counterparts: Two assumptions should be noted: (1) there is uniform mixing of the tracer with the dissolved orthophosphate pool (DIP) in the sample water, i.e., the specific activity ($^{32}P/^{31}P$) remains constant during the experiment, and (2) a steady-state is assumed between the particulate and dissolved phases. Such a two compartment transfer model for phosphate uptake is described below.

Calculations:

General: If A = activity (^{32}P)

A_t = activity at any time (t)

A_o = initial activity

then the disappearance of tracer from the soluble phase can be described by the equation:

$$A_t = A_o e^{-kt}$$

$$\text{or } k = \ln (A_t - A_o)/t$$

where k = rate constant.

A more general formula is $k = \ln (A_{t1} - A_{t2})/\Delta t$

To simplify the calculations, base 10 logs (Logs) can be used in place of base e logs (ln). This allows the use of semi-log paper. Thus:

$$k' = \log (A_{t1} - A_{t2})\Delta t \qquad (1)$$

Here k' = the slope of a semi-log plot of filtrate activity (y-axis) vs time (x-axis). This rate constant (k) is expressed:

$$k = 2.303 \times k' \text{ or } k'/0.4343 \qquad (2)$$

DIP turnover time (1/k) is the time required for the plankton to metabolize an amount of inorganic phosphate just equal to the quantity of phosphate in the water.

The rate constant (k) is a relative number which indicates the fraction of the DIP pool being transferred to particulate matter (mostly phytoplankton; some bacterial uptake; some clay adsorption, etc.) per unit time, i.e., as proportion per unit time or as 100 k which equals per cent per unit time. The quantity of DIP being taken up is calculated by multiplying the rate constant by the concentration of DIP in the water:

$$v = \text{uptake rate } (\mu g/l \cdot min) = k(DIP) \quad (3)$$

Knowing this and the amount of particulate P in the water we are able to estimate the turnover time of P in particulate form:

$$\text{Turnover time pp (min)} = (PP)/v \quad (4)$$

The two compartment transfer model as described above is applicable when dealing with the initial slope of the ^{32}P uptake curve because the specific activity of the tracer does not change. However, one of the problems associated with tracer experiments is the distinction between a one way process of uptake or loss, and a two way exchange. A number of early tracer studies were in error because only one-half of a two-way process was detected. Thus, what was reported to be uptake was really half of an exchange process (Pomeroy 1975).

Thus, when a steady state is reached in a tracer experiment (i.e., non-changing ^{32}P fractions), a two compartment exchange model was used which corrects for the change in tracer specific activity. The calculations are essentially the same except that instead of filtrate ^{32}P being plotted vs. time, the asymptote value for filtrate ^{32}P is first subtracted from filtrate $^{32}P_i$, i.e., $(A_t - A_\infty)$. It is these values which are plotted on semi-log paper vs. time.

$$\text{Then, the slope } (s') = \text{Log } [(A_{t1} - A_\infty) - (A_{t2} - A_\infty)]/\Delta t \quad (5)$$

$$s = 2.303 \times s' \text{ or } s'/0.4343$$

The resulting value of (s) is corrected for specific activity changes by multiplying (s) by the ratios of cell ^{32}P to filtrate ^{32}P to give k_1:

$$k_1 = \text{Log } [(A_{t1} - A_\infty) - (A_{t2} - A_\infty)]/\Delta t \times \frac{(A_o - A_\infty)}{A_o} = k \qquad (6)$$

Then $1/k_1$ is again equal to DIP turnover time, and k_1(DIP) = v = uptake rate ($\mu g \cdot min^{-1}$). At true tracer equilibrium k_1 should be equal to k (equations 2 and 3).

When filtrate ^{32}P was not entirely $DI^{32}P$, i.e., when $FU^{32}P$ was detected as part of filtrate ^{32}P, a three compartment model was used. In these calculations, $FU^{32}P$ was first subtracted from filtrate ^{32}P to give $DI^{32}P$. $DI^{32}P$ was then used to calculate the uptake constant as for the two compartment exchange model. Such calculations correct for biphasic kinetics, i.e., a nonlinear semi-log plot of filtrate ^{32}P vs. time.

Using the above information, I calculated the flux of inorganic phosphate into the algal and bacterial cells in North Gate Lake, and I estimated the concentration of $DI^{32}P$. These data are not directly available due to the lack of chemically detectable FRP.

$$\text{Thus, } k_1 = s \frac{(A_o - A_\infty)}{A_1} \text{ or } k_1 = s \left(\frac{\text{cell P}}{\text{total P}}\right) \qquad (7)$$

Since $S = k_1 + k_2$; when k_1 = rate constant for DIP uptake, (8) k_2 (the rate constant for DIP loss from the plankton) can be calculated (Hayes et al. 1952).

Diel Metabolism of Phosphorus

I investigated the diel differences in the metabolism of phosphorus at several depths in North Gate Lake using 3000 liter in situ plastic cylinders. For the detailed description of the cylinder construction, see Koenings (1976). In brief, the cylinders were polyethylene tubes 1 m in diameters and 4 m deep. The cylinders were open at the top and bottom and, as in the small bag experiments, were run in duplicate approximately 2 m apart. Samples were taken from the middle of the cylinders to reduce the effects of phosphate absorption on the walls of the cylinder. The methods used to collect samples were similar to those used in the small bag experiments, i.e., samples were taken at the surface through tygon tubing placed at depth prior to the beginning of the experiments. Samples were taken simultaneously in both cylinders at all depths (surface to 2.5 m in 0.5-m intervals). Samples were treated as in the small bag experiments except that filtering and extraction were carried out in the laboratory within 20 min of sampling.

To study phosphorus metabolism of the plankton and bacteria in the lake, one cylinder was labelled at 4 depths (surface, 0.5 m, 1.0 m, and 1.5 m) at dusk-dark (9 p.m.) and sampled the next morning. After sampling was complete, the second bag was labelled in the same manner at 9:00 a.m. and then sampled 10 hrs later before dusk. This enabled the determination of phosphate concentrations due to dark and light metabolism.

Environmental Constraints on $DI^{32}P$ and $FU^{32}P$ Kinetics

To determine the effect of light or photosynthesis on the $Part\text{-}^{32}P$, $FR^{32}P$, and $FU^{32}P$ fractions, I incubated the small bags at

light levels (depths) different from those from which the enclosed samples were taken. In addition, I covered the bags at the depth sampled with opaque plastic coverings. Finally, I incubated large plastic cylinders only in the dark and then only in the light and compared phosphorus fractions. The use of the above procedures allowed me to ascertain the effect of light and temperature on $FR^{32}P$ utilization and $FU^{32}P$ production by bacteria and phytoplankton.

The Separation of Bacterial and Algal Cycling of ^{32}P

To confirm strata by strata differences in biological activity (algal vs. bacterial), I used antibiotics to inhibit the growth and metabolism of both gram-negative and gram-positive bacteria through the use of streptomycin and penicillin G. This series of experiments (0.0 m to 2.5 m in 0.5-m intervals) was started at the same time that an untreated depth series was begun which served as a control.

Individual 4 liter polyethylene chambers were used at each depth with one series serving as a control and a second series serving as the experimental group. The results of these experiments were compared to those of the 3000 liter cylinders to determine if enclosure of the sample in small bags had affected any of the results obtained.

The rationale of using antibiotics effectively in algal cultures was reported by Spencer (1952). With 50 units ml^{-1} of penicillin G and 100 units ml^{-1} of streptomycin sulfate, bacterial activity was reduced for at least 7 days without affecting algal cultures. Spencer concluded that the ratio of bacteria to diatoms was increased tremendously in favor of the diatoms by this treatment. Rigler (1961) found that 100 units ml^{-1} of streptomycin and 100 μg ml^{-1} of penicillin was effective in reducing bacterial activity in zooplankton excretion

experiments. Hargrave and Geen (1968) used 50 μg ml^{-1} of penicillin G and 100 μg ml^{-1} of streptomycin to reduce bacterial activity in flagellated phytoplankton and zooplankton cultures without affecting uptake of ^{14}C by phytoplankton. Finally, Kaushik (1975) used only 30 μg ml^{-1} of each of these antibiotics to effectively reduce bacterial activity in river water.

I chose to use 50 μg ml^{-1} (80 units ml^{-1}) of penicillin G and 100 μg ml^{-1} of streptomycin sulfate to reduce bacterial activity. This was effective for periods greater than 7 days. It is important to remember that as Spencer (1952) concluded, the antibiotics might have a direct effect on the algae, but a more probable role lies in their indirect action. That is, the more the algae are dependent upon symbiotic bacteria for essential nutrients or growth factors, then the control of the bacterial flora may ultimately limit algal growth. Bacteria are normally found tenaciously attached to diatoms and may penetrate the gelatinous sheaths of blue-green algae.

The Utilization of ^{32}P Added as $FU^{32}P$ and $FR^{32}P$ by Bacteria and Phytoplankton

Filtrate ^{32}P was then added to a surface sample of phytoplankton (a composite of three 1-liter subsamples) at a 3:1 ratio of lake sample to filtered lake water containing ^{32}P. Since slight changes in turnover times of $DI^{32}P$ have been observed in the epilimnion as well as partial complexation of $FR^{32}P$ to COM through an iron bridge (Koenings and Hooper 1976), I incubated a control experiment consisting of a similar 3:1 dilution of lake sample and filtered lake water (without $FR^{32}P$ and $FU^{32}P$) with $DI^{32}P$ (i.e., stock ^{32}P or free $DI^{32}P$). This

series of experiments were undertaken at 0.0 m and 2.0 m to both test the ability of phytoplankton (0.0 m) and bacteria (2.0 m) to reuse algal FU^{32}P, and to evaluate a possible differential utilization of FR^{32}P compared to free DI^{32}P.

To test this reutilization further, I incubated a surface sample of plankton in a darkened 4-liter bag with DI^{32}P for 4 days. This time period is many fold greater than the turnover time of the sestonic phosphorus so that all phosphate within the system has been "turned over" by the plankton as well as equilibrating with any abiotic transformations, i.e. complexation with COM and iron.

Results and Discussion

Uptake of DI^{32}P and Formation of Part-^{32}P

To differentiate between algal and bacterial cycling of ^{32}P, and the importance of each at the specific depths tested, I added antibiotics to a series of depths (0.0-2.5 m in 0.5 m intervals). A second complete depth series served as controls.

In the antibiotic treated samples (which were inoculated with ^{32}P three hrs after the introduction of the antibiotics), phosphorus was taken up by the plankton less rapidly than in the untreated controls (Figure 15). This delay period in the uptake of ^{32}P increased with depth through to 1.0 m, the Secchi disk depth. Below this strata inorganic phosphorus was not converted to Part-^{32}P (compared to control levels) during the entire 7-day experimental period (Figure 15). Thus, in the antibiotic treated samples the FR^{32}P levels remained close to 80% of the total ^{32}P added (at 1.5 to 2.5 m) while in the untreated controls FR^{32}P represented less than 5% of the added ^{32}P. Obviously,

Figure 15. The effect of the addition of antibiotics (AB) on phosphate absorption by the seston present in 6 strata of North Gate Lake.

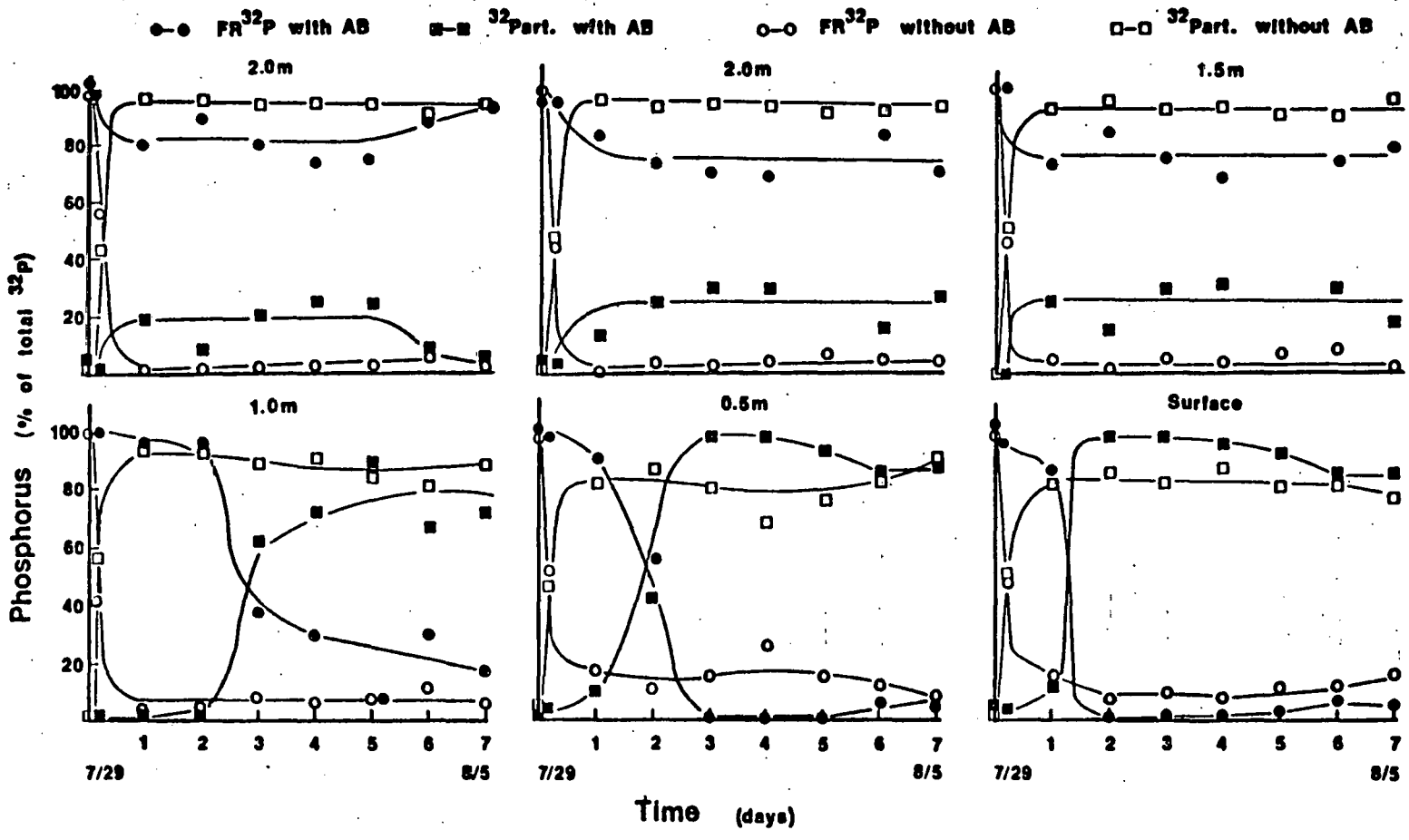

●–● FR 32P with AB
■–■ 32Part. with AB
o–o FR 32P without AB
□–□ 32Part. without AB
2.0m
2.0m
1.5m
1.0m
0.5m
Surface
Phosphorus (% of total 32P)
100
80
60
40
20
1 2 3 4 5 6 7
7/29
8/5
Time (days)

Figure 15.

the lower strata contain populations of aquatic bacteria that are metabolically inhibited by antibiotics. However, in the treated upper three strata (surface-1.0 m) $FR^{32}P$ is taken up rapidly, but the time until the asymptote is reached increases with depth incidating that bacteria become more important in the phosphorus dynamics of these strata with an increase in depth. In these antibiotic treated samples, unlike those of lower strata, phosphorus is still extracted from the water by the seston indicating activity of metabolizing phytoplankton.

In summary, a depth series from surface to 2.5 m contains decreasing amounts of metabolically active phytoplankton, with an increasing importance of aquatic bacteria. Below 1.0 m the organisms dominating the metabolism of phosphorus as bacteria, while above this depth phytoplankton increase in importance.

In the control (i.e., no antibiotics) small bag experiments, amounts of Part-^{32}P found in the surface phytoplankton-dominated strata were lower (82.6% and 81.3% of total ^{32}P) than the Part-^{32}P fractions in bacterial strata. Thus, 1.0 m (Secchi disk depth) had 87.6% Part-^{32}P while the bacteria-dominated depths of 1.5 m, 2.0 m, and 2.5 m contained 93.3%, 93.7%, and 95.3% Part-^{32}P (Figure 15). Accompanying this increase in Part-^{32}P with depth was a decrease in $FR^{32}P$. The amount of equilibrium $FR^{32}P$ in the photic zone equalled 12.1% at the surface, 15.06% at 0.5 m, but decreased to 8.65% at 1.0 m. Again, 1.5 m demarcated a further drop to 2.71% $FR^{32}P$ in the bacterial layer with 4.39% at 2.0 m, and 3.80% $FR^{32}P$ at 2.5 m.

In summary, it appears that the phytoplankton are turning over $DI^{32}P$ very rapidly and establishing a larger pool of cycling $FR^{32}P$ in the euphotic zone compared to the aphotic bacterial strata. In the

upper two strata active photosynthesis if taking place, during which $FR^{32}P$ is taken up very rapidly and incorporated into organic form. At the same time, an $FR^{32}P$ pool is maintained to provide adequate inorganic nutrients for further synthetic reactions. Thus, the evidence supports the observations of Schindler (1973), Lean (1975), Schindler et al. (1975), and Likens (1975), that plankton excretion is a primary mechanism responsible for the steady-state level of FRP in the epilimnion of nutrient-deficient lakes. This rapid cycling provides the inorganic phosphate necessary for the maintenance of the algal population. However, for substantial net growth of the plankton to occur additional input of new phosphate from allochthonous sources is needed.

Below the photic zone heterotrophic bacteria dominate the system, and a shift in phosphorus cycling strategy takes place. Here the bacteria take up nearly all the inorganic nutrient available (>93% Part-^{32}P) and more importantly maintain a smaller (<5% $FR^{32}P$ pool. Thus, the bacteria absorb the phosphate and retain it. When faced with the situation of a phosphorus-deficient substrate (e.g., colloidal organic phosphorus), bacteria resort to inorganic sources and strip phosphate from the water column. This phosphate is necessary to meet metabolic demands and is not returned to the dissolved phase. Thus, mineralization reactions (i.e., an increase in dissolved phosphate) will not be observed in bacteria-dominated communities limited by a shortage in substrate phosphorus. Thus, the evidence supports the hypothesis of Pomeroy (1975) that bacteria are not always major regenerators of FRP in natural waters, especially nutrient-deficient waters. Thus, heterotrophic bacteria may only act as regenerators of FRP when

presented with either substrates or an environment containing an amount of phosphate over and above that required for metabolic reactions. The immobilization of $DI^{32}P$ by the bacterial layer is similar to the results of other studies in both terrestrial and aquatic systems which have repeatedly demonstrated the immobilization of inorganic phosphorus and nitrogen by microbes associated with decomposing plant material with high C:P:N ratios.

Production of $FU^{32}P$

Antibiotic treated samples at 1.5 m and 2.5 m show little Part-^{32}P activity and high amounts of unabsorbed $FR^{32}P$. In these strata, $FU^{32}P$ was never detected. However, the antibiotic treated samples of the surface, 0.5 m and 1.0 m contained a major fraction of Part-^{32}P, low levels of $FR^{32}P$ and detectable amounts of $FU^{32}P$ on all dates sampled (Figure 16). The formation of $FU^{32}P$ is dependent upon the uptake of $FR^{32}P$ and its conversion into organic linkages. This argues that the formation of $FU^{32}P$ is biologically mediated in North Gate Lake.

In the control experiments at 1.5 m to 2.5 m, only a small amount of $FU^{32}P$ was found in the presence of a large fraction of Part-^{32}P (Figure 16), but $FU^{32}P$ did not increase over time. This is contrasted to the surface to 1.0 strata where $FU^{32}P$ did increase over time. If $FU^{32}P$ formation was the result of the continual process of death and decay (autolysis) of sestonic cells, $FU^{32}P$ should increase overtime at all depths. Thus, the formation of $FU^{32}P$ must be an active metabolic process that closely balances $FU^{32}P$ excretion and $FU^{32}P$ utilization. For example, in the upper three depths in the presence of actively metabolizing phytoplankton cells, $FU^{32}P$ excretion exceeds $FU^{32}P$ utilization and $FU^{32}P$ activity increases (Figure 16).

Figure 16. A comparison of the distribution of FU^{32}P in several strata in the presence and absence of antibiotics (AB). Between day 3 and day 4 the bags indicated by "dark" were covered with opaque plastic coverings to reduce photosynthesis. Those not covered served as controls.

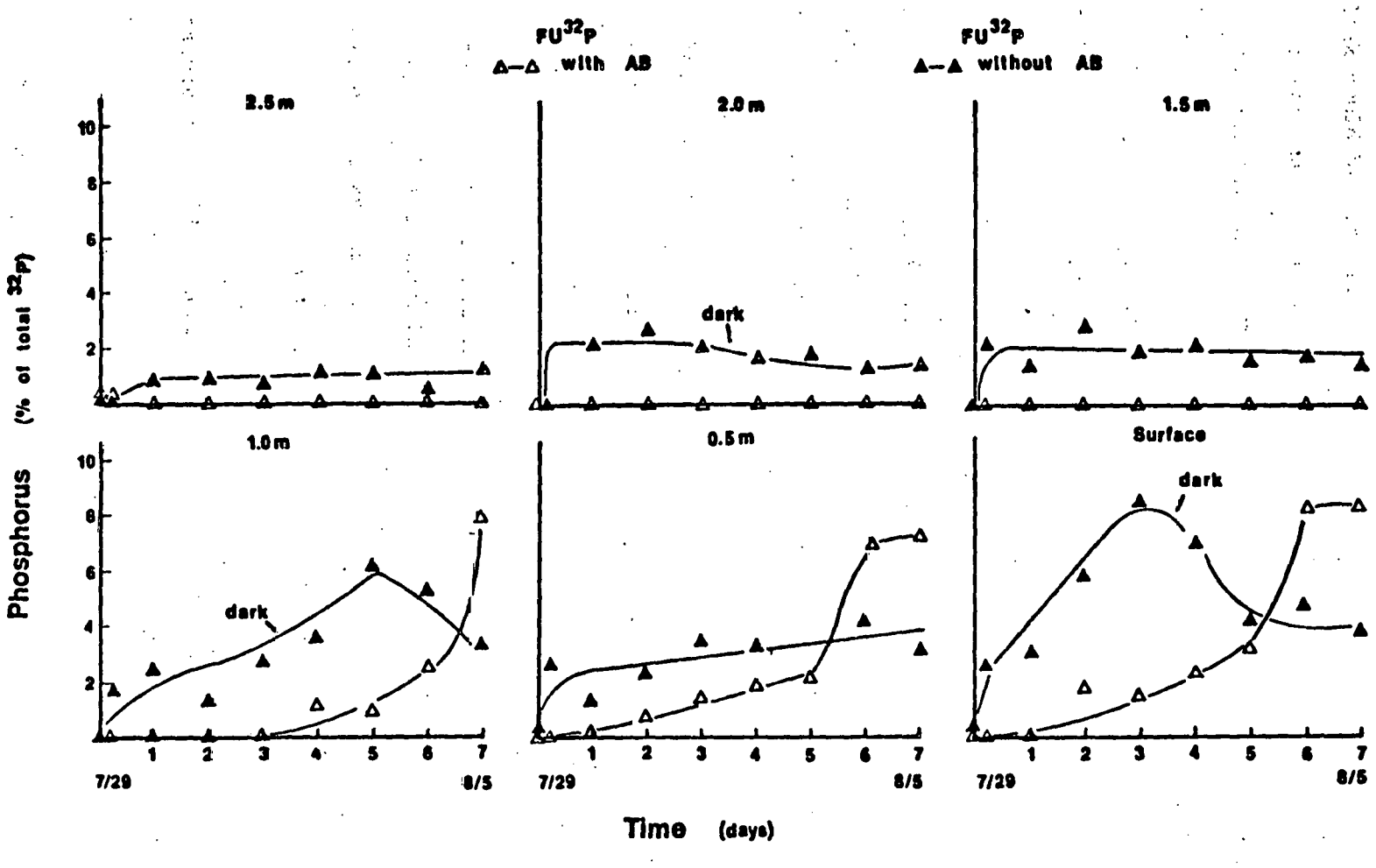

FU32P
Δ—Δ with AB
FU32P
▲—▲ without AB
2.5m
2.0m
1.5m
1.0m
0.5m
Surface
dark
Phosphorus (% of total 32P)
Time (days)
7/29
8/5

Figure 16.

Since the autotrophic layers actively produced $FU^{32}P$, an apparent link could be surmised to exist between the driving force of autotrophy, photosynthesis, and the active production of $FU^{32}P$. If so, a reduction of the photosynthetic process would tend to reduce $FU^{32}P$ production. This would have the effect of artificially magnifying the importance of reutilization reactions resulting in a reduction of the pool size of $FU^{32}P$. To test this in situ, I covered the 4 liter bags with an opaque black plastic outer bag at the surface, 1.0 m, and 2.0 m strata after the sampling period on day 3. This left 0.5 m, 1.5 m, and 2.5 m as control experiments (Figure 16). Thus, photosynthesis was reduced in the covered bags while the uncovered bags could function normally, thereby acting as control experiments.

The net excretion of $FU^{32}P$ decreased (utilization exceeded excretion) after darkening the bags at the surface and 1.0 m (after day 3 and day 5), while in the control at 0.5 m the pool size of excreted $FU^{32}P$ continued to increase (Figure 16). In the lower strata there appeared to be little effect on the $FU^{32}P$ levels, although even here a slight decrease may have occurred in the darkened 2.0 m bag. Thus, $FU^{32}P$ excretion in the euphotic zone can be characterized as an active process that appears to be accelerated by an energy yielding metabolic process (e.g., photosynthesis).

These light and dark experiments (Figure 16) suggest the dynamic nature of $FU^{32}P$ production, i.e., that a portion of the $FU^{32}P$ pool once excreted could be reutilized under appropriate conditions. Since it has been well established that the reutilization of $FU^{32}P$ (i.e., organic phosphorus) depends on the productivity of a phosphatase enzyme external to the cell membrane, the relationship between $FU^{32}P$

and enzyme levels in situ was also studied.

Acid Phosphomonoesterase Activity and the Production of $FU^{32}P$

A vast difference existed in $FU^{32}P$ levels in the antibiotic treated and control experiments (Figure 16), as well as in the enzyme levels in these two systems (Figure 17). Those strata which showed very little $FU^{32}P$ activity (antibiotic treated) until later (day 7-8) in the experiment were those which also possessed the largest amount of enzyme activity earlier in the experiment. This newly formed algal enzyme functioned to hydrolyze $FU^{32}P$, decreasing the fraction of $FU^{32}P$ appearing in the antibiotic treated experiment. In the control experiments lower levels of enzyme allowed a larger fraction of $FU^{32}P$ to accumulate. A functional relationship exists between excreted $FU^{32}P$ and enzymatic hydrolysis reactions. The formation of this enzyme results in the partial utilization of $FU^{32}P$ excreted by the seston. Thus, the net production of detectable $FU^{32}P$ in North Gate Lake is a balance between its production by an active metabolic process and its degradation by enzymatic hydrolysis.

The production of this enzyme in the antibiotic treated samples is interesting in that it was found solely in the particulate fraction. Indeed, unlike the open lake water where >68% of the enzyme activity was filterable, >95% of the new enzyme production was particulate. In addition, the filterable enzyme activity in the bag experiments (unlike the particulate activity) declined over time suggesting that filterable enzyme activity extends to periods less than 7 days. The pool of filterable enzyme is thus dependent upon continual renewal from the particulate fraction in order to be detectable.

The bacterial layer treated with antibiotics did not show this

Figure 17. A comparison of the distribution and production of acid phosphatase (acid PMase) in the presence and absence of antibiotics (AB).

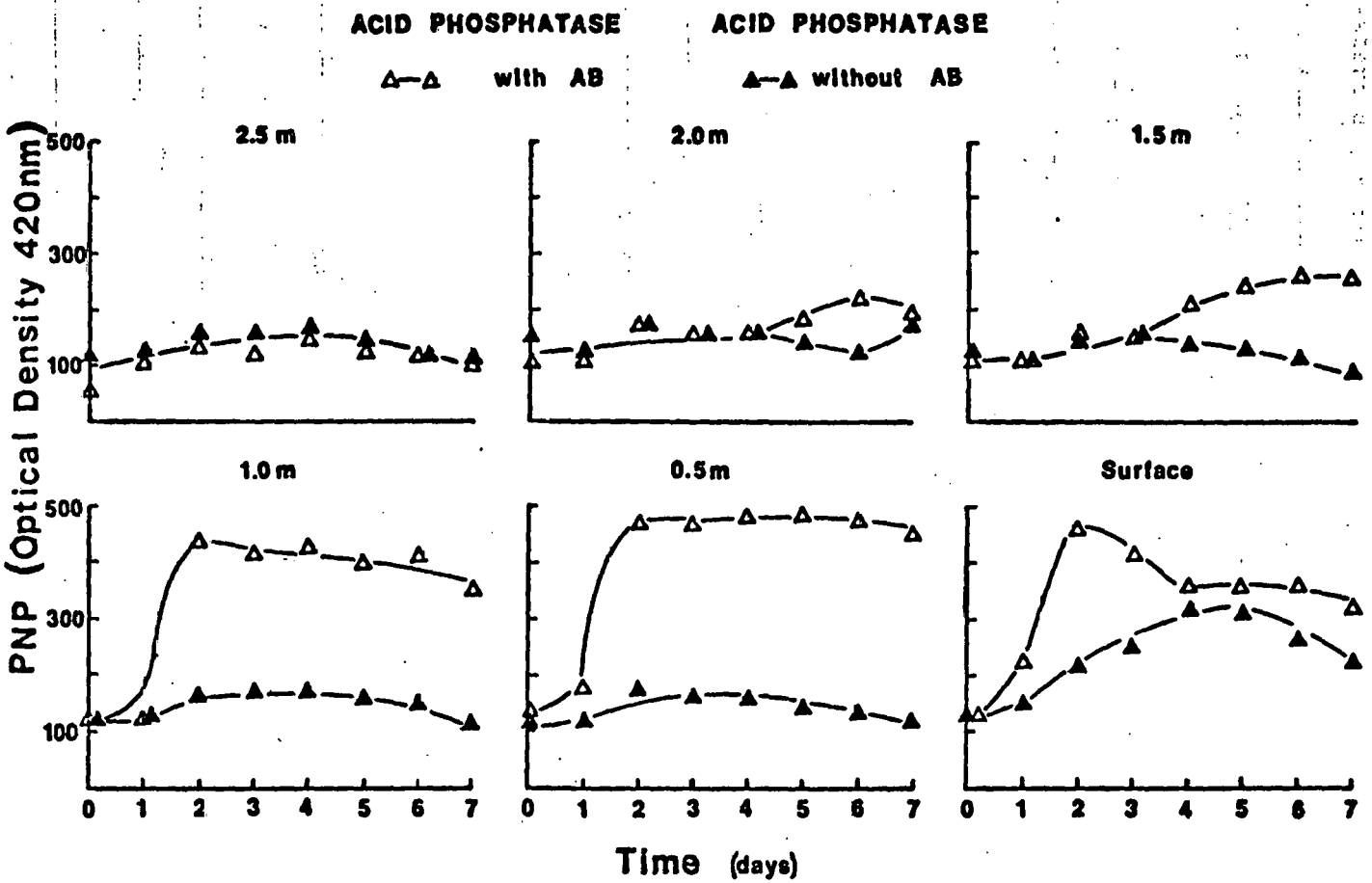

ACID PHOSPHATASE
△—△ with AB
ACID PHOSPHATASE
▲—▲ without AB
2.5 m
2.0 m
1.5 m
1.0 m
0.5 m
Surface
PNP (Optical Density 420nm)
500
300
100
0 1 2 3 4 5 6 7
Time (days)

Figure 17.

increase in enzyme activity since both control and treated experiments contained equivalent enzyme activity. The increase in acid phosphomonoesterase (PMase) activity was confined to the upper strata, and may be directly attributed to an increased synthesis by the algal population induced after treatment with antibiotic (i.e., bacterial inhibition).

In summary, $FU^{32}P$ synthesis was slow compared to $FR^{32}P$ equilibration with the Part-^{32}P pool, confirming a slow equilibration with the stable $FU^{31}P$ pool. However, in all cases studied, the 3000-liter cylinders and the 4-liter bags, by the time the first sample was taken (day 1) considerable $FU^{32}P$ was already present. It is evident that $FU^{32}P$ excretion, while not being quantitatively large, did take place very rapidly in North Gate Lake.

Cell autolysis is a potential source of $FU^{32}P$. However, if $FU^{32}P$ production was due solely to cell autolysis, then (1) $FU^{32}P$ should increase over time in the bacterial layers (not observed) as well as in the algal layers (observed) if bacterial autolysis controlled $FU^{32}P$ production and (2) if algal autolysis controlled $FU^{32}P$ production, $FU^{32}P$ should increase in both dark and light experiments (not observed) and not decrease as was observed only in the dark samples. These results agree with Richey et al. (1975) who concluded that algal autolysis plays only a minor role in filterable phosphorus production in Castle Lake.

Rates of $FU^{32}P$ Production

In the foregoing experiments, $FU^{32}P$ was detectable in the untreated controls by the first sampling period. So to determine the rates of $FU^{32}P$ production, the amount of $FU^{32}P$ was determined in

kinetic experiments after $DI^{32}P$ addition. In all experiments $DI^{32}P$ was quickly absorbed by the plankton (Figure 18), incorporated into cellular compounds, and a small fraction rapidly excreted as $FU^{32}P$. This metabolic $FU^{32}P$ quickly reached a maximum, but then decreased to a rather constant value (Figure 18). Thus, once leaked into the water, a portion of $FU^{32}P$ was biologically removed from solution leaving a fraction that is not as readily susceptible to seston utilization. This initial process (i.e., short-term leakage) is similar at all depths sampled. However, the rate of $FU^{32}P$ production decreases slightly with depth. At 0 m the rate constant equals 0.04667 min^{-1}, this falls to 0.04603 min^{-1} at 1 m and to 0.04088 min^{-1} at 2 m.

The initial appearance of short-term $FU^{32}P$ in the filtrate (<10 min) and its distribution over time suggests (1) that it is not far removed from the initial pool of cellular organic phosphorus initially labelled by absorbed $DI^{32}P$ and (2) that it is a low molecular weight compound (Saunders 1972; Fogg 1966). Even though the $FU^{32}P$ may be in a low molecular weight form that is more readily susceptible to enzymatic hydrolysis, this exponential production of filterable organic phosphorus by the seston in phosphorus deficient waters is somewhat of a paradox. The rate at which phosphorus is lost to the $FU^{32}P$ is nearly one-half the rate at which $DI^{32}P$ is absorbed. However, after 24 hrs, the bacterial layers (1.5 m, 2.0 m, 2.5 m) do not show a further net increase in $FU^{32}P$ (Figures 16 and 19), while in the euphotic zone (surface, 0.5 m, 1.0 m), $FU^{32}P$ shows only a slow increase. This long-term production of $FU^{32}P$ is depth dependent, and represents a net increase in only a small fraction of $FU^{32}P$ that is not susceptible to immediate algal utilization.

Figure 18. The short term, in situ production of FU^{32}P by the surface, 1 m, and 2 m plankton in North Gate Lake.

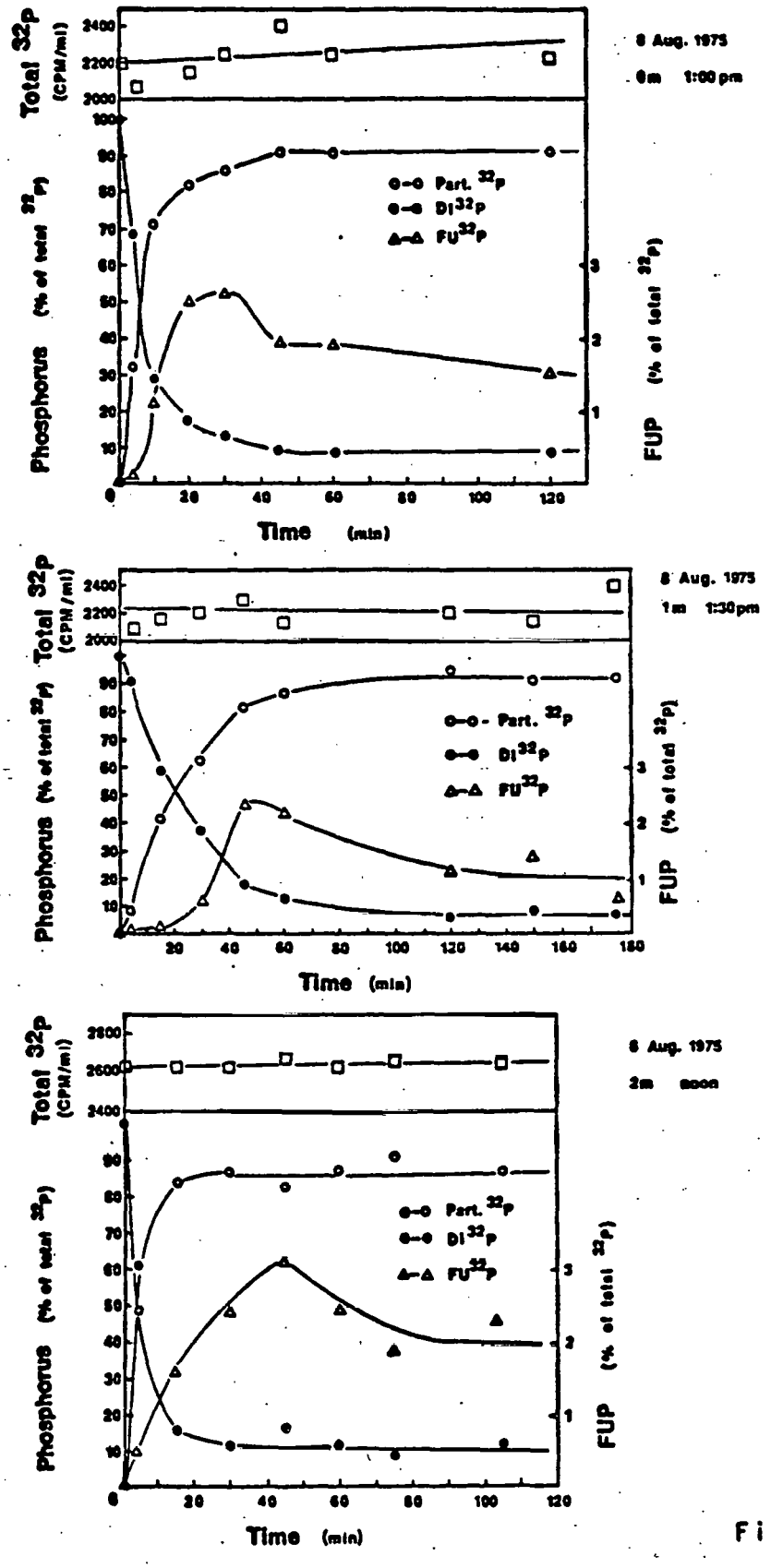
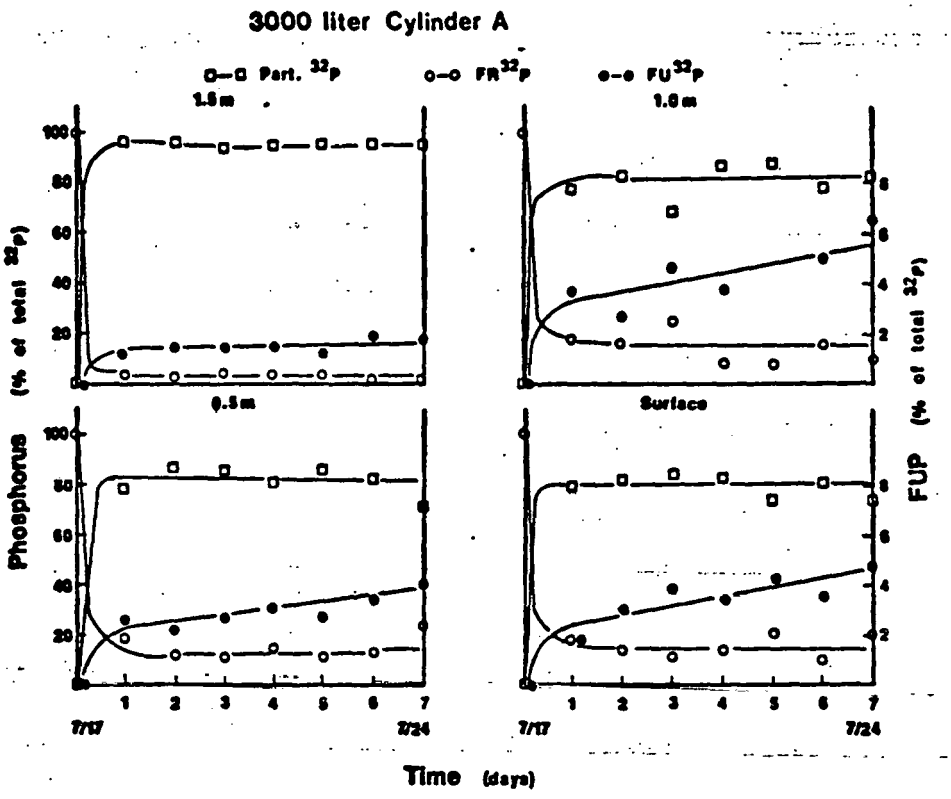

Total 32p (CPM/ml)
Phosphorus (% of total 32p)
FUP (% of total 32p)
Time (min)
8 Aug. 1975
8m 1:00 pm
o–o Part. 32p
●–● DI32p
Δ–Δ FU32p
8 Aug. 1975
1m 1:30pm
8 Aug. 1975
2m noon

Figure 18.

The rate of $FU^{32}P$ production (Figure 19) decreases from 0.00859 d^{-1} at the surface, to 0.00638 d^{-1} at 0.5 m, 0.00599 d^{-1} at 1.0 m and to 0.0 at 2 m. $FU^{32}P$ production averaged +0.00521 day^{-1} for all depths in the photic zone. At this rate of production (an assuming an equal rate of utilization), the FUP pool would have a turnover time ranging from 116 days to 290 days or an average turnover time equalling 192 days or nearly 6.5 months. This is further evidence that the rate at which the $FU^{32}P$ pool comes into isotopic equilibrium with the $FU^{31}P$ pool (if it ever does) is very slow. The production of long term $FU^{32}P$ is much slower than determined by the short term experiments of Watt and Hayes (1963) and Lean (1973). Similar short-term experiments in North Gate Lake also resulted in very fast rate constants (e.g., 0.04667 min^{-1}) for $FU^{32}P$ production. Thus, $FU^{32}P$ is produced very rapidly in time limited kinetic experiments, however, in long term experiments where reactions of production and of utilization are allowed to continue and integrate over time, the actual net production of $FU^{32}P$ is slight and the rate constant (0.00859 d^{-1}) correspondingly slow. Thus, short term and long term $FU^{32}P$ are not only qualitatively and quantitatively different, but also vary in rates of production. If short term excretion experiments are performed, the results would indicate a very rapid exponential productionof $FU^{32}P$. However, longer term experiments show that the initial exponential excretion rate is not maintained and the actual net production of $FU^{32}P$ is a slow linear process.

Finally, strata by strata differences in both the quantity and quality of excreted $FU^{32}P$ were found. These different strategies of metabolic ^{32}P cycling are particularly interesting in light of the

Figure 19. Phytoplankton (0.0 - 1.0 m) and bacterial (1.5 m) partitioning of ^{32}P into $FU^{32}P$, $FR^{32}P$, and Part-^{32}P over time in North Gate Lake.


3000 liter Cylinder A
Part. 32P
FR 32P
FU 32P
1.6 m
1.6 m
0.5 m
Surface
Phosphorus (% of total 32P)
FUP (% of total 32P)
7/17
7/24
Time (days)

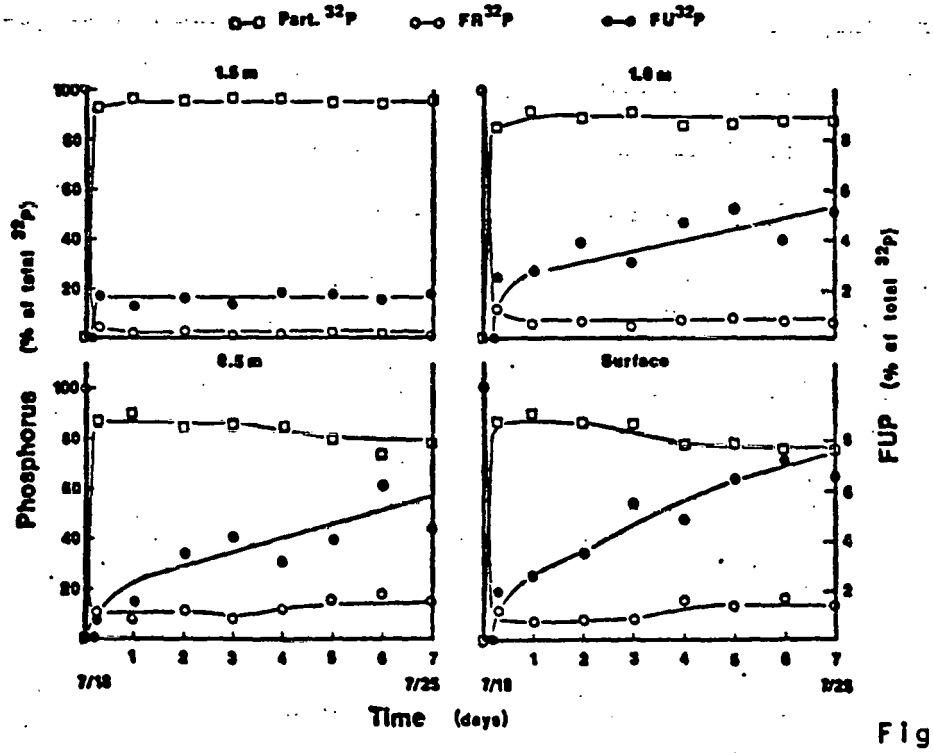

3000 liter Cylinder B
Part. 32P
FR 32P
FU 32P
1.6 m
1.6 m
0.5 m
Surface
Phosphorus (% of total 32P)
FUP (% of total 32P)
7/18
7/25
Time (days)

Figure 19.

potentially related differences in $FU^{31}P$ size distributions (Figure 10) of compounds making up the $FU^{31}P$ pool (Table 5) observed to exist between these same layers. The major pool of stable low molecular weight DUP in the surface strata is correlated with the production of $FU^{32}P$ by the plankton in that strata. However, since the bacteria at 2 m do not produce $FU^{32}P$, the major pool of $FU^{31}P$ (instead of being the lower molecular weight fraction) is found in the colloidal fraction (CUP), a high molecular weight form of detrital organic phosphorus.

Recycling of Filterable Phosphorus: $FR^{32}P$ and $FU^{32}P$

Levels of Part-^{32}P in the epilimnion of North Gate Lake showed a general increase with depth with a sharp increase below the Secchi disk depth (Figure 19). For example, in cylinder B values range from 82% at the surface to 88% at 1.0 m, but jump to greater than 96% at 1.5 and 2.0 m. The same general trend was seen in cylinder A which showed 79% Part-P at the surface and nearly 81% at 1.0 m. Here again Part-^{32}P increased to 95% of total ^{32}P below the Secchi disk depth. Thus, for the 7 days in each of three separate experiments less particulate ^{32}P was observed in the strata which contained populations of phytoplankton than was found in bacterial layers below the photic zone, due to the active recycling of phosphorus.

The recycling of phosphorus by the phytoplankton resulted in up to 16.9% of the phosphorus at the surface to 1.0 m being present as $FR^{32}P$ (average of 13.5%) while at 1.5 and 2.0 m only a maximum of 3.75% of the total ^{32}P was being recycled into $FR^{32}P$ (average of 2.91%). In addition, there was a small but measurable increase in $FU^{32}P$ over time and with depth. The values from the surface to 1.0 m were very close but decreased from 4.15% to 3.34% of total ^{32}P. At 1.5 and 2.0 m there

was again a sharp difference. Only 1.70-1.89% of total ^{32}P was present as $FU^{32}P$. Thus, the plankton in the photic zone maintained a large filterable pool of ^{32}P as $FR^{32}P$ (nearly 20% of total ^{32}P), while the bacterial layers below the photic zone maintained smaller pools of filterable ^{32}P (<5.0% of total ^{32}P).

Even though the quantity of excreted metabolic products are smaller, the bacteria appear to generate more $FU^{32}P$ compared to $FR^{32}P$ so that there is a general increase in the $FU^{32}P$:$FR^{32}P$ ratio with depth. Such a trend suggests that the phytoplankton appears to leak much more $FR^{32}P$ compared to a much more conservative excretion of $FU^{32}P$.

While $FU^{32}P$ slowly increases over time in the euphotic strata of North Gate Lake, the aphotic bacterial strata do not even show this increase. (Figures 15 and 19). This, combined with the changing $FU^{32}P$:$FR^{32}P$ ratio and the different amounts of $FR^{32}P$ excreted with depth suggests that excretion or turnover strategy for phytoplankton differs from that of the bacteria. Thus, cycling of filterable phosphorus back to the water column does not appear to be a function of bacteria whereas the bulk of regeneration of equilibrium $FR^{32}P$ appears to be the major responsibility of the phytoplankton.

Algal and Bacterial Usage of $FU^{32}P$

Apparent in the foregoing cylinder and bag experiments is the general trend of an exponential short term increase in $FU^{32}P$ (Figure 18) followed by partial reutilization, and a longer term, slow linear increase (Figure 19). Since long term $FU^{32}P$ does increase in the lake water, most of the material may be relatively resistant to immediate reutilization. Thus, these compounds may be recycled over a longer

Figure 20. The in situ reutilization of algal FU^{32}P by the phytoplankton (0 m) and bacteria (2 m) in North Gate Lake.

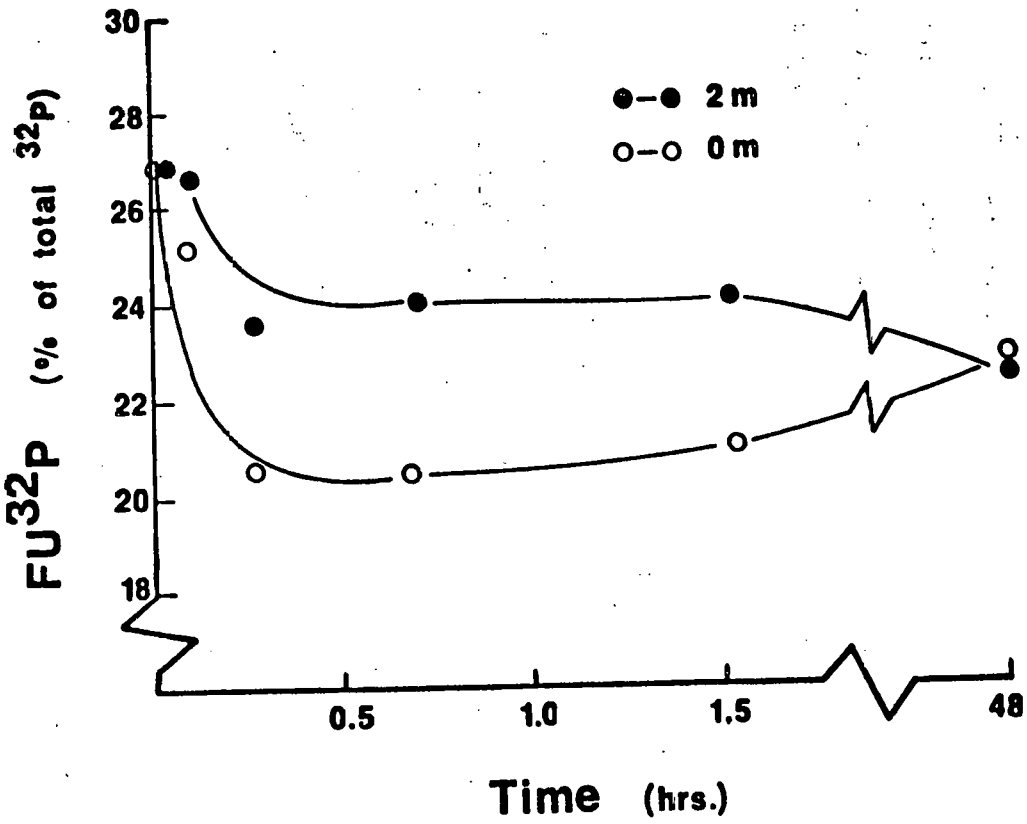

30
28
26
24
22
20
18
FU32P (% of total 32P)
●–● 2 m
o–o 0 m
0.5
1.0
1.5
48
Time (hrs.)

Figure 20.

period of time by bacteria, etc., but rapid seston utilization should be small. However, when production of $FU^{32}P$ was minimized by darkening selected samples, reutilization of $FU^{32}P$ did occur, suggesting that $FU^{32}P$ is not totally refractory material, i.e., a portion can be utilized by the seston.

Filtrate ^{32}P used as a source of $FR^{32}P$ and $FU^{32}P$ for further seston utilization experiments contained >72% $FR^{32}P$ and <28% of $FU^{32}P$. This confirms the existence of a larger pool of cycling inorganic phosphate compared to a smaller pool of $FU^{32}P$ as found in my earlier experiments.

The plankton within the surface strata utilized less than 25% of the $FU^{32}P$ supplied as a source of phosphorus within 30 minutes (Figure 20). This represents at 22.6% decrease in $FU^{32}P$ added originally from the filtered lake water, and a 3% net increase in newly produced $FU^{32}P$ (estimated from control experiments). The utilization curve (Figure 20) suggests that a portion of the $FU^{32}P$ was used very rapidly ($k = -0.02105\ min^{-1}$), while a greater fraction was refractory and was not further degraded for 2 days. This hydrolysis curve followed the same pattern reported by Johannes (1964) who looked at $FU^{32}P$ (produced by senescent algal cultures) utilization by growing phytoplankton cultures. He found up to 30% of the added $FU^{32}P$ was utilized within 5 hrs, which was the initial sampling period. Apparently, at least two functionally distinct pools of $FU^{32}P$ exists both in aged phytoplankton cultures and in natural waters as products of algal leakage.

Since $FU^{32}P$ utilization does not require a time scale drastically different from $FU^{32}P$ production, both processes influence the levels of

$FU^{32}P$ in lake waters. Indeed, the rate of the initial disappearance of $FU^{32}P$ ($k = -0.02105\ min^{-1}$) suggests that a portion of this fraction of filterable phosphorus can be used as a source of orthophosphate almost as quickly as $DI^{32}P$ ($k = -0.09442$).

At 2 m only 8.9% of the added algal $FU^{32}P$ was hydrolyzed and absorbed at a rate of $-0.01209\ min^{-1}$ (Figure 20). This fraction along with an estimated 2% increase in $FU^{32}P$ from new production results in a net utilization of 11% compared to algal utilization of 25%. Another difference in the two strata becomes apparent after utilization had reached a plateau (Figure 29). There was a net increase of $FU^{32}P$ in the surface experiment at 24 hr (as was observed for $FU^{32}P$ in several other experiments), but a net decrease or no change was observed in the bacterial experiment (as was observed in my earlier experiments) (Figure 19).

Since only 9-22% of the metabolically produced $FU^{32}P$ was reutilizable, 0.8-2% of the total ^{32}P is represented by reutilizable $FU^{32}P$ compared to a cycling pool of $FR^{32}P$ of 10-15% of total ^{32}P. It follows that $FU^{32}P$ does not serve as an important short-term source of phosphorus for algae. Since reutilization of $FU^{32}P$ plateaus, a considerable fraction must be considered relatively inert which increases over time adding very slowly to the pool of noncycling FUP.

Since both particulate and soluble PMase activity was always present in these bog waters, it seems reasonable to conclude that a major portion of excreted long-term $FU^{32}P$ does not consists of easily available simple phosphate esters, and a major fraction may in fact consist of complex, large molecular weight organic esters such as RNA or DNA (Table 5).

Apparent Reutilization of $FU^{32}P$ Due to Filtration Artifacts

To determine the effect of the filtration of bog water equilibrated with $DI^{32}P$ for 4 days on the concentration of $FU^{32}P$ and $FR^{32}P$ produced, I filtered the water and determined the quantity of $FR^{32}P$ and $FU^{32}P$. I then calculated the expected concentration of both phosphorus forms after adding 1 liter of the filtered water to 3 liters (4 liters total) of unfiltered lake water. I found that I could expect 137.0 cpm ml^{-1} of total ^{32}P and 39.0 cpm ml^{-1} of $FU^{32}P$. Thus, in the reutilization experiments, I could predict the levels of $FR^{32}P$ at 72% of the total phosphorus, and $FU^{32}P$ at 28% of the total phosphorus.

The initial values from unfiltered and filtered lake water of the reutilization experiment resulted in a total activity of 134.01 cpm ml^{-1}, and a soluble activity of 135.59 cpm ml^{-1}. This, of course, confirmed two things: that all the activity added was filterable as expected, and that 97.8% of the total label could be accounted for. Since I recovered 100% of the activity added in the filterable state, I concluded that I had not "reutilized" any $FU^{32}P$ at the time of the initial sample due to filtration. Furthermore, in the initial sample 36.0 cpm ml^{-1} was in the $FU^{32}P$ pool. This, of course, equals 26.5% of the total activity compared to a calculated value of 28% of total activity. Since recovery of the tracer was 100% for the filterable phase, filtration artifacts were not responsible for this error. Regardless of the difference found, it is important to note that all calculations of $FU^{32}P$ reutilization begin from the initial filtered value which automatically corrects for the fraction of the particulate ^{32}P that is really colloidal ^{32}P retained during the first filtration (Lean and Nalewajko 1976).

In acid bog lake water, reutilization of $FU^{32}P$ varies between 8.9-22.6% while in algal cultures experiments reutilization may approach 100% (Kuenzler 1970). The reason for high reutilization of $FU^{32}P$ results not from filtration artifacts but from using algal $FU^{32}P$ from lag phase cultures (Kuenzler 1970). This $FU^{32}P$ is potentially different from naturally produced $FU^{32}P$, given the environmental and physiological differences in the two populations, since the rate of production of extracellular products depends on physiological as well as environmental factors affecting membrane permeability (Hellebust 1974). Thus, results derived from batch cultures of phytoplankton may be of significance to natural systems only when the latter are under bloom conditions. Changes occur in the amount or kind of organic molecule excreted by algae under culture conditions. For example, Malewajko (1966) found that the excretion of dissolved organic carbon increased as the density of the algal culture increased. Orlova and Zlobin (1973) reported that the amount and the type of organic molecule excreted (i.e., amino acids) changed with the growth phase of an algal culture. During aging and autolysis, cells released all amino acids into the medium, but living, growing cells continuously excreted individual amino acids into the medium and selectively absorbed them from it. They concluded that (1) mechanisms of selected excretion and reutilization of amino acids exist to maintain an active balance of free and bound amino acids in the presence of a changing environment, (2) up to 33% of metabolites excreted during active metabolism are readily assimilated, and (3) the excretion of metabolites by algae is a normal physiological process.

Uptake Rate of ^{32}Phosphorus Added as DI^{32}P by Phytoplankton and Bacteria

Stable chemical analysis of North Gate Lake water indicated that for strata above the monimolimnion, phosphate was undetectable (i.e., <3 µg $^{31}PO_4$-P l^{-1}). This suggested that if phosphate is not limiting the plankton, it is at least in extremely short supply and is potentially being cycled very rapidly.

In situ radiochemical experiments were carried out to measure the rate of phosphate uptake at the surface, 1.0 m and 2.0 m strata. To reduce the influence of extraneous factors on the cycling rates of phosphate with depth (e.g., diel differences or day to day variations) all samples were taken on the same day and all experiments were started between noon and 1:30 p.m. with overlapping sampling periods. All determinations were carried out in situ with four one-liter subsamples at a depth equalling the total experimental sample of 4 liters.

As the stable phosphate was undetectable, the actual cycling was detectable only through the use of a tracer amount of marked atoms, i.e., $^{32}PO_4$-P. At the surface (1:00 p.m.) phosphorus was being turned over by the plankton at a rate of 0.09442 min^{-1} (Figure 21) or a replacement of dissolved phosphate every 10.59 min (turnover time). At 1 m (1:30 p.m.), the rate constant measured 0.03422 min^{-1} or a turnover time of 29.13 min. This represented an increase in the turnover time by a factor ~3.0 and may be due to three factors: (1) a decrease in the number of active plankton, (2) a decrease in temperature, and (3) a decrease in utilizable light. This trend was expected to continue into the 2 m depth (as indicated by the biological data (Figure 2) and the temperature regime but the rate constant (noon) surprisingly

Figure 21. The rate of decrease of filtrate ^{32}P representing the uptake of $DI^{32}P$ by the plankton in the surface, 1 m, and 2 m strata of North Gate Lake.

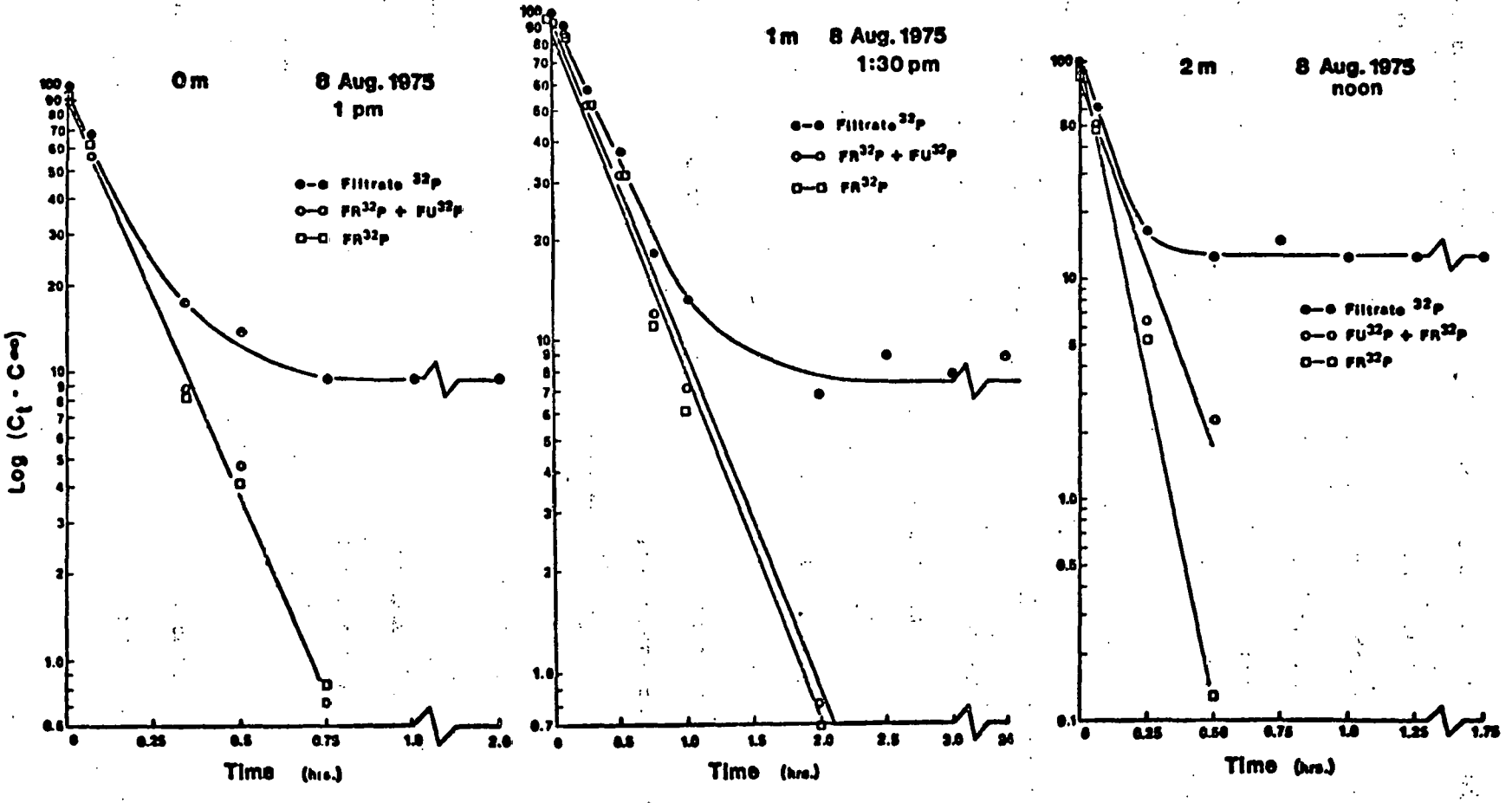

0 m
8 Aug. 1975
1 pm
1 m
8 Aug. 1975
1:30 pm
2 m
8 Aug. 1975
noon
Filtrate 32P
FR32P + FU32P
FR32P
Log (Ct - C∞)
Time (hrs.)

Figure 21.

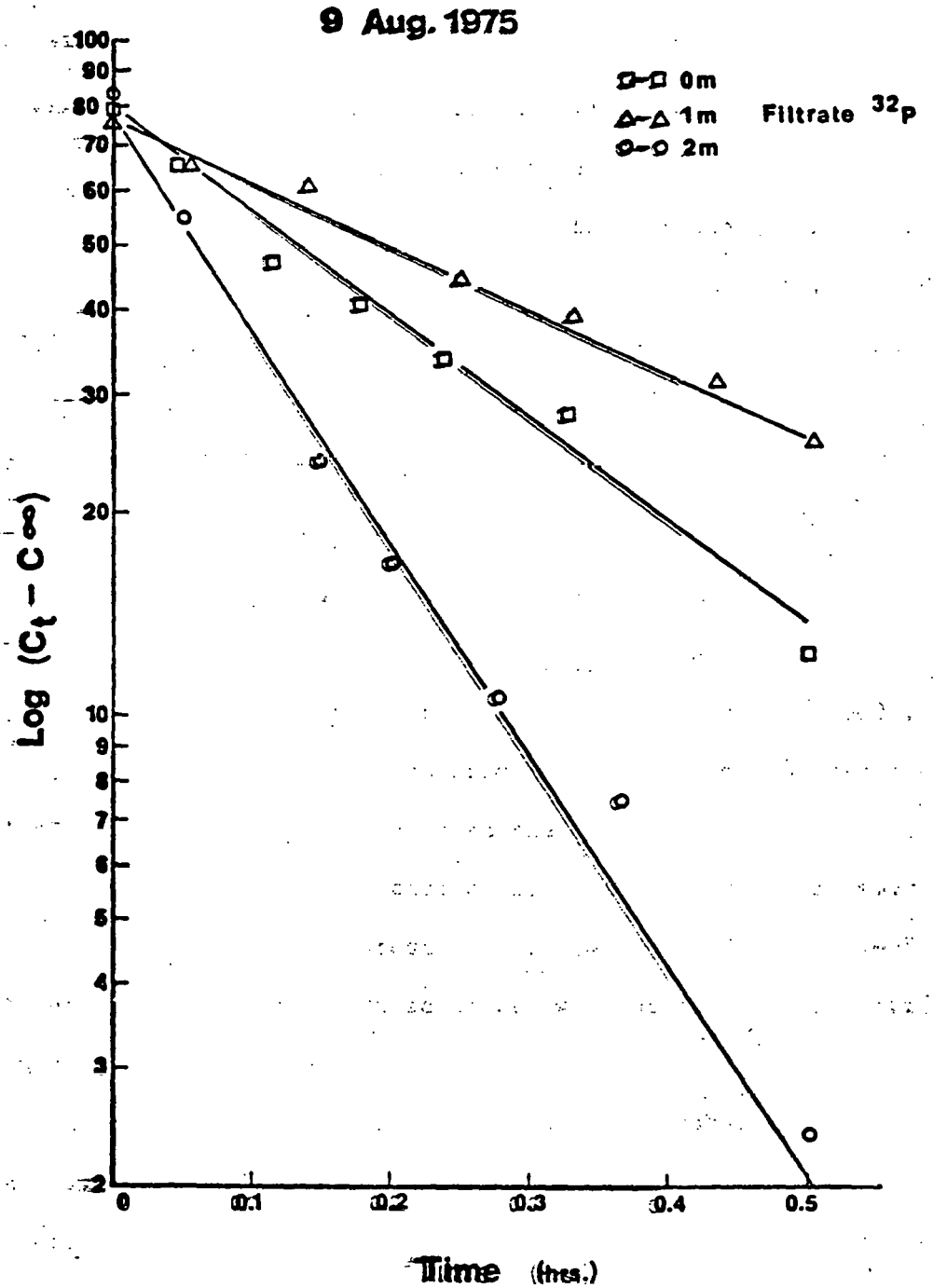
increased to 0.11253 min^{-1} with a turnover time of 8.89 min. This represented a decrease by 16% over that at the surface and by a factor >3 over that at 1 m (Figure 21). Thus, the trend of decreasing uptake rate with depth did not continue into the 2 m strata. Apparently, the heterotrophic bacteria at this depth are removing phosphate from solution at a rate greater than that of the autotrophic phytoplankton at the surface strata.

While this trend was observed whenever a depth series of uptake rates was carried out, turnover times varied slightly on a daily basis. One day after the above rates were measured another depth series was taken with little change if any in the weather conditions. The turnover time increased from 11.43 min (1:00 p.m.) to 17.39 min (1:00 p.m.) at the surface, but remained relatively constant at the lower depths (i.e., 29.13 min to 27.04 min at 1.0 m, and at 2 m from 8.89 min to 8.25 min). These continual short turnover times indicate the rapid cycling of phosphorus (suggesting that phosphate phosphorus is in extremely short supply) with the turnover time being the shortest for the bacterial layer at 2 m (Figure 22).

Effect of Light on DI^{32}P Uptake

To ascertain the effect of light on the uptake of phosphorus by the phytoplankton, a 1 m composite sample (4 1-liter subsamples) of plankton was held at 1 m and then transferred to the surface. After 15 min the uptake rate was measured (2:00 p.m.) which resulted in a turnover time of 20.96 min which was close to but less than the previously measured values of 29.13 min and 27.04 min. During the incubation period the temperature did not increase, indicating that low light levels at 1 m may be reducing the uptake rate of phosphorus. However, an

Figure 22. A direct comparison of the rate of uptake of $DI^{32}P$ in three strata in North Gate Lake.


9 Aug. 1975
0m
1m
2m
Filtrate ^{32}P
Log $(C_t - C_\infty)$
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
0
0.1
0.2
0.3
0.4
0.5
Time (hrs.)

Filgure 22.

equivalent experiment was carried out for a composite surface sample. It was incubated in situ at 1 m after a 15 min adjustment period. The turnover time (1:00 p.m.) equalled 11.83 min, which was in close agreement with the previously determined values of 10.59 min. To confirm the rates did not change due to peculiar environmental differences at these sampling times (as was shown to take place earlier) a composite sample of surface water was incubated at the surface (noon) during the same day. The turnover time equalled 11.43 min compared to 11.83 for the 1.0 m incubated surface sample. It was concluded that light did not control the uptake rate of $DI^{32}P$ in short term experiments under nutrient-short conditions.

Effect of Temperature on $DI^{32}P$ Uptake

Since the temperature change (decrease) from the surface to 1 m equaled 4-5°C, a Q_{10} of 2 would predict a decrease in the uptake rate of only 50%, but in fact the uptake rate decreased by over 300%. In addition, the temperature change from 1.0 m to 2.0 m was even greater (8-10°C) which alone should decrease the uptake rate by 100%. Yet the uptake rate increased by a factor of 3. Thus, the rate of removal of $DI^{32}P$ from solution was greatest at the lowest temperature (4-5°C). This suggests that a temperature effect was not the factor controlling the uptake rate.

The main determinate of the removal of phosphate in North Gate Lake appears to be both the quantity and type of seston at the depth sampled. Bacteria not only retain phosphate as Part-P but remove it from the water column at a very quick rate. It appears that the bacteria do not have sufficient phosphorus (substrates rich in phosphorus) to export a portion as mineralized phosphate. This would be in

agreement with the presence of an extremely phosphate-poor organic carbon pool in North Gate Lake. Thus, the bacteria are in competition with the plankton for phosphate and may in fact strip the water of available phosphate and decrease further the small pool of cycling inorganic phosphate in the euphotic zone, i.e., the bacteria layer is an inorganic nutrient trap.

Biphasic Kinetics

The differential cycling of phosphorus by bacterial and algal communities, and the differential amounts of both $FU^{32}P$ and $FR^{32}P$ in the filtrate of the ^{32}P kinetics experiments (Figure 23) is interesting in two ways. One, filtrate ^{32}P can no longer be considered purely $FR^{32}P$ (or as $DI^{32}P$) and second, the presence of a large fraction of $FU^{32}P$ compared to $FR^{32}P$ in the filtrate would cause a deviation of the ^{32}P filtrate curve from an exponential function. This is the cause of biphasic kinetics (i.e., a departure after log transform from log linearity) observed by Lean and Rigler (1974). If a population of organisms produces a significant fraction of $FU^{32}P$ compared to $FR^{32}P$ (regardless of absolute quantities), the biphasic kinetics will be observed. If filtrate ^{32}P is predominantly $FR^{32}P$ with little $FU^{32}P$, then monophasic kinetics will be observed.

In North Gate Lake the proportion of $FU^{32}P$ to $FR^{32}P$ was depth dependent (Figure 23). Thus, biphasic kinetics should also be depth dependent, and in the case of North Gate Lake, organism dependent. Bacterial communities absorb more $FR^{32}P$ than algal communities, have less $FR^{32}P$ at equilibrium (<5% total ^{32}P), and produce less $FU^{32}P$ (<3% of total ^{32}P) but the proportion of $FU^{32}P$ to $FR^{32}P$ is much greater (0.6 to 0.9) than algal communities. Algal communities

Figure 23. The depth-dependent ratio of $FU^{32}P$ to $FR^{32}P$ in the filtrate of uptake experiments at tracer equilibrium.

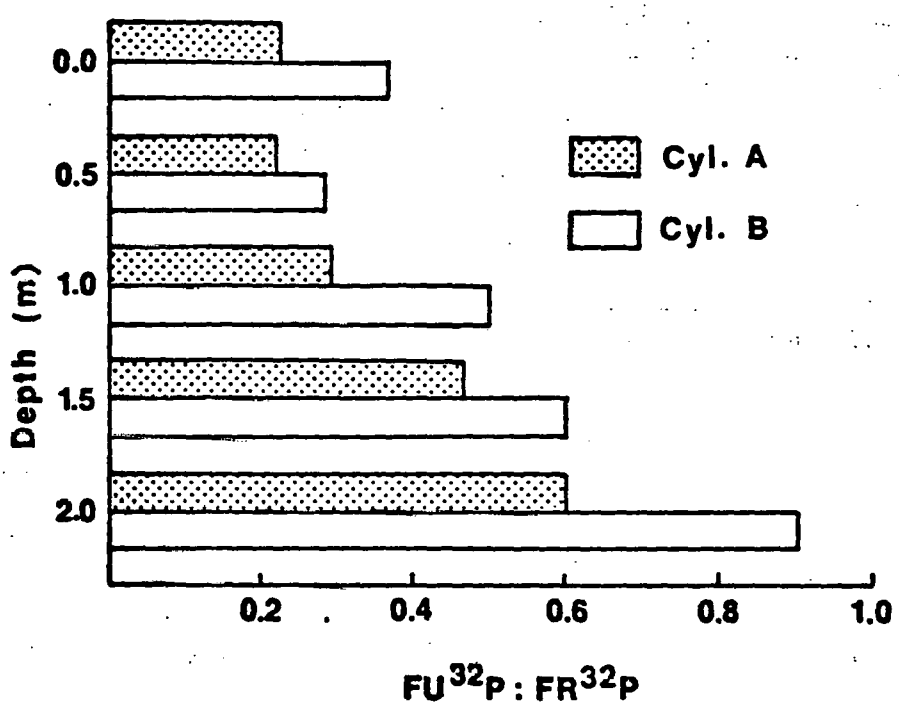


Figure 23.

excrete more $FU^{32}P$ over 7 days but in a short-term uptake experiment contribute around 2-3% of the total ^{32}P as $FU^{32}P$ while maintaining a much larger pool of cycling $FR^{32}P$ (12-15% of total ^{32}P) than bacterial communities. The FUP:FRP ratio is much lower at 0.20 to 0.35. Departure from log linearity after log transform would be expected to be more drastic in the bacterial layer in which filtrate ^{32}P is almost equally divided between $FU^{32}P$ and $FR^{32}P$, than in the algal layer where $FR^{32}P$ dominates the filtrate ^{32}P.

Such a trend is suggested in North Gate Lake. At the surface, 1 m, and 2m, there was an increasing difference between the slope of the log transform of the decrease in filtrate ^{32}P (i.e. $FR^{32}P + FU^{32}P$) and $FR^{32}P$ (Figure 21, Table 7). The ratios of the respective slopes decreases from 1.00 at the surface to 0.88 at 1.0 m, and to 0.55 at 2 m. At 2 m $FU^{32}P$ makes up close to 50% of filtrate ^{32}P causing the observed difference in slope and thus biphasic kinetics. Lean and Rigler (1974) consistently observed biphasic kinetics when filtrate ^{32}P contained a nearly 1:1 ratio of $FU^{32}P$ and $FR^{32}P$.

In summary, a subtraction of the $FU^{32}P$ concentration from filtrate ^{32}P to give $FR^{32}P$ results in an improved fit of the log transform curve to a linear model. This, of course, results in the better fit of the decrease in $FR^{32}P$ to an exponential nutrient uptake model, and is consistent with the hypothesis that a proportion of $FU^{32}P$ in filtrate ^{32}P causes biphasic kinetics.

Diel Changes in Inorganic Phosphorus Metabolism

In these experiments two large 3000 liter polyethylene cylinders were used. Temperature in the lake varied with every 0.5-ft. increment, so any disturbance of the natural layering of the water strata and its

accompanying plankton could be followed with a resistance thermometer. Temperature distributions in both bags did not vary by more than 0.2 degrees from that outside the chambers. Thus, I felt that the placement of the chambers did not affect the natural layering of plankton in the lake and the results of these chamber experiments represented the in situ reactions in the lake.

After nearly 50 times the turnover time of ^{32}P, different equilibrium distributions of ^{32}P were observed in the cylinders. These differences were depth related (as was seen for the ^{32}P uptake measurements) and were related to the time period of incubation (i.e., day - night).

In cylinder A (night 9 hrs) phosphate is taken up by the plankton and is held to a greater extent by the particulate fraction with increasing depth (Figure 24). At the surface 92.8% of the added tracer was particulate which increased to 94.7% at 0.5 m, 97.5% at 1.0 m and 98.8% at 1.5 m. However, in cylinder B (day 9 hrs) the same depths exhibited a lower amount of particulate ^{32}P. At the surface only 86.7% was Part-P, 0.5 m 87.4%, 1.0 m 84.9% and 1.5 m 93.5% (Figure 24).

It is interesting to note that at night (cylinder A), all strata (0m - 1.5 m) had Part-^{32}P fractions of >93.0%, however, after one daylight incubation period (i.e. day 24 hrs), this fraction decreased to <80.0%, at 0 m - 1.0 m, but remained greater than 93% at 1.5 m. The light apparently triggered a decrease in equilibrium Part-^{32}P in strata within the Secchi disk depth to values relatively consistent with those in light incubated cylinder B.

In cylinder A (night 9 hrs) the higher amount of Part-^{32}P is reflected in a lower equilibrium level of FR^{32}P. These values ran from

Figure 24. The amount of Part-^{32}P following dark and light in situ incubation periods at four strata in North Gate Lake. Times indicated are from the start of the experiment (the addition of tracer) to when the sample was taken, and include the day and/or night incubations for the cumulative time periods.

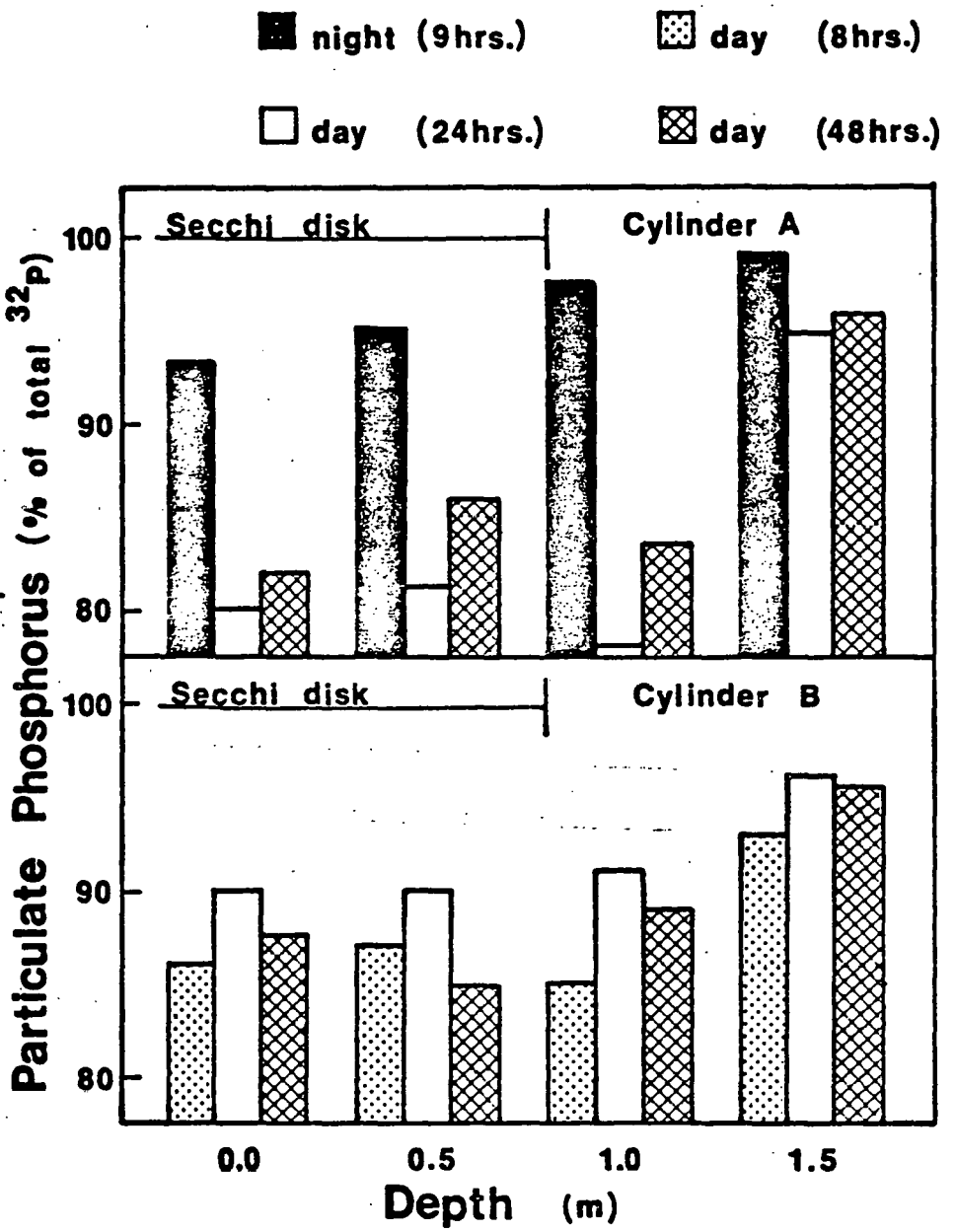

night (9hrs.)
day (8hrs.)
day (24hrs.)
day (48hrs.)
Secchi disk
Cylinder A
Secchi disk
Cylinder B
Particulate Phosphorus (% of total 32P)
100
90
80
100
90
80
0.0
0.5
1.0
1.5
Depth (m)

Figure 24.

Figure 25. The amount of filterable reactive ^{32}P in solution following dark and light in situ incubation periods in four strata in North Gate Lake. Times are from the start of the experiment (addition of tracer) to when the sample was taken, and include the day and/or night incubations for the cumulative time periods.

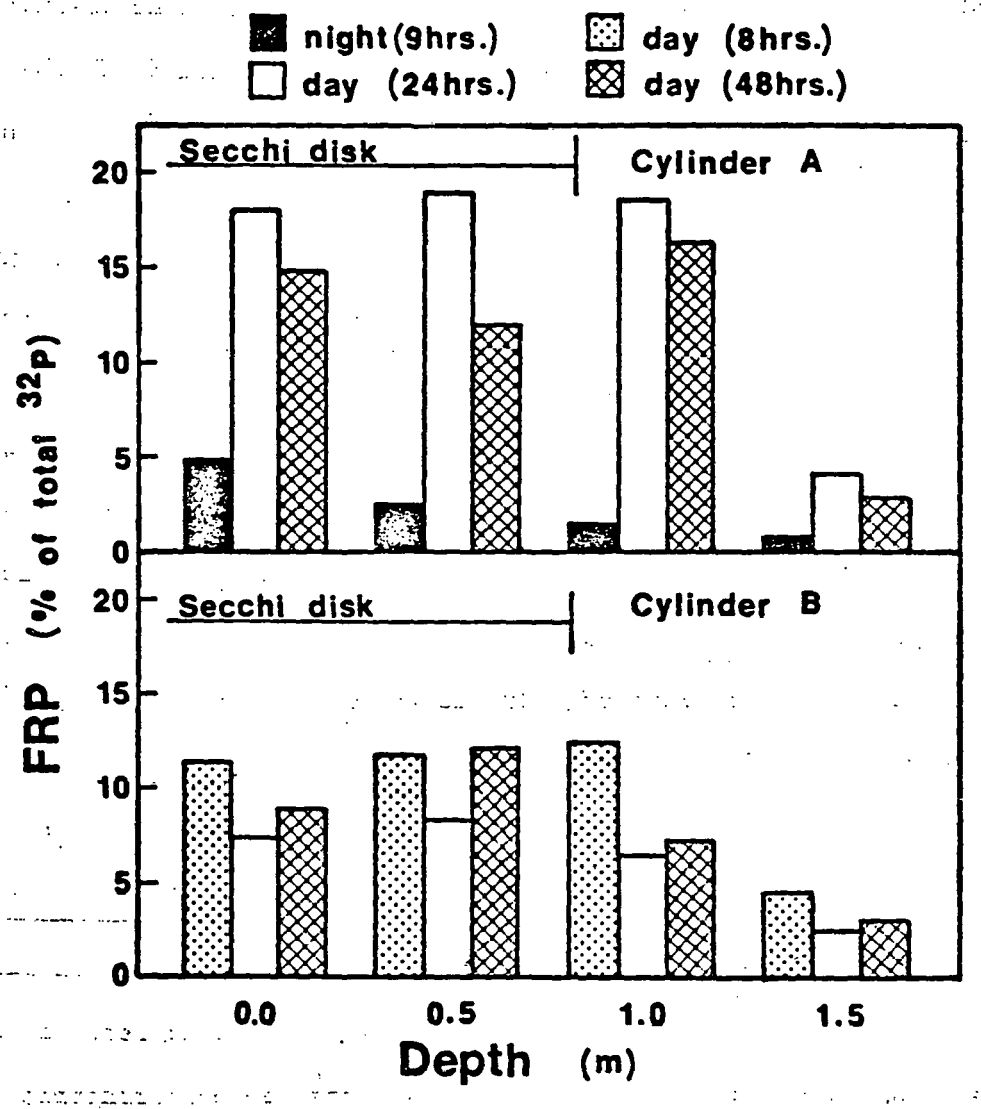

night(9hrs.)
day (24hrs.)
day (8hrs.)
day (48hrs.)
Secchi disk
Cylinder A
Secchi disk
Cylinder B
FRP (% of total 32P)
20
15
10
5
0
0.0
0.5
1.0
1.5
Depth (m)

Figure 25.

4.66% of total ^{32}P at the surface to 0.44% at 1.5 m (Figure 25). In the light (day 24 hrs) the amount of $FR^{32}P$ in equilibrium with the particulate fraction increased to 11.3% to 12.4% of total ^{32}P but remained low (4.6%) at 1.5 m.

After a further light period (day 48 hrs) the Part-^{32}P in the previously dark cylinder (A) was reduced to 80.8% to 85.8% of total ^{32}P and the $FR^{32}P$ fraction increased to around 12-16% of total ^{32}P. This occurred only for those strata within the Secchi disk depth. The layer at 1.5 m continually remained at >95% Part-^{32}P and 2.8% $FR^{32}P$. The same trend with depth was noted for the cylinder (B). Thus, the strata within the light remained around 85% Part-^{32}P while at 1.5 m the Part-^{32}P amounted to 95% of total ^{32}P. It is apparent that the phytoplankton cycle a considerable amount of $FR^{32}P$ back to the water column compared to bacterial cycling reactions.

Since $FR^{31}P$ was not detectable in North Gate Lake, the distribution of $FR^{32}P$ at tracer equilibrium is the only evidence for diel differences in the concentration of orthophosphate as a result of plankton metabolism. In nutrient deficient waters, it would not be unexpected to find higher FRP levels during the day and lower levels at night.

Algal and Bacterial Uptake Rates of $DI^{32}P$ and $FR^{32}P$

Earlier experiments (Table 2) and Koenings and Hooper (1976) have demonstrated that a colloid of COM and iron has the ability to reduce the concentration of free DIP by forming a COM-iron-phosphate colloid. Thus, DIP is converted to FRP. While the formation of such a complex enables FRP to remain molybdate reactive as DIP, the colloid prevents FRP from reacting as DIP in tests (e.g. anion exchange experiments) designed to measure just DIP.

Thus, the utilization of phosphate added as $DI^{32}P$ by algae may not be equal to the algal absorption of colloid bound $FR^{32}P$. Thus, $DI^{32}P$ may not accurately measure the cycling of $FR^{31}P$ in North Gate Lake.

In a control experiment (diluted with filtered bog water), $DI^{32}P$ was used by the phytoplankton from the surface strata at a rate less than that of an undiluted sample (Figure 26; Table 7). Thus, dilution itself did have an impact on sestonic turnover time of inorganic phosphorus. Turnover times ranged from 10.59 min to 17.37 min in undiluted samples, compared to 21.09 min in the diluted control sample.

In the samples to which $FR^{32}P$ was added the uptake rate by the sestonic fraction was $-0.00461\ min^{-1}$ at the surface, and $-0.00494\ min^{-1}$ at 2 m. This increased the turnover time of reactive phosphorus to 217 min (surface) and to 203 min at 2 m. Thus, the turnover time for $DI^{32}P$ equalled 21 min while the turnover time for $FR^{32}P$ was over 3.5 hr (Figure 27). Since the FRP analysis measures colloidal orthophosphate as well as free orthophosphate, it is not surprising to find that $FR^{32}P$ is not biologically equivalent to $DI^{32}P$. The primary mechanism responsible for this discrepancy is the time dependent complexation of free $DI^{32}P$ to COM and iron to form $FR^{32}P$. Thus, colloid bound $FR^{32}P$ is not as biologically available as is DIP but is detectable chemically as free orthophosphate.

Not only are the turnover times of $DI^{32}P$ and $FR^{32}P$ different by a factor of 10, but a greater fraction of $FR^{32}P$ compared to $DI^{32}P$ remains unutilized by the time of apparent tracer equilibrium. If the asymptote level of filtrate ^{32}P represents an equilibrium between Part-^{32}P and $DI^{32}P$, then the rate constants k and k_1 should be equal (see Methods).

Figure 26. A comparison of the decrease of ^{32}P added as $FR^{32}P$ and $DI^{32}P$ to samples of surface and 2 m water from North Gate Lake.

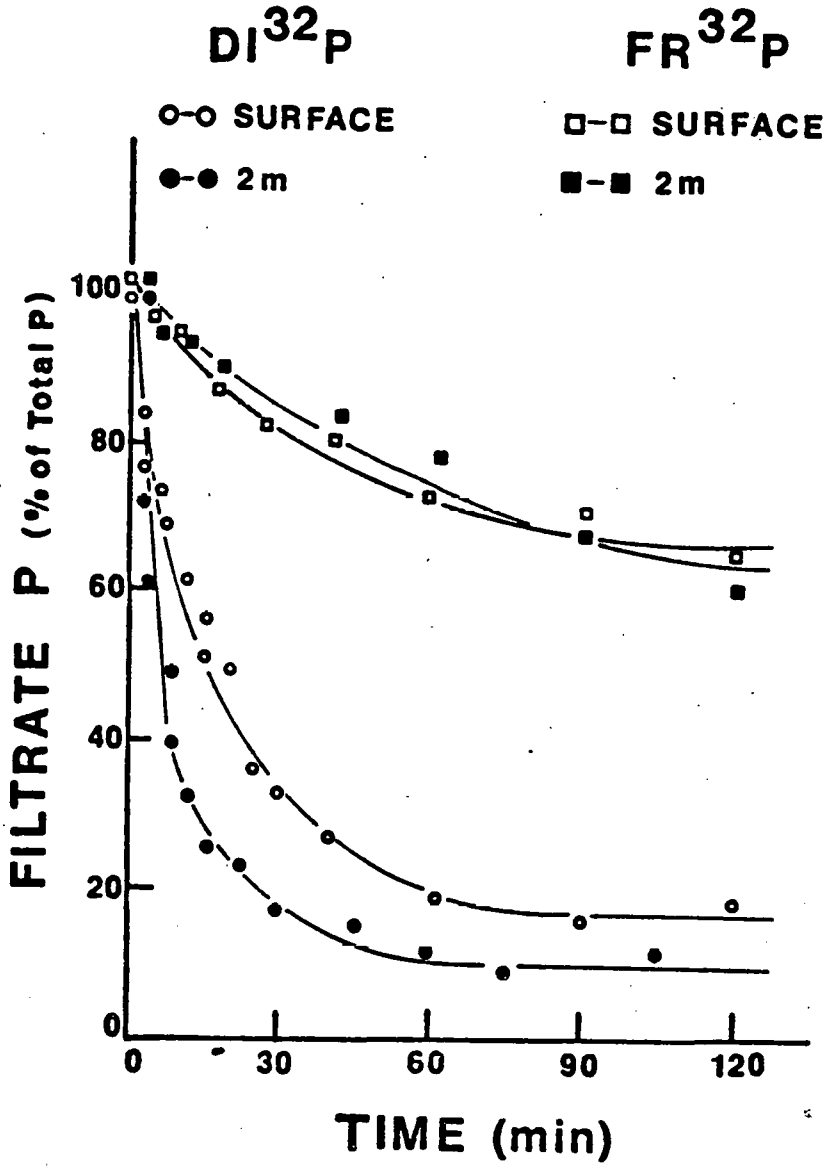

DI 32P
FR 32P
O-O SURFACE
●-● 2m
□-□ SURFACE
■-■ 2m
FILTRATE P (% of Total P)
100
80
60
40
20
0
0
30
60
90
120
TIME (min)

Figure 26.

Table 7. A comparison of the rate constants for $DI^{32}P$, $FR^{32}P$, and filterable ^{32}P uptake in three strata in North Gate Lake.

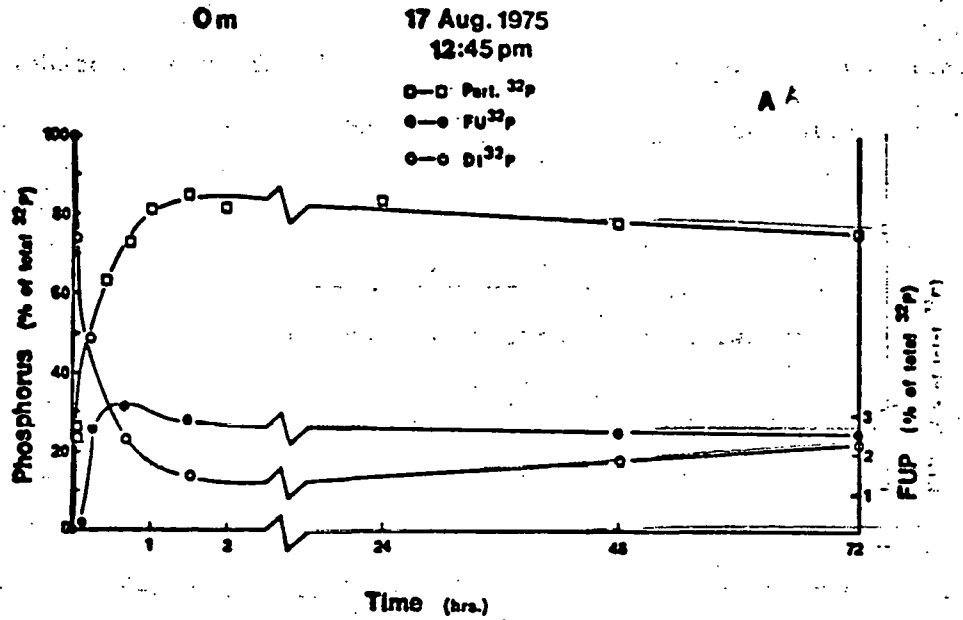
Table 7.

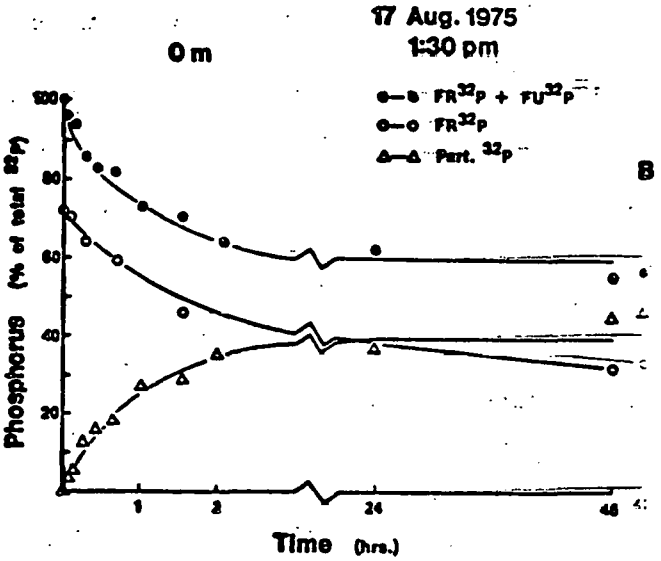
Depth	Tracer	Date	Slope (s)	Filtrate ^{32}P Rate Constant (k_1)	Filtrate ^{32}P Turnover Time (min)	Filtrate Reactive ^{32}P Rate Constant (k_1)	Filtrate Reactive ^{32}P Turnover Time (min)	Part-^{32}P Rate Constant (k_2)	Part-^{32}P Turnover Time (min)
0 m	DI^{32}P	8-9	-0.10491	-0.09442	10.59	-0.09400	10.64	-0.01049	95.33
		8-10		-0.05749	17.39				
		8-12		-0.08751	11.43				
		8-12		-0.08450	11.83				
1 m	DI^{32}P	8-9	-0.03732	-0.03433	29.13	-0.03887	25.73	-0.00299	334.45
		8-10		-0.03699	27.04				
		8-12		-0.04771	20.96				
2 m	DI^{32}P	8-9	-0.12503	-0.11253	8.89	-0.20098	4.98	-0.01250	80.0
		8-10		-0.12136	8.24				
0 m	DI^{32}P	8-17	-0.05578	-0.04741	21.09	-0.04869	20.54	-0.00837	119.47
0 m	FR^{32}P	8-17		-0.00374	267.38	-0.00461	216.70		
2 m	FR^{32}P	8-17		-0.00382	262.04	-0.00494	202.63		

However, if the asymptote level of filtrate ^{32}P actually represents an unutilizable fraction of $FR^{32}P$ then k will not equal k_1. By such an analysis the rate constants k and k_1 were found to be vastly different only for the $FR^{32}P$ experiments. Thus, the asymptote level of $FR^{32}P$ is in fact an unutilizable portion of $FR^{32}P$ (Figure 26). At equilibrium in the surface $DI^{32}P$ experiments, $DI^{32}P$ represented from 8-10% of the total ^{32}P, and at 2 m was equal to 2.12 to 2.95% of total ^{32}P. However, at tracer equilibrium using $FR^{32}P$, 65-70% of the total ^{32}P remained as unutilized $FR^{32}P$. Thus, the phytoplankton and bacteria used from 85 to 95% of the $DI^{32}P$ before equilibrium, but only 35% of $FR^{32}P$. If $DI^{32}P$ comes into equilibrium with stable free orthophosphate, then obviously a significant quantity of $FR^{32}P$ is not free orthophosphate.

In summary in North Gate Lake colloid bound orthophosphate reacts as orthophosphate in the routine chemical test for FRP, but it is not measured by techniques or by biological assays which are designed to measure free orthophosphate. The portion of $FR^{32}P$ that is not available to algae represents the difference that exists between chemical estimates of FRP and radiochemical estimates of FRP by use of $DI^{32}P$. At tracer equilibrium in North Gate Lake, $DI^{32}P$ represented a maximum of only 20% of $FR^{32}P$ at the surface and 6% of $FR^{32}P$ in the 2 m strata. In comparison, Kuenzler and Ketchum (1962) found 4% of FRP as DIP, and Rigler (1966) found 1-10% of FRP as DIP. Thus, both authors using ^{32}P concluded that the acid molybdate test overestimates the concentration of orthophosphate in natural waters. In light of such studies, Likens (1975) questions the utility of continuing the chemical estimates of molybdate reactive phosphorus (i.e. FRP). However, what is actually measured by the ^{32}P technique is a small subfraction of filterable

Figure 27. The uptake and distribution of ^{32}P added either as $DI^{32}P$ (a) or $FR^{32}P$ in surface (b) and 2-m (c) water at tracer equilibrium. Experiments were performed after dilution of unfiltered lake water with filtered lake water at a ratio of 3:1.


0 m
17 Aug. 1975
12:45 pm
Part. 32P
FU32P
DI32P
A
Phosphorus (% of total 32P)
FUP (% of total 32P)
Time (hrs.)


0 m
17 Aug. 1975
1:30 pm
FR32P + FU32P
FR32P
Part. 32P
B
Phosphorus (% of total 32P)
Time (hrs.)

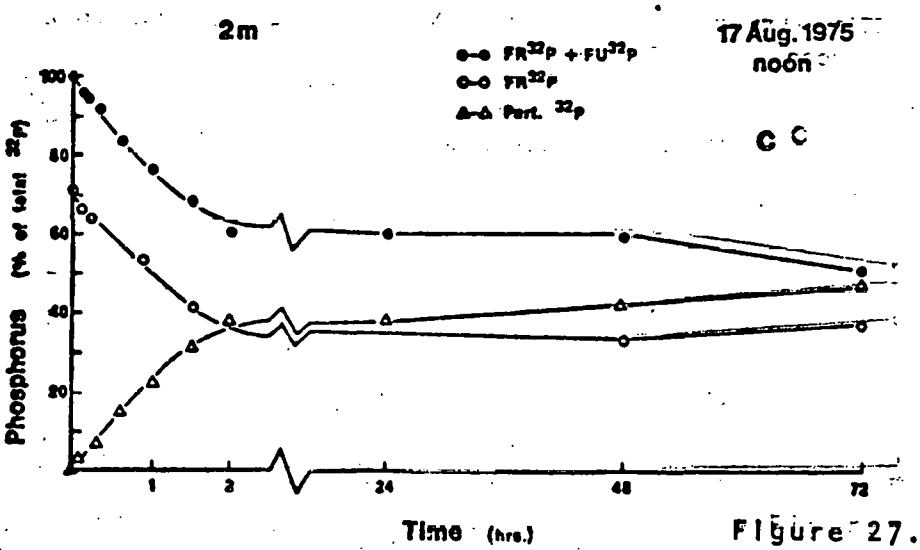

2 m
17 Aug. 1975
noon
FR32P + FU32P
FR32P
Part. 32P
C
Phosphorus (% of total 32P)
Time (hrs.)

Figure 27.

orthophosphate namely "free" dissolved orthophosphate. The chemical test for FRP does measure orthophosphate but simply overestimates free orthophosphate (i.e. DIP).

Radiophosphorus and Stable Phosphorus Equilibria

If an element (phosphorus) is cycling between two or more phases of a system (i.e., FUP, Part-P, FRP), and its radioisotope is introduced into the system, then the specific activity ($^{32}P/^{31}P$) will be equal in each phase after the radioisotope has reached equilibrium, i.e., its steady-state distribution among the phosphorus fractions. In other words the radioisotope will distribute itself in proportion to the stable form already present in the system (e.g. Figure 21). It follows then that the steady state levels of tracer measure only the biologically active pools of filterable phosphorus that are readily used by the metabolizing seston. Any differences in ^{32}P levels (when at steady state) with their stable counterparts can be ascribed to non-metabolically active forms of stable phosphorus within these fractions. If aquatic systems do not realize ^{32}P-^{31}P equilibrium, the measurable fractions of stable phosphorus must consist of sizeable percentages of detrital or non-metabolically active phosphorus, i.e., refractory forms. The greater the amount of detrital phosphorus or refractory phosphate, the more unequal will be the specific activity of any fraction and the less appropriate the estimation of ^{31}P flux from ^{32}P data.

When introduced into North Gate Lake water, DI^{32}P is rapidly taken up by the phytoplankton and partitioned into three compartments. For example when tracer equilibrium is achieved in the surface strata after 60 min (Figure 21), Part-P represents 90%, FRP 8% and FUP 2% of

total ^{32}P. In all experiments, most of the phosphorus is retained by the phytoplankton with a considerable pool of rapidly cycling $FR^{32}P$ and a lesser amount of $FU^{32}P$. The bacterial layer at 2 m partitioned ^{32}P a little differently with 94% Part-P, 2% FRP and 4% FUP. In experiments lasting for up to 10 days these equilibrium levels changed slightly only at the surface due to the production of $FU^{32}P$.

However, it is important to note that only tracer equilibrium was established within the metabolically active forms of phosphorus within the time period of 1½ weeks. For example, surface $FU^{32}P$ levels averaged 5% of the total ^{32}P, but $FU^{31}P$ levels in the same strata averaged 55% of the total ^{31}P. Thus, $FU^{32}P$ and $FU^{31}P$ differ by a factor of 10. Obviously, a majority of $FU^{31}P$ is not labelled during this experimental period. This is consistent with the hypothesis that stable $FU^{31}P$ is not turning over fast enough to be considered a major factor in the phosphorus cycle in the euphotic zone of North Gate Lake. Thus, the cycling of metabolic FUP (^{32}P) is substantially different than that exhibited by stable (^{31}P) phosphorus, because stable FUP appears to be dominated by forms of less utilizable phosphorus.

Experiments using $DI^{32}P$ and $FR^{32}P$ (an $FR^{31}P$ analog), show that FRP consists of fractions that do not cycle as does DIP (Table 7). If this holds for other lake systems, estimates of turnover times of ^{31}P fractions based upon the results of ^{32}P studies will be in error, and phosphorus transfer work based upon ^{32}P studies cannot be used to indicate the flux of forms of stable phosphorus. Calculations of stable phosphorus fractions and flux rates have been made using ^{32}P distributions at tracer equilibrium and total ^{31}P values (Watt and Hayes 1963; Lean 1973; and Likens 1975). However, this study

demonstrates that ^{32}P data should be used with caution in calculating stable phosphorus fractions, and flux rates because only tracer equilibration occurs, not the required ^{31}P-^{32}P equilibria.

CONCLUSION

The Formation and Importance of Sestonic FUP

Phosphate in North Gate Lake is in very short supply (undetectable) and cycles very rapidly through the biota as indicated by radiotracer kinetics. Phytoplanktoners take up $DI^{32}P$ (turnover time 10-29 min) and excrete a larger portion of $FR^{32}P$ (8-10% of total ^{32}P), than $FU^{32}P$ (2-3%) at tracer equilibrium. $FR^{32}P$ then increased over a 7-day period to nearly 15-20% of the total ^{32}P, while $FU^{32}P$ increased to nearly 8% of total ^{32}P. The bacterial layer removed $DI^{32}P$ at a quicker rate than the surface phytoplankton. Turnover of $DI^{32}P$ was from 5 to 9 min, while a smaller fraction of $FR^{32}P$ compared to the surface strata remained at tracer equilibrium. At 2.0 m $FR^{32}P$ represented 2-3%, and $FU^{32}P$ 2% of total ^{32}P. Neither fraction increased significantly at the end of 7 days. Thus, the distribution of $FR^{32}P$ showed a maximum in the surface which decreased with depth (Figure 28). Particularly noticeable is the sharp drop in $FR^{32}P$ at 1.5 m and below where the phytoplankton are nearly nonexistent and bacteria dominate the system. The $FU^{32}P$ pools generally follow the same trend indicating the excretion of $FU^{32}P$ by the plankton only in the photosynthetic zone.

In contrast to $FU^{32}P$, $FU^{31}P$ does not show a depth-related difference (Figure 28, Table 3). Only the depth distribution of dialyzable organic P ($DU^{31}P$) resembles that of metabolic $FU^{32}P$ (Figures 10 and 28). This disequilibrium found between $FU^{31}P$ and $FU^{32}P$ argues that a considerable portion of $FU^{31}P$ is not recently derived from particulate metabolic phosphorus. Further evidence for the detrital nature of $FU^{31}P$ comes from the unusually high C:P ratios found only for the colloidal

Figure 28. A summary of the in situ distribution of ^{31}P (IV) and ^{32}P (I, II, III) fractions at tracer equilibrium for several experiments in North Gate Lake. The FRP fraction in the ^{31}P pool is undetectable.

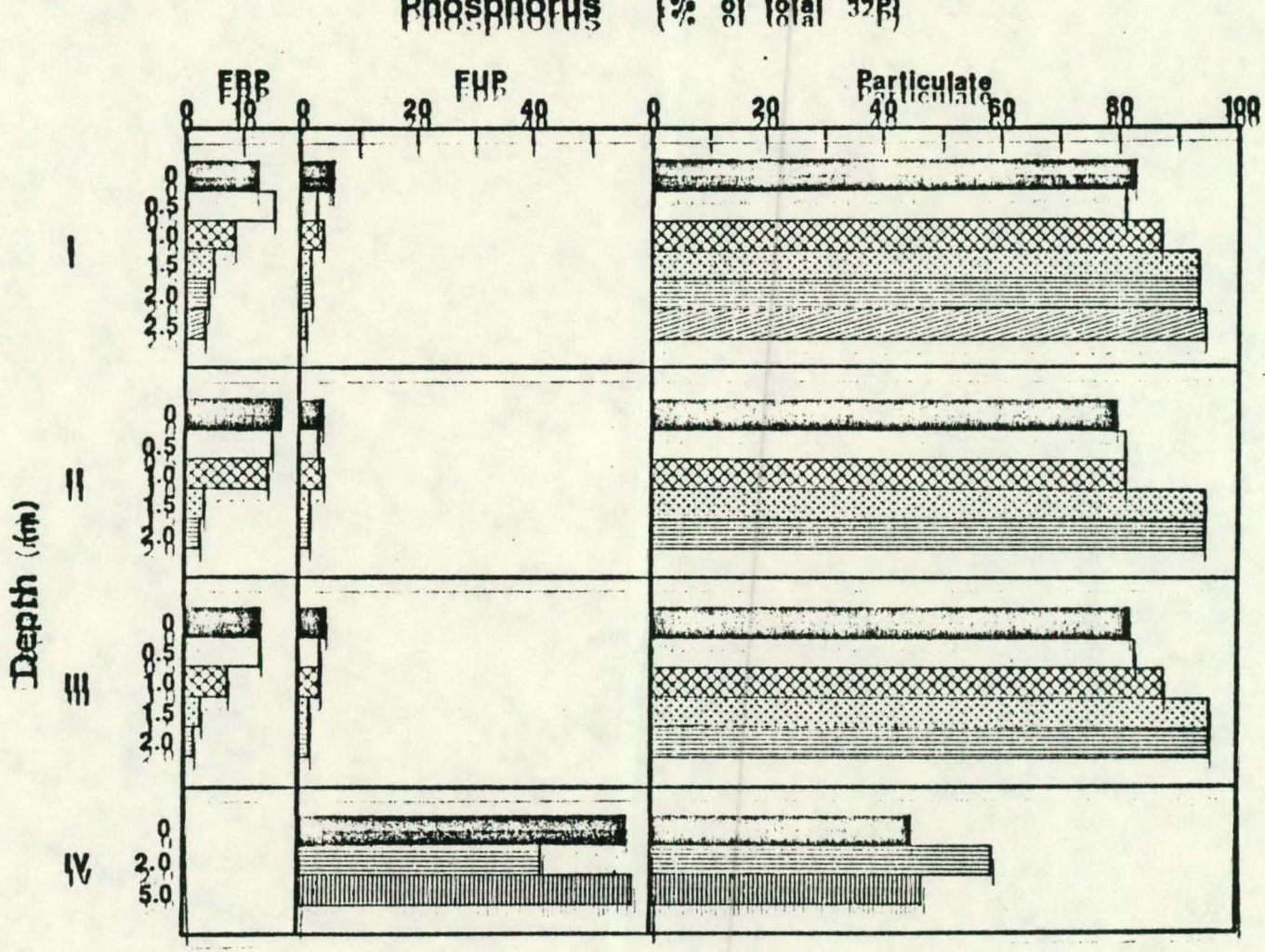

Phosphorus (% of total 32P)
FRP
FHP
Particulate
0
10
20
30
40
50
60
80
100
Depth (m)
I
II
III
IV
0
0.5
1.0
2.0
2.5
5.0

Figure 28.

organic phosphorus (CUP) fraction (Figure 11) which dominates $FU^{31}P$.

Particulate ^{32}P follows an inverse trend to $FR^{32}P$ and $FU^{32}P$, i.e., increasing with depth. The heterotrophic bacteria below 1.5 m extract $DI^{32}P$ from the water and do not cycle it back to the water (i.e., a lower level of $FR^{32}P$) compared to the autotrophic phytoplankton. The phytoplankton through a balance of uptake and excretion maintain a pool of $FR^{32}P$ that can be continually used as a source of phosphate for metabolic needs under conditions approaching a steady-state. The direct release of FRP and FUP from the plankton is very important in the rapid turnover of phosphorus in North Gate Lake. This is especially significant in the euphotic zone where FRP excretion greatly exceeds the production of unuseable FUP.

Since phosphorus is rapidly liberated during autolysis (Golterman 1975), particulate detrital organic matter should consist of a high proportion phosphorus-poor compounds. Decomposed organic matter derived from the Sphagnum mat is also a source of this material. North Gate Lake does contain a considerable pool of detrital colloidal organic carbon with very high C:P ratios. If insufficient phosphorus is available from such organic compounds for bacterial use, inorganic phosphate is stripped from the water to provide for cellular maintenance reactions. The heterotrophic bacteria in these phosphate-poor waters are not regenerators of either DIP or sources of FUP. Because of such a conservative use of phosphorus by the bacteria, the 2 m layer acts as a temporary trap for inorganic phosphate. For this reason, and because of the 2 m strata is the top of the thermocline, phosphorus levels are the highest in the lake with the exception of the monimolimnion (Figure 7). More importantly, the heterotrophic bacteria cannot be

considered efficient dissolved nutrient regenerators since they produce particulate organic phosphorus, and recycle very little of either filterable form. This has been observed by Pomeroy (1975) and Johannes (1964, 1974) who have stressed that bacteria are not the only nor even the major agents of nutrient regeneration especially in nutrient deficient systems.

The production of $FU^{32}P$ by the phytoplankton occurs very rapidly ($\leq$3 min) and can be expected to occur in nutrient deficient waters. In this environment internally stored phosphate (as polyphosphate) is low, so that the $DI^{32}P$ can be quickly absorbed, transferred to the metabolic organic P pools, and a small fraction then excreted or leaked as $FU^{32}P$. Such rapid leakage of recently labelled ^{14}C organic material has been observed by Saunders (1972) and Dunstall and Nalewajko (1975), and consists of both low and high molecular weight substances. Fogg (1966) found that glycolic acid was a significant fraction of the DOC excreted by 23 species of phytoplankton. Since glycolic acid is an immediate product of photosynthesis (Bassham et al. 1968) (produced by the splitting of a diphosphate sugar), leakage of a small portion of the considerable pool of organic phosphorus esters within the photosynthetic cycle should not be unexpected. Indeed, Bassham et al. (1968) found that accompanying leakage of ^{14}C labelled glycolic acid out of isolated chloroplasts was 3-phospho-glyceric acid, dihydroxyacetone phosphate, fructose-1,6-diphosphate (sugar phosphate), pentose monophosphates (nucleotides), and several other phosphate esters. The ratio of these (but not all) phosphate compounds in the supernatant to that in the chloroplast was greater in

some cases to that of glycolic acid. Later studies using pre-labelled intact Chlorella pyrenoidosa found a considerable portion of $^{32}P_i$ and bound pohsphates in the medium after 30 minutes.

The low molecular weight compounds leaked from the chloroplasts are from the same classes of compounds occurring in algal culture filtrates. Krause and Stegman (1968) and Phillips (1964) found orthophosphate, nucleotides or poly nucleotides, and perhaps phosphorylated carbohydrates (sugar phophates) in sea water containing algae equilibrated with ^{32}P. These are the same compounds that are hydrolyzed by extracellular PMases and are effectively used as sources of DIP by algae.

Since FU^{32}P appears within 3 min after taking up DI^{32}P in North Gate Lake, it is apparent that metabolic FUP is derived from the pool of particulate phosphorus not far from the photosynthetic pathway. PMase hydrolyzable material (Table 5) was in greatest concentration in the surface strata of North Gate Lake as was the low molecular weight, low C:P ratio FU^{31}P (Figure 10) suggesting that a portion of FUP is derived from the particulate metabolic pool (i.e., overlapping C:P ratios) (Figure 11). In addition, a portion of the FU^{32}P pool was reutilizable as would be predicted by the presence of an acid PMase whose substrates include sugar phosphates and nucleotides (Figure 20). The activity of this enzyme was found to be greatest above the thermocline (Figure 12), which argues for a sestonic origin and a functional role in obtaining phosphate from excreted FUP.

However, a major portion of the total P pool (Figures 7 and 10), in North Gate Lake is filterable but not nondialyzable and represents

a colloidal or a high molecular weight fraction of FUP. This form of FUP (i.e., CUP) has a high C:P ratio and increases with depth (surface 5.8% of total P; 2 m 32.2%; 5 m 34.6%) while metabolic DUP (lower molecular weight) decreases (surface 42.9%; 2 m 11.5% 5; 5 m 13.8%). The production of $FU^{32}P$ by the plankton results in a turnover time for metabolic $FU^{32}P$ of 6.5 months on the average. This in no way represents the turnover of $FU^{31}P$ in North Gate Lake since tracer equilibrium was far from achieved. The data indicate that most of the $FU^{31}P$ pool is refractory and does not serve as a ready source of phosphorus for the plankton in North Gate Lake. Thus, FUP in acid bog lakes cannot be included in productivity forecasts from total phosphorus. When FUP is subtracted from total phosphorus, the prediction of productivity from total phosphorus begins the match the recognized trophic status of bog lakes (Hutchinson 1957, Vollenweider 1968).

Phosphorus Equilibria: Comparison of ^{32}P and ^{31}P Fractions

During the summers of this study (1973-75), FRP remained undetectable in the lake. However, at tracer equilibrium 6-10% of the $DI^{32}P$ tracer [turnover time (TT) = 5-29 min] remained filterable for time periods exceeding by several fold that of the turnover times for both particulate and dissolved phosphate. This can be considered the steady-state pool of phosphate-phosphorus that is maintained by the plankton through a balancing of excretion and uptake rates. These same low levels of equilibrium ^{32}P were noticed by Rigler (1956) with 97% particulate P with 2.9-5.2% of the ^{32}P remaining filterable (TT=10 min). Lean (1973) found that usually less than 4% of the total ^{32}P added remained as filterable ^{32}P. These values agree very well and may be

representative of Canadian shield temperate soft water lakes. Hallmann and Stiller (1974) found that less than 10% of the ^{32}P added to Jordan River or Lake Kinneret water remained in a filterable form, but turnover times were much longer (TT 13 hrs and 1.80 hrs, respectively). Peters (1975) looking at central European lakes, found from 0-7% of the tracer in solution at tracer equilibrium (average TT equalled 27 min). In Lago Maggiore during 1973-74, equilibrium filterate ^{32}P averaged 44%. This was much higher than expected and Peters (1975) proposed that unlike North American lakes, a higher level of filterable phosphorus available to the algae exists in European lakes. However, stable phosphorus analyses were not performed to confirm this hypothesis.

Where direct comparison of steady-state tracer levels with stable phosphorus distributions are available (Table 8), it can be seen that in the time periods of these experiments (which in all cases were followed for time periods several fold greater than that of the dissolved phosphate turnover time) tracer equilibrium levels were not established at levels equal to that of the equivalent stable counterpart. Thus, the ratios of the representative radiophosphate fractions (e.g., FUP; FRP) are not equivalent to the same stable fractions (Table 8). The fraction which was out of phase to the greatest extent (which caused the remaining calculated disequilibrium) is the same in all cases, the filterable fraction

An average of 34% of the total stable phosphorus in Canadian shield lakes is filterable (Rigler 1964), but less than 10% of the ^{32}P at equilibrium is filterable. Thus, it appears that like North Gate Lake and seawater, a considerable portion of filterable phosphorus is not being cycled as rapidly as DI^{32}P and the equilibrium level of

Table 8. The fractional distribution and the ratios of stable (^{31}P) and radiotracer (^{32}P) phosphorus at tracer equilibrium in marine and freshwater systems.

Table 8.

Form	Part-P	FRP	FUP	FUP:FRP	FUP:Part-P	System	Source
$\%^{31}P$	75.8	8.9	15.3	1.71:1	0.20:1	Marine	Watt and Hayes (1963)
$\%^{32}P$	84.0	12.0	4.0	0.33:1	0.048:1		
$\%^{31}P$	64.5	5.9	28.7	4.68:1	0.44:1	Freshwater	Rigler (1964); Lean and Rigler (1974)
$\%^{32}P$	93.0	3.5	3.5	1.00:1	0.038:1		
$\%^{31}P$	45.0	6.0*	49.0	8.17:1*	1.09:1	Freshwater	Present work
$\%^{32}P$	82.5	12.0	5.0	0.44:1	0.061:1		

*Assumes a minimal detectable concentration of 3 µg PO_4-P l^{-1} since $^{31}PO_4$-P was undetectable this ratio

the tracer is below that of the soluble total phosphorus; i.e., FUP is not turning over as quickly as orthophosphate. Thus, the pool of utilizable phosphorus is a small fraction of the filterable phosphorus pool.

If as found in central European lakes a higher level of filterable phosphorus (^{31}P) were cycling as rapidly as $DI^{32}P$, then the ^{32}P asymptote could be higher (Peters 1975), which may in turn (if proven correct) indicate a lower level of FUP and a higher level of FRP in these systems. The greater the difference in the percentage of filterable ^{32}P from that compared to filterable ^{31}P, the greater the amount of noncycling phosphorus; and the more important it becomes to determine the asymptotic values of filterable ^{32}P and ^{31}P.

In North Gate Lake (as in the other studies) $FU^{31}P$ levels vastly exceed the amount of $FR^{31}P$ (Figure 9). However, when looking at the metabolic (^{32}P) pools of phosphorus (Figure 28 and Table 8), it becomes apparent that $FR^{32}P$ levels are much greater than $FU^{32}P$. Evidence such as this argues that stable ^{31}P distributions do not represent the metabolic cycling of phosphorus because ^{31}P fractions are dominated by refractory compounds which mask the actual cycling. Thus, the size of the chemically determined pools of phosphorus may have little relationship to their actual importance in the phosphorus cycle.

The Abiotic Formation of Colloidal Organic Phosphate

Olsen (1967) emphasized the importance of colloidal phosphorus to the understanding of phosphorus cycling. In short he felt that "significant progress in limnological phosphate research will depend

on a perfect differentiation of the liquid as distinguished from the colloidal state." Olsen (1958, 1964) emphasizes the importance of colloidal phosphorus and emphasizes the contribution of sediment clays to this fraction. Olsen (1967) suggested that adsorption to colloidal clay might account for the observations of Rigler (1966) concerning chemical (i.e., FRP analysis) over estimates of DIP. He reasoned that upon addition of small amounts of orthophosphate (e.g., DIP) adsorption would take place especially if the colloids were in an oxidized state.

Evidence exists that FRP does not equal free DIP in natural waters. In addition, it is apparent that the acid hydrolysis of FUP is not the reason for this discrepancy. However, the adsorption of phosphate to organic colloids may well account for this anomaly.

The evidence for this hypothesis is as follows. Rigler (1968), working with anion exchange resins observed that while $DI^{32}P$ was removed efficiently (96%) by the resin, FRP (42%) was not. He noted that FRP was initially taken up by the resin like $DI^{32}P$, but with time it increased in the effluent along with FUP. However, $DI^{32}P$ was still removed from solution. The explanation was that large organic molecules would react with the resin exhausting the exchange capacity at which time the organics plus attached FRP would appear in the effluent. However, $DI^{32}P$, it was thought, could still penetrate the pores of the resin and be removed from solution.

Koenings and Hooper (1976) demonstrated that the organic colloids of North Gate Lake were too large to penetrate the apparent "pores" of the resin and would likely coat the outside of the resin bead. This is supported by the observations of Eliassen et al. (1965) who found that the organic colloids in the water coated the resin particles and

reduced the number of exchange sites. This presents another explanation of continued removal of $DI^{32}P$ by the resin. The active sites of the organic colloid may be still active which would in addition to the resin remove $DI^{32}P$ from the lake water as was shown with the XAD-2 resin experiments in this study.

Jones and Spencer (1963) demonstrated that ion exchange resins could not remove all the phosphate from natural sea water (33% removal) while phosphate removed from artificial sea water equalled 98%. In addition, they concluded (as did Chamberlain and Shapiro 1973; Kuenzler et al. 1963; Murphy and Riley 1962; Edwards et al. 1965 and Strickland and Solorizano 1966) that it was difficult to be confident that any increase in FRP was the result of the acid hydrolysis of organic phosphorus, so that the dissociation of inorganic complexes of phosphate should be considered as the possible cause of the effects observed (Koenings and Hooper 1976).

Westland and Boisclair (1974) found that river water salted with DIP and extracted by ion exchange did not result in 100% recovery of the salted DIP upon elution. They attributed the losses to the brown adsorbed material that was irreversibly bound to the resin. Substances such as lignins have many polar sites capable of binding to the resin. Again Blanchar and Riego (1975) suggest that colloidal phosphates that pass through a 0.45 μ membrane react with acid molybdate and are reported as DIP. However, DIP salted and non-salted river water analyzed by ion exchange resulted in only 74% recovery of FRP while distilled water standards resulted in a recovery of 97%.

Similar results were obtained by Koenings and Hooper (1976) who showed that ion exchange extraction of phosphate standards resulted in

in 99% removal, while samples containing FRP complexed to COM was removed at 82% and 67% depending on contact time of the FRP with the oxidized complex. If the potential complexing COM-iron colloid was removed by in situ dialysis, 99% of FRP was recovered as DIP. Thus, it was reasoned that COM is directly responsible for the nonrecovery of FRP as DIP, and that the FRP chemical method measures both bound and free orthophosphate. Koenings and Hooper (1976) demonstrated the actual formation of this colloidal complex through the use of both radioisotopes and stable chemistry in natural lake water.

Abiotic complexing may be responsible for the underestimation of free DIP compared to FRP measurements (Rigler 1968), but Lean and Rigler (1974) also reported that the biota may be responsible for a filterable ^{32}P component that does not react as orthophosphate. This indicates that both colloid formation through interaction of phosphate with COM and iron (DIP → FRP), and the formation of $FU^{32}P$ may interfere with $DI^{32}P$ uptake kinetics. However, abiotic fixation does not occur rapidly enough to affect short-term tracer studies [the time dependent phenomenon found by Koenings and Hooper (1976) and Fried and Dean (1955)]. However, after enough time for equilibration with the lake solids (particulate and colloidal), the newly formed $FR^{32}P$ is utilized much less rapidly than $DI^{32}P$ (Figure 26).

Evidence of the time dependent formation of colloidal $FR^{32}P$ in situ is available from my tracer studies. $FR^{32}P$ levels equalled 5-10% of total ^{32}P at tracer equilibrium after 1-2 hrs. but increase to 15-20% of total ^{32}P after 5-7 days. Colloidal $FR^{32}P$ formation will be greater when phosphate levels are low. In such systems complexed phosphate may be a significant fraction of FRP, but in phosphate-rich systems the

influence of colloidal-bound phosphate may be insignificant.

The significance of colloid bound phosphorus

While experimental evidence could account for the discrepancy between FRP and DIP, the more important question remains to be answered: can the biota use this complexed phosphate? In a standardized bioassy procedure Walton and Lee (1972) suggest no difference in the usage of FRP and orthophoaphate by algae. However, a high level of DIP was used and this led the authors to conclude that these results might not apply to low nutrient systems. This is supported by Fried and Dean (1955) who found that the more phosphate added to an ion exchange system the less of the total present was adsorbed. Thus, at a level of FRP sufficient to saturate colloidal exchange sites, an insignificant fraction of the FRP may exist complexed to the colloid. At under-saturating levels of FRP a higher level of the phosphate may exist in a colloidal form.

When $DI^{32}P$ is added to a saturated system, it remains as $DI^{32}P$, but when $DI^{32}P$ is added to an unsaturated system, a significant fraction over time forms colloidal FRP. In a saturated system simulated by Koenings and Hooper (1976), COM reduced the formation of bound phosphate, however, in an unsaturated system (such as in this study) colloidal reactive phosphate dominates the inorganic phosphorus fraction. This causes $FR^{32}P$ cycling to deviate from that obtained with $DI^{32}P$.

In North Gate Lake the formation of COM-iron bound phosphate increased the turnover time of the orthophosphate from 5-10 min to $\sim$ 3.5 hrs, and also reduced the amount of phosphorus available to the plankton. $DI^{32}P$ at tracer equilibrium averaged close to 15% of total

^{32}P at the surface, and 2.5% at 2 m, while $FR^{32}P$ at tracer equilibrium averaged close to 65-70% of ^{32}P at both depths. Thus, while 85-97.5% of the $DI^{32}P$ was absorbed by the seston, only 30-35% of the $FR^{32}P$ was absorbed (Figure 26, Table 7). Jackson and Schindler (1975) found that less than 5% of the total ^{32}P was associated with sediment humic acid-iron associations, but they believed even at this level that this binding had considerable ecological significance. It was believed to lower the availability of phosphate to aquatic algae.

Finally, FUP formation may provide a second phosphorus component that can react with the COM-iron complex. Hesse (1973) found that phytic acid reacts with the same minerals as phosphate which reduces the action of the hydrolytic enzyme phytase. Simkis (1964) reports that organic phosphorus esters such as adenosine triphosphate and glycerophosphate (in addition to pyrophosphate) can react with calcium in the same manner as orthophosphate. Chondroitinsulphate did not produce this action, so it was concluded that polysaccharides are not responsible for this reaction. This phenonmenon is reminiscent of Shapiro's (1957) results of yellow organic matter adsorbing to iron colloids, reducing iron precipitation. The importance of complex formation of metals with phosphate esters and pyrophosphate is that these potential components of the FUP pool may, like phytic acid, become less susceptable to the action of hydrolytic enzymes such as PMase. This would reduce the availability of the phosphorus in the FUP to the seston.

Abiotic complex formation in natural waters involving either

oxidized clay of COM-iron colloids, decreases the cycling rates of both inorganic and organic phosphorus. Since complex formation is a time dependent process, the results from both short term $DI^{32}P$ kinetic and $FU^{32}P$ utilization experiments may not reflect the actual cycling of stable phosphorus.

The diffusion layer gradient and its importance to phosphorus metabolism

The movement of inorganic phosphorus out of and into the seston occurs very rapidly in North Gate Lake. Two forms of phosphorus are involved in the transfer. The first is DIP which is removed from the soluble phase at rates approaching 15% min^{-1}. At the same time phosphorus is being returned to the soluble phase from the particulate pool. Thus, excretion is a very important process in maintaining adequate inorganic phosphorus in the dissolved phase. During darkness the phytoplankton absorbed phosphate to a greater extent than during the day when FRP was released (Figure 25). Apparently, the photosynthetic reactions are accompanied by a much more rapid leakage of phosphate due to a more rapid turnover of cellular phosphorus. In pelagic nutrient poor systems such a reaction may actually induce changes in detectable FRP concentrations on a diel basis (Figure 25).

The second form of phosphorus transferred from the particulate phase to the aqueous phase is FUP. The FUP pool consists of low molecular weight compounds of recent metabolic origin leaked by the plankton, and larger molecular weight compounds of colloidal size. A portion of the $FU^{32}P$ pool can be utilized by the seston as a source of phosphorus but >75% is refractory (Figure 20). In addition, a net production of FUP was observed in the phytoplankton strata in the light but a net

Figure 29. Phosphorus metabolism within the microenvironment of a phytoplankton or bacterial cell. FUP = filterable unreactive phosphorus, PMase = Phosphomonoesterase, PDase = Phosphodiesterase, RNase = Ribonuclease, DIP = dissolved inorganic phosphate.

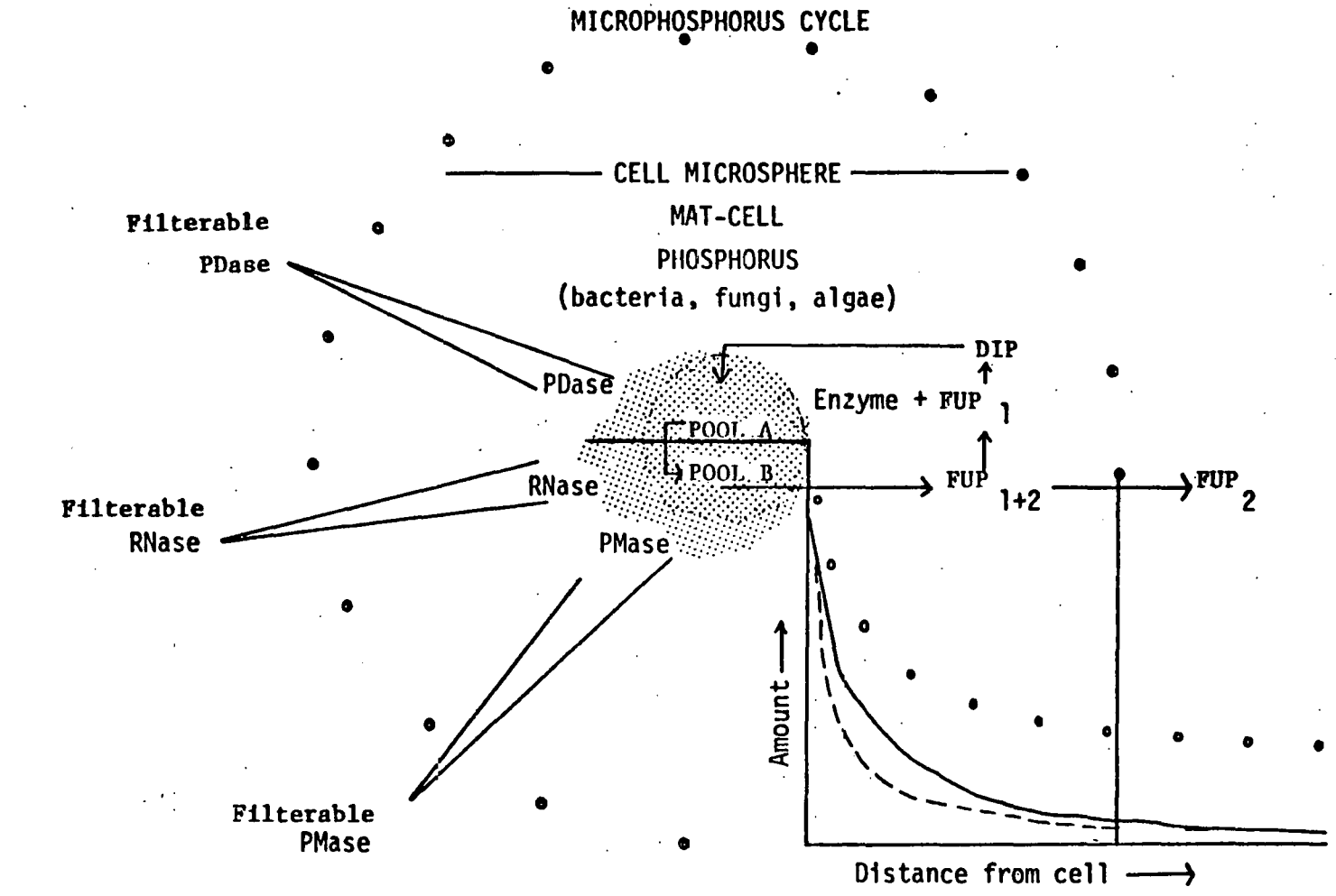

MICROPHOSPHORUS CYCLE
CELL MICROSPHERE
MAT-CELL
PHOSPHORUS
(bacteria, fungi, algae)
Filterable
PDase
PDase
DIP
Enzyme + FUP 1
POOL A
POOL B
RNase
FUP 1+2
FUP 2
Filterable
RNase
PMase
Amount
Filterable
PMase
Distance from cell
DIP
FUP2;enzyme
FUP1

Figure 29.

reutilization occurred in the dark. Thus, the FUP pool is partially controlled by the competing metabolic reactions of production and selective utilization.

The question now becomes why do cells when faced with an already nutrient deficient intra- and extracellular status excrete or form refractory FUP? The model (Figure 29) of the microphosphorus cycle of a nutrient deficient cell is an attempt to explain the presence of two differentially utilizable pools of FUP, and the presence of particulate and soluble enzyme activity.

A cell has a micro-diffusion layer surrounding it which can determine its ability to compete for nutrients with other cells (Hulbert 1970). Schumacher and Whitford (1965) showed that the uptake of ^{32}P was three times faster in the presence of a current which reduced the size of the diffusion layer. Whitford (1960) determined the thickness of this layer to be approximately 0.25 mm. The diffusion of nutrients into and out of this layer was dependent on the current speed, water temperature, and the size of the molecule. Larger organic molecules diffuse much slower than small inorganic nutrients. It is within this layer that the cell exerts its maximum effect by both extracting and excreting inorganic and organic molecules. The reactions and the concentrations of reactants and products within this microenvironment is under the control of the cell. For example, the excretion of dissolved organic carbon will increase the viscosity of this micro zone which may reduce the sinking rate of the organism. This increased viscosity will also decrease the rate of diffusion affecting the rate of exchange of nutrients, metabolites and other compounds between the cell and the water. This is especially valid for large organic molecules (e.g.,

enzymes) which diffuse much slower than smaller inorganic molecules (e.g., DIP).

Malamy and Horecker (1964) found that several hydrolytic enzymes important to cellular metabolism are located in a compartment between the cell wall and the cell membrane of E. coli including PMase. This enzyme functioned to make available the phosphate of phosphomonoesters when the inorganic phosphate of the medium was exhausted. During such a reaction the liberated phosphate did not equilibrate with the external phosphate pool, but was selectively removed from solution. The factor limiting the uptake was the diffusion of inorganic phosphate through the compartment (i.e., diffusion layer) containing PMase.

Periplasmic enzymes and binding proteins (e.g., phosphate binding protein) used in substrate transport include over 26 macromolecules including the enzymes DNase, RNase, and alkaline PMase (Heppel 1971). Hydrolytic periplasmic and truely extracellular enzymes in bacteria are considered to be analogous to the lysosomal enzymes of mammalian cells, but instead of an internal location are compartmentalized on the cell's outer membrane and in the micro-diffusion layer.

These same enzymes are known to be extracellular in algal cells as well (e.g., Kuenzler 1970, Aaronson 1971, Kuenzler and Perras 1965, Berman 1970), and have been found in solution in the open water and interstitial water of the Sphagnum mat in North Gate Lake. Acid PMase (a marker enzyme for the location of mammalian lysosomes) was found by Lien and Knutson (1973) in Chlamydomonas reinhardti to be attached to both sides of the cell's outer membrane. It is this enzyme along with alkaline PMase that increases in activity in algae during nutrient deficient conditions. One would expect the lake water to dilute the

extracellular enzyme concentration, and the enzyme products to the point where they would be useless, e.g., far below the K_m of the enzyme. Such is the case for the acid PMase in North Gate Lake where the K_m is from 23 to 140 μM PNPP or a minimum of 2.2 mg l^{-1} of organic PO_4-P. The substrate concentration in the lake is ∿7 μg l^{-1} PO_4-P. However, the diffusion layer provides a mechanism for preventing enzyme, and enzyme product and/or substrate dilution by maintaining these molecules at the cell surface. Thus, the role of extracellular enzymes becomes one of digestion of intracellular organic phosphorus compounds, and the resorption (recapture) of the products (Heppel 1971), and the supply of phosphate from external sources as well as internal sources when phosphate becomes deficient in lake water.

Increased production of FUP has generally been observed in viable cells of algae and bacteria when phosphate becomes limiting (e.g., Kuenzler 1970, Johannes 1964). This is also the time when PMase activity increases suggesting a possible correlation of purpose. That is that the hydrolytic enzyme may be acting on excreted FUP. A functional relationship has been established between external and internal phosphorus pools and the activity of PMase in algae and bacteria (Rhee 1973, Fitzgerald and Nelson 1966, Harold 1963). The internal polyphosphate pool decreases following nutrient depletion from the medium. Once the internal as well as the external pool is reduced to a minimum value, the cell produces PMase. It stands to reason that the function of the newly produced enzyme would be to provide inorganic phosphate for the cell.

When faced with nutrient deficient conditions (carbon, nitrogen or phosphorus), Euglena gracilus, Pseudomonos aeruginosa, and Escherichia

coli degrade a portion of the cellular protoplasm. This progressive degradation represents a mechanism for providing the cell with breakdown products for utilization in continued maintenance of basic metabolic processes (Bertini et al. 1965; Maruyama and Miziuno 1965, 1966; Natori et al. 1966; and MacKelvie et al. 1968). The external diffusion barrier creates a compartment adjacent to the external surface of the plasma membrane in which the concentration of a metabolite may be different from either the intra- or extracellular pool and from which partial recapture or reutilization may occur (Robbie and Wilson 1969).

In a similar manner algae and bacterial cells in a phosphorus deficient environment (as in North Gate Lake) may use external PMases to degrade excreted organic phosphorus esters (FUP_{1+2}) (Figure 29) and absorb the liberated inorganic phosphate. Over time a portion of the organic phosphate may be lost from the diffusion layer and appear as FUP_2 (Figure 29). FUP_2 would have to pass through the enzyme layer to become free in the water column accounting for the refractory nature of a considerable portion of the free FUP pool (Figure 20). Thus, the appearance of FUP during phosphorus deficient conditions represents a net loss from the microdiffusion layer due to either the inability of the enzymes to degrade it, or simple inefficiency. This loss over time is also a source of the considerable pool of free enzymes found in North Gate Lake.

In phosphorus poor waters, the plankton have very little phosphorus stored as polyphosphate. It is this pool of internal cell phosphorus that receives absorbed phosphate (Rhee 1973). If this pool is small then the phosphorus absorbed will be rapidly transferred from it (pool A) to the organic phosphorus fraction (pool B) e.g., RNA (Figure 29). It is

from this pool that short term FUP first arises (Figure 18). Such a rapid production of FUP would not be expected to take place in nutrient sufficient waters since the absorbed phosphate ($DI^{32}P$) would have to equilibrate with a very large pool of stored polyphosphate before being excreted as an organic compound. Extracellular leakage of FUP may follow carbon excretion since comparative studies of extracellular production by plankton samples indicate that such release is higher in olitotrophic (phosphate poor) than in eutrophic (phosphate rich) systems (Saunders 1972).

Several other important predictions can be made from this model. For example, if you have sufficient polyphosphate phosphorus, the PMase would be expected to be considerably reduced, the uptake kinetics would be linear, and the uptake rate of $DI^{32}P$ would follow photosynthesis. There would be a diel difference in uptake rate with a high rate in the day time falling to a minimum at night. In addition, since this would be a phosphorus sufficient system, a diel difference in FRP concentration would not be expected. In contrast, a phosphorus deficient system would have a considerable pool of active PMase, exponential uptake kinetics, and very little diel difference in uptake rates. However, the FRP might well be expected to change on a diel basis with a minimum at night.

Finally, the relative importance of the process of algal autolysis (i.e., death and decay) compared to excretion by viable cells in the production of FUP may in part be determined by the nutrient status of the system studied. In a nutrient deficient system the zooplankton graze heavily on the phytoplankton so that actual algal autolysis is

unimportant (Lean 1975). However, in a nutrient sufficient system the algae may not be as digestible by the zooplankton or may simply bloom and die. In this case the contribution of death and decay to the production of FUP may be very significant (Minear 1975). Thus, in nutrient sufficient systems zooplankton grazing, plankton excretion are less important, whereas bacterial action and algal autolysis are more important in nutrient regeneration compared to nutrient deficient systems where the opposite holds true.

Phosphorus Flow in North Gate Lake

A preliminary model of phosphorus flow in the surface of North Gate Lake has been developed (Figure 30). Of particular importance are the pool size differences representing the disequilibrium that was found to occur between ^{32}P and ^{31}P fractions. For this reason only approximate values for rates of transfer of phosphorus between compartments can be assigned. The width of the line represents the importance of the inter-compartment transfer whereas the size of the compartment is in relation to the fraction of each pool at apparent equilibrium.

The most important transfer between compartments takes place between the $DI^{32}P$ pool and the Part-^{32}P compartment. This rate is measured in minutes (e.g. 10% min^{-1}) with a calculated turnover time of 4-9 min depending on the depth sampled. Since the internal pool of stored polyphosphate is low in these phosphate deficient waters, absorbed $DI^{32}P$ is immediately transferred to organic linkages, and a very small portion (2-3% of total ^{32}P) is very quickly leaked back into solution as $FU^{32}P$ (fraction 1). A portion of this product is immediately

Figure 30. The cycling of phosphorus by the seston in North Gate Lake. Circled areas are proportional to the amount of phosphorus in each fraction at tracer equilibrium. Arrows indicate the magnitude of phosphorus flow.

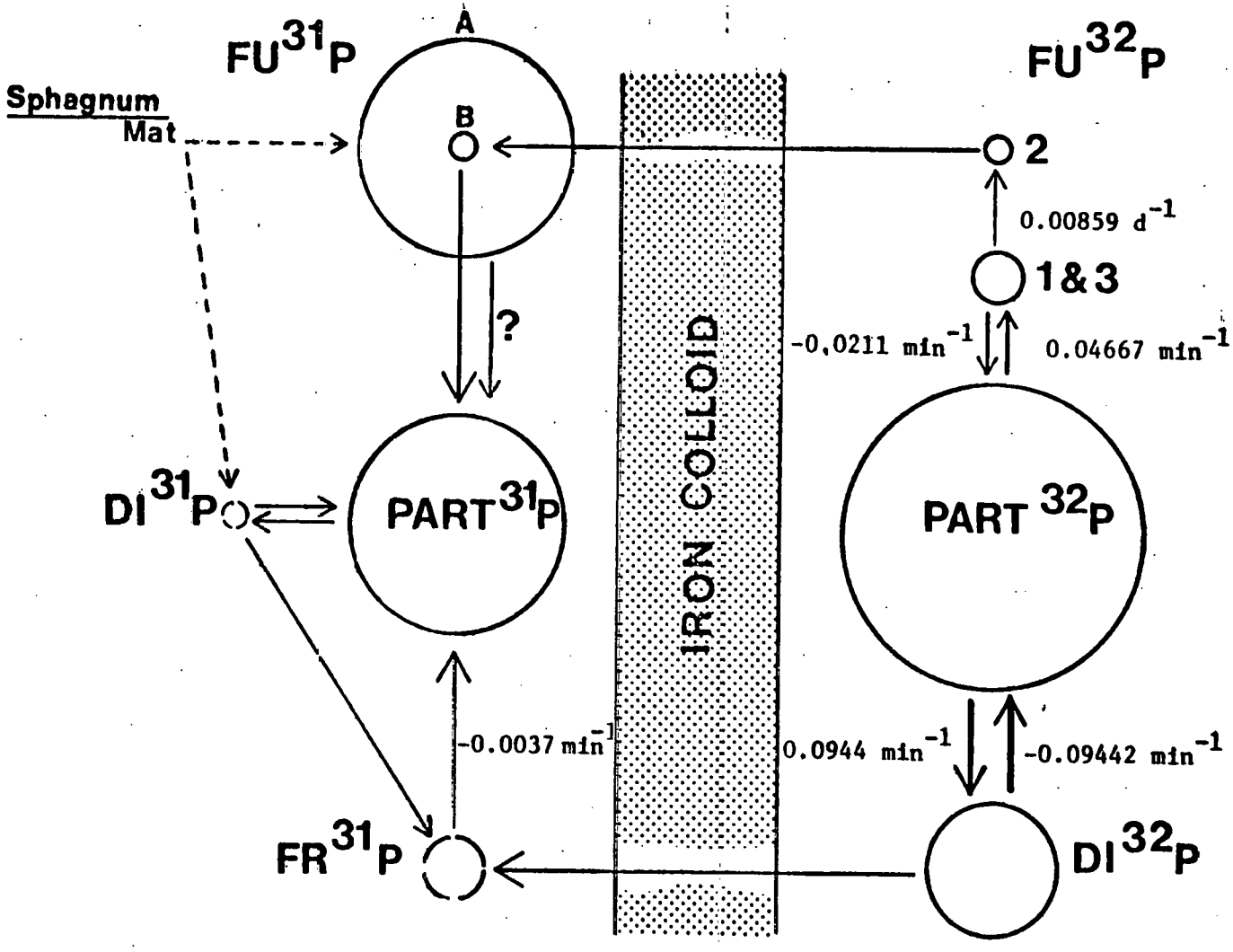

FU 31P
A
Sphagnum
Mat
B
FU 32P
2
0.00859 d-1
1&3
?
-0.0211 min-1
0.04667 min-1
IRON COLLOID
DI 31P
PART 31P
PART 32P
-0.0037 min-1
0.0944 min-1
-0.09442 min-1
FR 31P
DI 32P

Figure 30.

reabsorbed at a rate almost equivalent to its production. This can also be measured in minutes. However, the major source of $FU^{32}P$ (which is still a minor fraction of $FU^{31}P$) comes from long term excretion measured in weeks with a turnover time as long as 6.5 months (fraction 2). Again a portion (10-25% depending on the depth) can be reused (fraction 3) within minutes. It is obvious that given an available organic substrate the plankton can use it as a source of phosphate just as effectively as DIP.

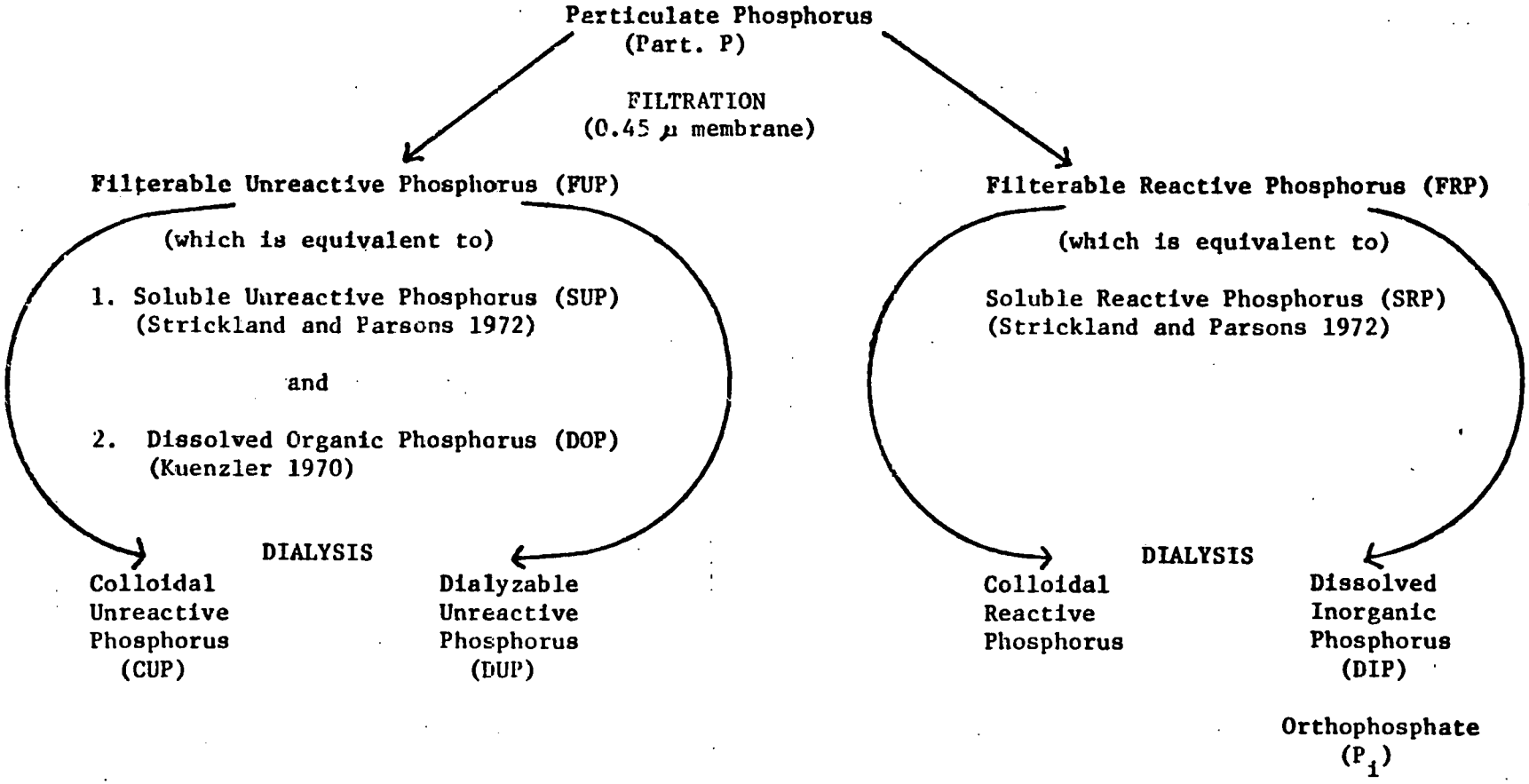
However, $FU^{32}P$ never approaches equilibrium with the larger $FU^{31}P$ pool (up to a 10-fold difference). Thus, cycling $FU^{32}P$ represents a small portion (B) of the $FU^{31}P$ pool. It is this minor fraction that interacts with the Part-^{31}P compartment. The major fraction of the $FU^{31}P$(A) is of colloidal size and is very refractory. Its turnover time can be measured in months, perhaps as long as one year.

An undetectable pool of $FR^{31}P$ was found in the euphotic zone, and any added $DI^{32}P$ is slowly bound by a COM-iron colloid to form $FR^{32}P$. There appears to be competition between the seston and the colloidal fraction for orthophosphate. Through mass action, free $DI^{32}P$ is converted to bound $FR^{32}P$. This bound $FR^{32}P$ can still be used by the plankton (algae and bacteria) as a source of inorganic phosphate, but instead of a turnover time of minutes as with $DI^{32}P$, the turnover time increases to nearly 3.5 hrs. However, even though the $FR^{32}P$ is not free, it is still responsive to the acid molybdate test, and is only partially available to the plankton. Up to 60% of $FR^{32}P$ is removed by binding from the utilizable pool of orthophosphate. Thus, one-half to two thirds of the total molybdate response is not biologically available

to the plankton within the time period of my experiments.

Since this is a nutrient deficient system which would emphasize the importance of the colloidal fraction, the actual importance of "bound" phosphate would certainly be less important in eutrophic or nutrient sufficient systems, especially those systems low in COM and iron or calcium. In those systems the FRP test may well give a very reliable estimate of free DIP.

APPENDIX


Particulate Phosphorus
(Part. P)
FILTRATION
(0.45 μ membrane)
Filterable Unreactive Phosphorus (FUP)
(which is equivalent to)
1. Soluble Unreactive Phosphorus (SUP)
(Strickland and Parsons 1972)
and
2. Dissolved Organic Phosphorus (DOP)
(Kuenzler 1970)
DIALYSIS
Colloidal Unreactive Phosphorus (CUP)
Dialyzable Unreactive Phosphorus (DUP)
Filterable Reactive Phosphorus (FRP)
(which is equivalent to)
Soluble Reactive Phosphorus (SRP)
(Strickland and Parsons 1972)
DIALYSIS
Colloidal Reactive Phosphorus
Dissolved Inorganic Phosphorus (DIP)
Orthophosphate (P_i)

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