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*Nutrient Cycling and Productivity
of Dystrophic Lake-Bog Systems
(Part A)*

Technical Progress Report

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SCOPE OF WORK

December 1, 1972 - September 30, 1975

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1. CONTRACT ACTIVITIES

During the above period, work on the project was carried on by a senior doctoral student (J. P. Koenings) under supervision of the principal investigator. In January, 1973, the Principal Investigator (F. F. Hooper) accepted an appointment as a member of the Atomic Safety and Licensing Board Panel. To avoid possible conflict of interest I agreed not to ask for a further renewal of this contract while serving as a member of the ASLB Panel. From January, 1974, until January, 1975, I was on sabbatical leave from the University and devoted approximately 50% of my time to ASLB Panel work. With the abolition of AEC in 1975, I made inquiries of the Nuclear Regulatory Commission regarding the status of the conflict of interest requirement. Dr. Marvin Mann, in answer to my letter of February 21, 1975, advised that I would be free to seek renewal of my contract with ERDA since the two agencies were now under different administration. My request for renewal was not made last spring but I requested additional special research support agreements because sufficient funds remained in my contract to perform the field work planned for the summer of 1975.

2. WORK PERFORMED (SUMMARY)

All the work undertaken during the above period was related to Subproject B (phosphorus cycling) and Subproject D (iron cycling).

SUBPROJECT B - PHOSPHORUS CYCLING

Mat-Water Phosphorus Exchange

BACKGROUND

The mat labeling experiment in 1971 (see Progress Report, 1972, and Appendix C) indicated the following:

1. The ^{32}P label applied to the surface of the Sphagnum of the mat moved into the lake as a filterable organic phosphorus component which persisted in the open water for at least three weeks. There was no demonstrable change in concentration and no evidence of uptake into the biota. During this time no activity passed into the plankton of the open water, thus there

was little, if any, breakdown of this material. This finding raised three questions about these compounds: (A) Why they were resistant to breakdown; (B) their source; and (C) their impact upon the phosphorus cycle.

2. Adding ^{32}P to the mat interstitial water rather than to Sphagnum produced a labeled particulate phosphorus which was soon incorporated into the biotic cycle of the system and was found in the zooplankton and phytoplankton immediately, as would be predicted from other labeling studies.

Hence, uptake by Sphagnum appeared to produce refractory organic phosphorus which tended to accumulate and possibly occlude the phosphorus cycle of the system.

3. The input of mat label phosphorus compounds to the open water of the lake was not always at the surface, but entered the thermocline and also the lower hypolimnion. Although the finding of this new pathway of phosphorus further complicated the phosphorus cycle of the system, it gave a potential explanation for the high concentration of phosphorus in the monimolimnion, and suggested that the meromixis found in the system might arise by means of density flow from solutes produced in the mat system.

4. Inflow of the material from the bog mat to the lake water occurred almost exclusively during periods of precipitation. Flushing of the mat by rain and by increase in the water table level thus appeared to be the physical process responsible for lakeward transport of mat phosphorus.

PROGRAM IN 1974-1975

Although the Principal Investigator did not participate in this work during the summer of 1975, it was carried on by Jeffery P. Koenings, senior doctoral graduate student employed on the project. He directed the continuation of Subproject B with the help of other graduate students. Since no additional funds were provided for the project in 1974, the 1974-1975 work was with a reduced budget.

Work under Subproject B consisted chiefly of a follow-up on the questions raised in paragraphs 3 and 4 above. We initiated studies to determine the extent of leaching of phosphorus and iron compounds from the mat during periods of rainfall. These studies verified that there was considerable leaching of phosphorus after each rain (Appendix C). We also undertook extensive measurements of precipitation on the mat surface together with studies of mat temperature changes, changes in total insoluble phosphorus, as well as changes in organic carbon. Corresponding measurements were made at the mat-water interface zone so that lake water changes might be related to hydrological events in the mat. The data gathered indicated large changes in concentration during the summer and further suggested that the evapotranspiration of the vegetation of the mat was responsible for a flow of lake water into the mat at 2 m during periods of warm dry weather. Thus in effect there appears to be a recycling

cell operating at the mat-water interface with a flow of phosphorus lakeward during periods of rainfall, and a return flow of dilute lakewater into the mat during periods of high evaporation and transpiration by bog vegetation.

The investigations relating to the mat-water exchange were continued in 1975 by Ms. Laura Broom, a candidate for a Master of Science degree in the School of Natural Resources. Ms. Broom will use the 1974-1975 data in preparation of her thesis "Hydrological Factors Influencing Transport of Mat-Phosphorus in the Cycle of a Bog Lake System." Ms. Broom's material is being assembled for her thesis but results are not yet available for a written report. However, it is clear that her data verify the above suggestion (paragraph 3) that phosphorus flows into the lake water along temperature contours, and therefore may transport phosphorus to deeper strata. For her work, additional lysimeters were installed in the bog mat and careful measurements were made of flows during the summer of 1975 in the mat and mat-water interface zone. A local ^{32}P labeling experiment at the mat zone provided all the critical data needed as proof of the proposed movement.

SUBPROJECT D - IRON CYCLING

Studies reported in earlier projects have indicated that the methodology widely used in iron studies does not properly characterize the chemical and colloidal state of iron in acid bog lakes. After finding the proper methods for analyzing the chemical and physical state of iron in our system (see Appendix A) it was necessary to undertake a series of in situ field experiments and laboratory tests of field samples to clarify the relationship of colloidal organic matter (COM) and various forms of iron and phosphorus in the North Gate Lake system.

The results of these studies are reported in Appendix B of this report. They indicate that the COM plays a major role in the changes taking place in iron and phosphorus during the seasonal cycling in the lake basin at the time of the overturns. With COM present, a fraction of the ionic (dialyzable) iron and phosphorus from the anaerobic zone is transported into the oxygenated zone as a colloidal iron-phosphate-organic acid complex but most remains free as SRP (soluble reactive phosphorus). Thus with COM present, a large fraction of available phosphorus enters the photosynthetic zone. Without COM, a high percentage of colloidal inorganic phosphate is formed which is not biologically available. The effect of COM is to mask the ionic properties of colloidal ferric iron and retard the formation of nonreactive ferric iron. Thus masking of iron makes possible the transport of phosphorus from the anaerobic water to the aerobic water in a form utilizable by photosynthetic organisms.

APPENDIX A

IN SITU EXPERIMENTS ON THE DISSOLVED AND COLLOIDAL
STATE OF IRON IN AN ACID BOG LAKE

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Abstract

A sampler and an analytical and characterization scheme were developed to investigate the chemical and physical state of iron in a highly stained acid bog lake. Iron fractions were chemically separated by reactivity to bathophenanthroline (BPN) after specific digestion procedures. Physical separation involved anaerobic in situ filtration (450 nm), and in situ dialysis (4.8 nm). This resulted in three size classes: particulate, colloidal, and dissolved.

Unlike pH 6-8 lakes, inorganic, nonreactive species of iron [e.g., $\text{Fe}(\text{OH})_3$] represent minor amounts of the total iron in North Gate Lake. In situ radiochemical analyses revealed that colloidal sized reactive ferric iron predominates in the oxygenated epilimnion, while dialyzable or dissolved ferrous iron increases with depth. Unlike iron, organic matter is present in a non-transient colloidal state. In acid (pH 4-5) bog lakes ferric iron is not colloidal as an inorganic complex (OH^-), but colloidal as reactive Fe^{+3} by complexation with the colloidal organic acids.

After thermally induced mixing with anaerobic waters, ferric iron is reduced and dissolved. However, some of the BPN reactive iron remains colloidal suggesting the presence of a residual ferrous organic complex.

Introduction

Because of their high contents of particulate and colloidal organic matter (COM), low pH, and extremes of Eh; bog lakes present complex systems for measurement of the physical and chemical states of iron. Sampling and analytical difficulties have created some uncertainties regarding the iron species present at specific depths in the water column.

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Inorganic iron may be present in the epilimnion of oxygenated lakes (pH 6-8) as Fe(III) hydroxides in the form of either a precipitate or a peptized sol (Hutchinson 1957; Shapiro 1964). Shapiro (1964) demonstrated the ability of organic acids to hold iron in apparent solution at pH levels greater than 5 as peptized sols. However, at lower pH, ionization of organic acids decreases, the rate of oxidation of iron becomes independent of pH, and the solubility of all ionic forms of iron increases. Low pH, combined with low oxygen tensions and a reducing (COM) environment, may permit large amounts of ionic iron to be present without inorganic (hydroxyl ion) complexation in bog lakes. In addition, the low ionic strength of bog lake water, as indicated by the low conductivity ($20-30 \mu\text{mhos cm}^{-1}$ at 18°C) reduces the coagulative formation of particulate forms of iron. Under these conditions, solution size, oxidation state, and organic acid complexation of ferrous and ferric iron may be quite different from that reported in lakes of neutral or basic pH.

Low dissolved oxygen levels, high CO_2 , low pH, and the presence of reducing organic and inorganic compounds favor formation of ferrous iron. Decomposing organic residues (including "humic acid") have been reported to reduce ferric iron to ferrous iron or form ferrous-organic complexes (Bloomfield 1952). Shapiro (1966) demonstrated the reducing power of colored organic acids on ferric iron from natural waters, and Hem (1960) demonstrated the reducing power of tannic acid toward ferric iron in artificial laboratory systems. Finally, divalent sulfur compounds (e.g., H_2S) found in lake waters are able to reduce ferric iron (Stumm and Morgan 1970). All the above are encountered in highly colored acid bog water (pH 4-5) and tend to increase the solubility of iron by favoring the ferrous state. The purpose of this paper is to define more precisely the physical and chemical states of iron in acid bog lakes, and to determine the in situ relationship of iron to COM in differentially oxygenated lake waters.

In the summer of 1972 I attempted to fractionate and characterize the iron compounds in the epilimnion and hypolimnion of North Gate Lake to assess the effects of oxidizing and reducing environments on potential COM-iron complexation. A sampler and analytical scheme were devised to enable the measurement of the forms of ferrous and ferric iron without interferences. The naturally existing physical state of iron at various depths in the epilimnion was determined by an in situ radiochemical and dialysis study on a column of lake water contained in a polyethylene cylinder.

I wish to thank Drs. F. F. Hooper, J. Shapiro, J. W. McMahon, and P. Kilham for both critically reviewing preliminary and final drafts of the manuscript and offering their encouragement. I also wish to thank Dr. Robert E. Gordon, Director of the University of Notre Dame Environmental Research Center, for providing facilities and use of the Notre Dame tract.

Study Area and Methods

These experiments were undertaken in North Gate Lake, a small, highly-colored, meromictic lake located within Section 37 of T45N, R42W, Gogebic County, Michigan. The lake is one of numerous bog lakes on a 2,225 ha reserve owned by the University of Notre Dame. It has a surface area of approximately 0.4 ha, and a maximum depth of 8 m. The open water is surrounded by a Sphagnum mat which extends into a black spruce forest.

Measurements of the oxidation state and speciation of iron in lakes is difficult because (1) the two oxidation states of iron and their various complexes may occur simultaneously at a given depth, (2) both the oxidation state and coordination are very sensitive to dissolved oxygen, and (3) the oxygen content of samples is easily altered during collection, storage, and analysis. Bathophenanthroline (BPN) was used for the measurement of ferrous iron. BPN does not react with any known metal ion (including Fe^{+3}), except Fe^{+2} , to give a colored complex that can be extracted by n-hexyl alcohol at pH 4.0. This extraction procedure isolates the colored complex from its aqueous solution and thereby increases sensitivity and extends the specificity to the point where there is no known interference by either metal cations or common anions (Smith *et al.*, 1952). The red complex is extracted into n-hexyl alcohol (10 ml), after neutralization of the various digests, is diluted with ethyl alcohol to a set volume (50 ml), and the absorbance determined on a Klett-Summerson colorimeter at 540 nm using a 4-cm light path length (Fig. 1). All samples were duplicated and each digestion procedure contained a blank. Standards were run with each experiment.

Since Fe(II) is unstable in the presence of oxygen, preservation of samples from time of collection has been necessary. Preservation with acid has the side effects of accelerating the reduction of Fe(III) to Fe(II), releasing iron from organic complexes, and dissolving inorganic ferrous salts (McMahon 1967; O'Connor *et al.*, 1965). Thus, only total ferrous iron was measurable. Sunlight also interferes with the determination of ferrous iron by differentially reducing Fe(III) compounds.

To circumvent these interferences, a plastic in situ filtration sampler was used which yielded 200 ml of a 0.45 μ membrane filtered sample. Samples were collected with four airtight, light-proof samplers from the surface (0-5 cm), the top of the thermocline (2 m), the middle of the anaerobic hypolimnion (5 m), and the monomolimnion (7.5 m). Sampler containers were first filled with a mixture of helium (99.05%) and isobutane (0.95%) and then transferred in a pressurized condition to the lake. At each depth sampled, a container was attached to a 0.45 μ membrane filtering apparatus and to filtration tubing connected to a vacuum pump. The apparatus was submerged in the water and the stopcocks opened. The slight positive pressure of the gas in the sampler cylinder removed air in lines leading to the membrane and to the vacuum pump. The apparatus was then lowered to the desired depth and filled with lake water by vacuum filtration. After quickly raising the sampler to near the surface,

Fig. 1. Analytical scheme used to characterize iron species in North Gate Lake.

the stopcocks were closed while the apparatus was still submerged to prevent the introduction of oxygen. Sampler cylinders were returned to the laboratory, under a slight negative pressure, and a gas-filled balloon was fitted to the upper stopcock. The stopcock was then opened, pressurizing the sampler. Gas from the balloon entered the cylinder during the removal of aliquots from the lower stopcock.

Four analyses were performed on duplicate 25-ml aliquots from a series of depths (Fig. 1, 1-4). All samples were analyzed within 20 min from time of collection. Analysis 2 measured total ferrous iron (acid + BPN), and analysis 4 measured total iron (reductant + acid + heat) (Golterman and Clymo 1969). Two further analyses were performed on samples without acid digestion. Analysis 1 consisted of the addition of BPN directly to a sample, followed by color development and hexanol extraction (McMahon 1969b). Since acid dissolves insoluble ferrous salts (O'Connor *et al.*, 1965), and releases Fe(II) from organic complexes (McMahon 1969a), the addition of BPN without acid measures only the BPN reactive form of ferrous iron (Fe^{+2}). Analysis 3 consisted of adding only a reducing agent (hydroxylamine hydrochloride) followed by BPN, color development, and extraction. This measured total reducible iron which includes BPN reactive ferrous iron, in addition to reactive ferric iron which requires reduction alone to bring it to the BPN reactive state. The term nonreactive ferric iron is used here to describe all forms of iron that require hot acid digestion plus reduction for release to the BPN reactive state. This is similar to the procedure used by Stumm and Lee (1961) to measure colloidal ferric oxyhydroxide.

In summary, the analytical scheme was as follows:

Analysis 1 = BPN reactive ferrous iron
Analysis 2 = total ferrous iron
Analysis 3 = total reducible iron
Analysis 4 = total iron

Results from these four analyses were used as follows in calculations of iron species:

Analysis 1 = BPN reactive ferrous iron
Analysis 2 - Analysis 1 = nonreactive ferrous iron
Analysis 3 - Analysis 1 = reactive ferric iron (result A)
Analysis 4 - Analysis 2 = total ferric iron (result B)
Result B - Result A = nonreactive ferric iron

The above categories can be described as dissolved, colloidal, etc., if the water has been appropriately pretreated. The effectiveness of the above characterization scheme in differentiating between dissociated and undissociated iron as well as between ferrous and ferric iron was tested by analyzing freshly prepared iron standards of known concentration. Whereas these compounds do not exist in nature, they do demonstrate the reactivity of known forms of iron to the proposed reaction scheme from which natural lake iron was

subsequently classified. The replicability of the BPN method was excellent. Eight samples containing 10 μg of iron were analyzed for total iron with a standard error of 0.02 μg .

Samples were taken on 19 July and again on 21 July using in situ anaerobic filtration at the four sampling depths in the center of the lake. Samples were taken at the same depths on 24 July and on 24 August using a modified Hale sampler with acid-washed 300 ml BOD bottles. These samples were transported to the laboratory with minimal exposure to light or atmospheric oxygen, and were filtered through 0.45 μ membranes in the presence of air. The analytical and characterization schemes described above were applied to the sets of samples collected by the two different methods. Physical and chemical parameters of the water were monitored continuously from 12 May to 10 August (including conductivity, pH, alkalinity, temperature, dissolved oxygen, total iron). These parameters did not change after the first of June. A stable thermocline (2 m) develops early in North Gate Lake with chemical and physical parameters remaining nearly constant until the fall overturn. The day to day variations were small. This permitted comparison of samples collected in the same location but analyzed with different procedures on successive dates. Duplicated experiments agreed very well and were averaged to show the naturally existing species of iron in different strata of the lake and the effects of aeration (aerobic filtration) on the species of iron present.

An in situ radiochemical experiment using ^{59}Fe was performed to determine both the natural physical state and the movement of iron in acid bog waters. $^{59}\text{FeCl}_3$ (1.0 mCi) was carefully added to the surface (7.0 cm) of a column of lake water contained in a polyethylene cylinder 1 m in diameter and 8 m deep. The addition of the tracer increased the amount of iron in the surface water by less than 1%. The cylinder contained a series of glass sampling tubes spaced at 1-m depth intervals allowing samples to be withdrawn from the center of the tube without disturbing the water column. An evacuated bottle was used to draw samples to the surface through connecting tygon tubing. A volume of water equal to that of the tubing volume was discarded prior to sampling. Samples were taken to the laboratory and filtered. Total and filterable activity of samples were measured by drying triplicate 5 ml subsamples on planchets, and counting on a low background beta counting system. Rates of movement of the tracer were equal to the slope of the least squares regression estimate for the change in ^{59}Fe activity with time after correcting for isotopic decay. In addition a total radioactive iron budget was calculated for the plastic cylinder in order to determine any loss of the tracer out of the epilimnion over the time period of the experiment.

To characterize further the physical state of the iron, I installed sealed sections of acid-cleaned (10% HCl) cellulose dialysis tubing (a pore diameter of 4.8 nm or a molecular weight cut off for proteins of >12,000) containing distilled water inside the cylinder at 1-m intervals five days after the tracer was introduced. I monitored activity within the tubing five days later by withdrawing triplicate 5-ml samples, evaporating to dryness and counting. The time period of five days used for quantitative equilibrium of the dialysate

with the surrounding water was determined using $^{59}\text{FeCl}_3$ and FeCl_3 standard solutions at 20° and 5° C.

The physical size fractions used were (1) materials $>0.45\ \mu$ were particulate, (2) those $<0.45\ \mu$, but $>4.8\ \text{nm}$ were colloidal, and (3) those $<4.8\ \text{nm}$ were truly soluble or dissolved.

Results

STANDARD IRON SOLUTIONS

All solutions were analyzed at pH 4-5 with $400\text{--}500\ \mu\text{g liter}^{-1}$ of iron present. Ferrous ammonium sulfate reacted as ferrous iron (100%) and as BPN reactive ferrous iron (95%) (Table 1). Less than 5% reacted as nonreactive ferrous iron. Ferric ammonium sulfate reacted as ferric iron (100%), and was calculated to be a nonreactive ferric iron compound (88%). Ferric chloride reacted in a similar manner. These ferric iron compounds, unlike the ferrous compound, did not react as free dissociated (hydrated) iron, but required acid digestion plus reduction to be reactive to BPN. The ability of ferric iron to form incompletely dissociated inorganic complexes with sulfate and chloride is well known (Cooper 1937; Rabinowich and Stockmayer 1942; Stumm and Lee 1960). More importantly, ferric oxyhydroxide reacted as did the previous two inorganically complexed forms of iron, that is, as nonreactive ferric iron. The above results are from freshly prepared standards. Aged solutions (3-5 days) of ferric chloride and ferric oxyhydroxide yielded recoveries as nonreactive ferric iron of 96%.

Table 1. Iron standards characterized by the proposed analytical scheme showing the separation of differentially reactive iron species. (Values are in percent of total iron.)

	Iron Standards			
	BPN Reactive Ferrous Iron	Non- Reactive Ferrous Iron	Reactive Ferric Iron	Non- Reactive Ferric Iron
(1) ferric oxyhydroxide	0.0	0.0	16.0	84.0
(2) ferric chloride	0.0	0.0	16.0	84.0
(3) ferric NH_4 sulfate	0.0	0.0	12.0	88.0
(4) ferric perchlorate	0.0	0.0	100.0	0.0
(5) ferric citrate	0.0	0.0	100.0	0.0
(6) ferric EDTA	0.0	0.0	100.0	0.0
(7) ferrous NH_4 sulfate	95.0	5.0	0.0	0.0

Ferric perchlorate reacted as reducible ferric iron, and was entirely reactive ferric iron that required reduction alone to react with BPN. The perchlorate anion does not form an inorganically bound complex with iron to the degree of sulfate, chloride, and hydroxide. Thus, reactive ferric iron represents iron more in the completely dissociated Fe^{+3} state. However, reactive ferric iron need not be in the free state, as iron organically complexed (e.g., ferric EDTA, and ferric citrate) also was present in the reactive state. Thus, iron in the ferric reactive state is primarily dissociated iron (Fe^{+3}) present either as free (hydrated) or as iron in colloidal organic complexes. Since free (hydrated) ferric iron would be dialyzable, colloidal reactive iron present in natural lake waters is more likely in organic complexes or perhaps as very weak inorganic colloids. Thus, if colloidal and reactive, the iron may be present in an organic complex with COM, but if colloidal and nonreactive, the iron is more likely to be present as an inorganic colloid, e.g., ferric oxyhydroxide.

The rationale is, then, that although dissociated iron will react with BPN without acid, inorganically complexed iron will not react until the appropriate acid digestion procedure renders the iron reactive. The above classification scheme linked with physical separation of iron forms (i.e., dissolved vs. colloidal) by in situ filtration and dialysis yielded a characterization scheme for acid bog lake iron.

LAKE IRON

Levels of iron in North Gate Lake were in the highest range of values for the adjacent northeast Wisconsin lakes studied by Juday et al. (1938). Many of the lakes of this region are highly stained. In North Gate Lake yellow colloidal sized organic matter comprises up to 85% of the total organic carbon, and 90-100% of the total iron is 0.45 μ membrane filterable (Table 2). This large fraction of filterable iron suggests that the presence of 30-40 mg liter⁻¹ colloidal organic matter combined with low pH and conductivity prevents formation of large iron polymers. Total filterable iron increased from

Table 2. Size classification of ^{59}Fe activity following addition of tracer to a water column enclosed in a plastic cylinder in North Gate Lake (A), and stable iron distribution in North Gate Lake using in situ dialysis (B).

Depth	A		B	
	Percent Total Filterable	^{59}Fe Activity Dialyzable	Percent Total Iron Filterable	Dialyzable
Surface	91-100	14-15.5	90-100	8
1 m	84- 92	22-25	-	-
2 m	80	55-63	99	45-60

264 $\mu\text{g liter}^{-1}$ at the surface to 1161 $\mu\text{g liter}^{-1}$ in the monomolimnion, and BPN reactive ferrous iron, measured after in situ filtration, increased from 73 $\mu\text{g liter}^{-1}$ (28% of total iron) to 1078 $\mu\text{g liter}^{-1}$ (93% of total iron) (Table 3). Oxygen was absent below 2 m, and iron was present almost exclusively as BPN reactive ferrous iron. Reactive ferric iron on the two dates sampled represented 78-90% of the ferric iron in the surface water. At the surface an average of 119 $\mu\text{g liter}^{-1}$ was present (45% of the total iron). Of interest is the failure to find large amounts of nonreactive Fe(III) (e.g., ferric oxyhydroxide) at any depth sampled. Values ranged from 26 $\mu\text{g liter}^{-1}$ to 79 $\mu\text{g liter}^{-1}$, but this represented less than 10% of total iron. At the surface 55% of the iron was in the ferric state, compared to 6% in the hypolimnion. BPN reactive iron and reactive ferric iron (i.e., from acid free analysis) increased from 73% in the epilimnion to 95% in the hypolimnion. The small remaining fractions (27% to 5%) in these two strata were acid digestible nonreactive iron [Fe(II) and/or Fe(III)].

The reducing COM in the hypolimnion does not form a more stable complex with ferrous iron than the BPN-Fe⁺² complex. The predominant form of Fe(II) was BPN reactive, contrary to Gjessing (1964) who postulated the presence of Fe(II) in organic complexes that was reactive only after hot acid digestion of highly colored, pH 4-6, Norwegian waters. The oxygenated epilimnion contains principally reactive ferric iron (84% of ferric iron) and little ferric oxyhydroxide. The 2-m stratum which had a dissolved oxygen content of 0.50 mg liter⁻¹ also contains reactive ferric iron, and thus, appears to be the zone in which the downward diffusing dissolved oxygen first oxidizes Fe(II) diffusing upward from the anaerobic hypolimnion.

In the low pH range (4-5) of the surface water of North Gate Lake, the reactive form of Fe(III) is the predominant form of iron. However, even though environmental conditions exist in these waters which allow Fe(III) to exist chemically in a reactive state, stable iron analysis and radiochemical data presented below indicate that this is in colloidal form.

LABELING EXPERIMENT

Working on highly stained lake waters from Alaska, Barsdate (1970) found that 80% of the cobalt and 68% of the manganese (in addition to the organic acids) were retained by dialysis bags. He believed that this was due to the formation of complexes and associations between metals and the highly dispersed organic colloids. To make an in situ test of this hypothesis in North Gate Lake, ⁵⁹FeCl₃ was carefully added to the surface water contained in the polyethylene cylinder, after it had been installed in the lake and it had been determined that thermal equilibrium had been established with the surrounding water (Fig. 2). Slow exchange with the 1-m stratum took place until cool weather and wind mixed the upper two layers (Day 5). Slow vertical leakage then occurred from the 1-m layer through the thermocline for the remainder of the 11-day experiment.

Fig. 2. Tracer (^{59}Fe) cylinder experiment in which the changing thermal regime of the lake water (A) caused the movement of ^{59}Fe added to the surface stratum to lower strata (B), and the total tracer budget for the epilimnion of the cylinder (C).

Table 3. Distribution of the natural species of dissolved iron in North Gate Lake following in situ filtration (A), and the distribution of dissolved iron species in the same strata following aerobic filtration (B).

Depth (m)	BPN Reactive Ferrous Fe(+2) Iron		Nonreactive Ferrous Fe(II) Iron		Reactive Ferric Fe(+3) Iron		Nonreactive Ferric Fe(III) Iron		Total	
	A	B	A	B	A	B	A	B	A	B
0 (surface)	73	42	46	19	119	93	26	140	264	294
2 (metalimnion)	426	182	36	16	84	241	49	168	595	607
5 (hypolimnion)	634	366	3	126	8	160	32	24	677	676
7.5 (monomolimnion)	1073	610	4	228	0	279	79	37	1161	1154

Marshall and LeRoy (1972) showed that ^{59}Fe equilibrated with the particulate inorganic iron of a South Carolina reservoir within 24 hours, and that on the average 65% of the iron was particulate. However, in the surface waters of North Gate Lake, 91-100% of the total activity was filterable and only 15.5% of the filterable activity was dialyzable after 11 days. At 2 m, up to 63% of the total activity was dialyzable. The percentage increase in the dialyzable activity fraction from the surface to 2 m (15.5% to 63%) was similar but lower than the increase in BPN reactive ferrous iron (28% to 72%) between these two strata and reflects increasing reduction and release of the colloidal iron to a dissolved state with increasing depth (Tables 2 and 3).

The organic matter in North Gate Lake does not require sorption to colloidal sized inorganic ferric iron to be nondialyzable. Along with the COM, the iron activity of the surface waters was colloidal, but did not react as an inorganic colloid. Thus, the iron species of the surface waters [primarily reactive Fe(III)] may be colloidal due to complexation with COM. However, at 2 m ferrous iron shows significantly less affinity for the colloidal state and appears to be in a dissolved ionic state.

Colloidal Fe(III) of the surface water moved down in the cylinder and was reduced to dissolved iron [Fe(II)]. This movement was caused by cooling of the surface water, and thermal equilibration between the 1-m and surface strata (Fig. 2). During the cooling period (Days 2-5) the rate of loss (-0.210×10^8 cpm day $^{-1}$) of activity from the surface agreed well with the increase of $+0.185 \times 10^8$ cpm day $^{-1}$ at 1 m prior to mixing. These rates increased to -0.546×10^8 cpm day $^{-1}$ and $+0.576 \times 10^8$ cpm day $^{-1}$, respectively, during mixing (Days 5-6). The combined rate of loss of tracer from the surface (0.756×10^8 cpm day $^{-1}$) agreed with the increase at 1 m (0.759×10^8 cpm day $^{-1}$). On Day 6, the recovery of added tracer was 102%. After thermal equilibration (Day 6) no further loss of tracer took place at the surface layer through Day 11, at which time ^{59}Fe activity at the surface layer represented over 50% of the added iron. Following mixing, activity was lost from the 1-m stratum at the rate of -0.0953×10^8 cpm day $^{-1}$. This rate was significantly lower than the rate of loss at the surface (Days 2-5). However, unlike the surface to 1 m mixing, the loss was not balanced by total rate increase at other depths, [2 m ($+0.0190 \times 10^8$ cpm day $^{-1}$), surface ($+0.0346 \times 10^8$ cpm day $^{-1}$), and 3 m ($+0.0012 \times 10^8$ cpm day $^{-1}$)], suggesting that 42% of the loss from 1 m had become concentrated between 1 and 2 m. The unaccounted for tracer loss from 1 m, 0.0405×10^8 cpm day $^{-1}$, for the 5-day period following mixing equals 0.2025×10^8 cpm which is 8% of the total ^{59}Fe added. This loss held at the top of the thermocline appears to be responsible for the slight under-recovery (92%) calculated for the total iron budget at the end of the cylinder experiment.

During the entire experiment, 80-100% of the tracer was filterable at all depths sampled. Thus, in this acid bog lake particulate polymers of iron constituted a minor fraction of the total iron and removal of iron from the epilimnion did not occur by precipitation and sedimentation. The predominant

form of ferric iron is colloidal. As the colloidal ferric iron moved to deeper layers, it was reduced to dissolved ferrous iron. However, a colloidal form of iron (II) appeared to be presented at 2 m since 72% was BPN reactive ferrous iron and only 63% dissolved iron. The slight increase in activity observed below the thermocline at 3 m may be an artifact caused by the preferential movement of tracer down the walls of the cylinder.

COMPARISON WITH AEROBIC FILTERED SAMPLES

To demonstrate the changes in iron species produced by the introduction of air (dissolved oxygen) to the lake water, aerobically filtered (0.45 μ membrane) samples were characterized by the same reaction series used to characterize in situ filtered samples (Table 3B). Since these samples were not and could not be subsamples of the anaerobic samples, but were from the same water layer, exact $\mu\text{g liter}^{-1}$ comparisons are not valid. However, I feel comparison of the relative forms of iron can be made, as shown by the high degree of similarity between the iron species and the total iron present within each of the duplicated aerobic and anaerobic experiments. Although the duplicates within each of the aerobic and anaerobic experiments were very similar, there were large differences between the aerobic and anaerobic results which cannot be attributed to spatial or temporal variations in the depths sampled.

Exposure to air during filtration (1-5 min) results in major shifts in iron species. In the oxygenated waters of the epilimnion, aerobic filtration caused BPN reactive Fe(II), nonreactive Fe(II), and reactive Fe(III) to decrease. These decreases were accompanied by an increase in nonreactive Fe(III) (perhaps ferric oxyhydroxide). Aerobic filtration of samples alters the reactivity of iron, and indicates that the filterable iron in the surface water is in a chemical state which is changed by further aeration. In the partially oxygenated water at 2 m, filtration brought about a decrease in BPN reactive Fe(II), and nonreactive Fe(II) and an increase in reactive Fe(III) and in nonreactive Fe(III). Aerobic filtration of completely deoxygenated water from 5 and 7.5 m resulted in similar responses (Table 3). In the case of anaerobic monomolimnion (7.5 m), 41% of BPN reactive Fe(II) was lost by aeration, 20% appeared as nonreactive Fe(II) and 24% appeared as reactive Fe(III). Nonreactive Fe(III) was not formed. In the two equally deoxygenated strata (5 m and 7.5 m), the percentage of BPN reactive ferrous iron lost by aeration was identical (43%) and the percentages of nonreactive Fe(II) and reactive Fe(III) that appeared were very similar. Since responses to aeration in these strata were similar, whereas the response of strata with different oxygen content (surface and 2 m) differed considerably, it would appear that the in situ oxygen content rather than a depth related factor regulates changes that take place upon aeration. Thus, the trend appears to be a successive conversion of iron to a more oxidized and nonreactive state upon aeration of samples from different depths.

Discussion

When lake stratification is destroyed, any dissolved Fe^{+2} in the hypolimnion is oxidized by oxygen. As the pH is usually greater than 6.0, the Fe^{+3} ions that are formed precipitate as $\text{Fe}(\text{OH})_3$ (Golterman 1973). Thus, in the oxygenated epilimnion of neutral to basic pH lakes, ferrous iron is not detectable and iron cannot be in true solution above $17 \mu\text{g liter}^{-1}$. Since lakes contain iron above this level, Shapiro (1967) proposed, and demonstrated in laboratory studies, the capacity of concentrated or chemically extracted organic acids or their salts to hold large amounts of iron in apparent solution. He concluded that the mechanism of "solubilization" of iron at high pH and redox potential is the formation of a protected sol of colloidal inorganic ferric iron. Peptized sol formation has become widely accepted as the mechanism through which organic matter complexes with iron (Hutchinson 1957; Stumm and Morgan 1970).

Acid bog lakes are potentially different systems than those on which a majority of the iron studies have been done. The presence of a large amount of COM, high CO_2 levels, low conductivity and alkalinity, and an acid (4-5) pH may alter iron species and relationships previously reported. Recently Elder (1975) demonstrated in theory that an acid pH alone may drastically de-emphasize the importance of hydroxyl ion as a ligand for metal ions. A ten-fold change in the hydroxide concentration of the water can produce changes in species of several orders of magnitude. This is true even for iron whose non-hydroxyl coordination is commonly considered negligible. Thus, in acid bog lakes ligands other than hydroxyl ion may be important in complexing iron when dissolved Fe^{+2} is oxidized to Fe^{+3} ions.

Plumb and Lee (1973) have shown that sample pretreatment in laboratory studies may change the natural iron-organic matter system, and I have demonstrated that drastic changes in iron species are caused in particular by aerobic laboratory filtration. In situ experimentation was used to minimize any effect on iron species due to sampling. The results demonstrate the undisturbed chemical and physical variability of iron in anaerobic and aerobic strata of a soft highly stained acid bog lake system.

These in situ stable and radiochemical studies support the laboratory findings of a colloidal form of iron, only for oxygenated epilimnetic waters where ferric iron predominates. Unlike neutral pH lakes, colloidal iron consists of many reactive species of iron II and III, and ferric iron reacting as a colloidal inorganic complex [e.g., sol of $\text{Fe}(\text{OH})_3$] is notably lacking. By chemical reactivity about 84% of ferric iron is potentially dialyzable reactive $\text{Fe}(\text{III})$. Since the reactive ferric iron is not dialyzable, the colloidal size of this species can be explained by complexation with pre-existing COM. The ligands present in COM appear to be important in complexing reactive iron at the low pH's encountered in bog lake waters. Although colloidal reactive ferric iron is the major form of iron in aerobic strata, ferrous iron is also present, not in trace amounts, but up to 45% of the total iron. High amounts

of ferrous iron can be maintained in partially oxygenated water by photoreduction of complexed reactive Fe^{+3} . Other organic reactive Fe^{+3} compounds, e.g., ferric citrate and ferric EDTA, have been shown to be photoreduced to a moderately stable ferrous compound if not subjected to oxidizing conditions (Jones and Long 1952).

The most prevalent forms of ferric and ferrous iron present in this study were those not requiring acid digestion to be reactive to BPN, an indication of a lack of inorganic $[\text{Fe(III)}]$ and $[\text{Fe(II)}]$ iron polymers. The hydroxyl ion concentration in North Gate Lake is up to 10,000 times less than that in pH 8 lakes from which most of the iron data have been derived. In these low pH lakes, the inorganic complexation of hydroxyl ion to iron [i.e., Fe(OH)_3] appears to be less important. That is, ferric iron is in a colloidal reactive state through complexation with the colloidal sized organic ligands (COM) rather than through inorganic (OH^-) complexation. This latter form would be nonreactive ferric iron, a form present in minor quantities. Thus, instead of organic matter sorbing at the surface of colloidal inorganically complexed iron [sol of Fe(OH)_3] as in pH 8 lakes, iron in the reactive state (Fe^{+3}), is complexed by COM in pH 4-5 highly stained bog lakes. This form of iron once reduced could account for the residual Fe^{+2} -colloidal BPN reactive iron found in strata that had recently become anaerobic.

Thus, I feel that in pH 4-5 lake waters the iron species present and their reactivities are not adequately explained by sol formation. The chemical environment of stained (COM) acid bog lakes differs from pH 6-8 lakes in which iron is soon precipitated as an acid digestable inorganic complex $[\text{Fe(OH)}_3]$ and ferrous iron is not usually present. I, therefore, propose two mechanisms of iron-organic matter complexation. The first exists in pH 4-5 highly colored lake waters where reactive ferric iron forms a colloidal complex with the COM as primary ligand. "Solubilization" of iron at acidic pH may take place through maintaining Fe^{+3} in a colloidal organic state without Fe(III) sol formation. The second mechanism occurs in pH 7-8 lake waters where nonreactive ferric iron forms an inorganic colloid with hydroxyl ion as the primary ligand. This sol could then be maintained in the soluble state by adsorption of organic matter to the iron colloid. Since flocculation and subsequent precipitation of iron has been linked to phosphate elimination from trophogenic waters of neutral to basic lakes, the interaction of iron to phosphate might also differ in acidic bog lakes. This is the subject of further research.

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APPENDIX B

THE INFLUENCE OF COLLOIDAL ORGANIC MATTER (COM) UPON IRON
AND IRON-PHOSPHORUS CYCLING IN AN ACID BOG LAKE

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APPENDIX B

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Abstract

The interrelationship between iron and phosphorus, and the relationship of these elements to colloidal organic matter (COM) was studied in a Michigan acid bog lake in which the overturns are incomplete, by means of (1) chemical characterization, (2) filtration and in situ dialysis, and (3) in situ experiments with labeled components.

Bathophenanthroline (BPN) reactive ferrous iron existed in true solution (dialyzable) in constantly anaerobic monimolimnetic water in contrast to the aerobic epilimnion, where reactive ferric iron was of colloidal size. Intermediate depths contained oxygen only during the spring and contained two forms of ferrous iron reactive to BPN. One was dialyzable ferrous iron (Fe^{+2}), and a second was colloidal Fe(II) which may have originated through in situ reduction of colloidal reactive ferric iron, and may be present as a COM-Fe^{+2} complex.

The amount of COM present had an important influence upon the fate of dialyzable Fe^{+2} (92% of total iron) and dialyzable orthophosphate (85% of total phosphorus) present in anaerobic strata prior to aeration. Aeration with soluble organic matter present resulted in the formation of colloidal nonreactive ferric iron (84% of total iron) and 65% colloidal phosphate while aeration with 26 mg liter^{-1} COM resulted in only 36% colloidal, nonreactive ferric iron and 19% colloidal phosphate. COM appears both to mask the cationic properties of colloidal ferric iron, and retard the formation of nonreactive Fe(III) .

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Introduction

The organic acids found in natural waters may alter the cycle of biologically active metals by means of various complexation reactions (covalent or electrostatic). Iron and phosphorus both accumulate in the anaerobic zones of stratified lakes and are recycled in the trophic waters during overturns. During cycling they may undergo a variety of changes in physical and chemical states so as to become associated with each other as complexes or precipitates (Hutchinson 1957) and both may become associated in one way or another with colloidal as well as larger organic particles. In this paper we will describe the influence of colloidal yellow organic matter (COM) upon the association between iron and phosphorus in an acid bog lake, and point out the indirect consequences of this interaction upon productivity.

The formation of complexes between iron and organic matter has been reported to occur in many lakes and has been suggested as the way iron is maintained in solution within aerated zones (Shapiro 1964) and is thereby a means of sustaining primary production (Schelske *et al.*, 1962). Through complexation reactions, COM may, in effect, mask the cationic properties of iron (Koenings unpublished). Stumm (1967) and Hem and Cropper (1959) believe that if the typical organic materials in natural waters such as tannins and phenols form complexes with ferrous iron at all, they form stronger complexes with ferric iron than with ferrous iron. On the other hand, Gjessing (1964) postulated the existence of predominantly Fe(II)-organic complexes in acid (pH 4-6) highly colored Norwegian lakes, which are reactive only after hot acid digestion. However, Shapiro (1966) has shown that the boiling and acidification used by Gjessing may reduce ferric iron to ferrous iron. McMahon (1969) suggested an acid-free method of determining ferrous iron based upon its reactivity with bathophenanthroline that results in the measurement of free Fe^{+2} . Using a combination of acid-free analysis and physical separation methods, Koenings (unpublished) suggested that two forms of reactive Fe(II) may be present in bog lakes: first, a dialyzable reactive form that appears to be ionic, and second, a nondialyzable Fe(II) form which is less stable than the Fe(II)-bathophenanthroline complex, and thus reacts as ionic Fe^{+2} . One purpose of the present investigation was to provide further evidence concerning these alternative relationships.

Precipitation of inorganic phosphate by iron has been reported frequently (Stumm and Morgan 1970), and is believed to limit the availability of phosphorus in trophogenic water (Hutchinson 1941; Mortimer 1941). Recently, Schindler *et al.* (1972), presented evidence indicating the absence of ionic iron in lake sediments. He pointed out that if naturally occurring organic-iron complexes in lakes behave like soil humic compounds, trace elements (e.g., Fe and Zn) released from reduced sediments would be sorbed onto organic acids.

To clarify the coordination state between iron, phosphorus, and organic acids in various zones of the lake and under varying conditions of aeration,

and to test the hypothesis that COM may influence productivity through interaction with these elements, we studied the interrelationship between iron, phosphorus and organic matter in North Gate Lake, a highly acid bog lake in Gogebic County, Michigan (T45N, R42W), utilizing a combination of in situ and laboratory techniques. North Gate has a surface of approximately 0.4 ha and a maximum depth of 8 m. It is surrounded by a mat of Sphagnum and an extensive black spruce bog forest. Other limnological features of the lake and details of the phosphorus cycles have been described elsewhere (Hooper unpublished; Hooper and Imes 1972; Koenings and Hooper 1973).

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Methods

MECHANICAL AND CHEMICAL SEPARATION

Mechanical separation of particulate and of colloidal forms of iron and phosphorus from water was made by filtration through a $0.45\ \mu$ membrane and by dialysis. This gave three size fractions: (1) materials held by a $0.45\ \mu$ membrane (here referred to as particulate), (2) suspended materials passing through a membrane but not through $4.8\ \text{nm}$ dialysis tubing (termed colloidal), and (3) dialyzable substances (termed dissolved). Filtration studies have shown that suspended iron particles range from less than $0.01\ \mu$ to greater than $15\ \mu$, and average $1\text{--}2\ \mu$. Hence filtration through a $0.45\ \mu$ membrane does not separate dissolved from suspended iron, but this separation can be made by dialysis. The above physical classification scheme of materials is similar to that given by Salutsky (1959).

In situ dialysis of anaerobic lake water was accomplished by suspending dialysis bags filled with anaerobic distilled water in the lake for 5 days. Cellulose dialysis bags were used which had a pore size of $4.8\ \text{nm}$ or a molecular weight cut off (in regard to proteins) of 12,000. To remove contaminants, the bags were first soaked in dilute HCl acid for 24 hours, and rinsed with distilled water. To define suitable conditions for quantitative dialysis of dissolved iron, $400\ \mu\text{g liter}^{-1}\ \text{FeCl}_3$ was added to a series of dialysis bags containing oxygen-free water and the bags were suspended in laboratory containers in light at 20°C and in the dark at 5°C . After 5 days, iron concentrations inside and outside the bags were not significantly different and 98% or more of the added iron was recovered. Solutions containing known amounts of orthophosphate (K_2HPO_4), ^{59}Fe (FeCl_3), and ^{32}P (K_2HPO_4) were treated in the same manner with identical results. From these tests, 5 days was judged to

be adequate time for quantitative dialysis.

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 were used both to separate and identify orthophosphate from other phosphorus containing compounds, and to determine recoveries of the phosphate from the experimental waters. The gradient elution system consisted of 0.0 M to 3.0 M acid + 3 M salt (2:1 v/v). The resin was changed from its supplied 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 cm x 7 cm, and were eluted at 1.0 ml/min 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).

With this system phosphate could be separated from other forms of phosphorus, and by comparison to the position on the elution curve of known orthophosphate the relationship between soluble reactive and orthophosphate in the water could be determined. Also, since the colloidal organic acids adsorbed irreversibly to the column (with any sorbed iron) the effect of this material on phosphate recovery could be determined.

Bathophenanthroline (BPN) was used for the measurement of ferrous iron. BPN reacts with no known metal ion (including Fe^{+3}), other than Fe^{+2} to give a colored complex that can be extracted by n-hexyl alcohol at pH 4. This extraction procedure for the isolation of the colored complex from its aqueous solution increases the sensitivity and extends the specificity to the point where no known interference by either metal cations or common anions occurs (Smith et al., 1952). All samples were duplicated and each digestion procedure contained a blank. Standards were run with each experiment.

Iron fractions are defined as follows: iron requiring acid treatment plus a reductant before reacting to BPN was considered to be predominantly inorganically complexed Fe(III), and is called nonreactive Fe(III). This procedure is similar to that used by Stumm and Lee (1961) to digest colloidal ferric oxyhydroxide. If reactive to BPN with reduction but without acid digestion, it is called reactive Fe(III), i.e., iron in the dissociated Fe^{+3} state. Iron reacting with BPN without acid or reductant is termed BPN reactive Fe(II). Reactive Fe(II) may or may not be dialyzable. If reactive and not dialyzable, it is presumed to be in a colloidal complex less stable than a BPN- Fe^{+2} complex. Thus, reactive dialyzable Fe(II) is termed ionic or dissolved Fe^{+2} , whereas reactive nondialyzable Fe^{+2} is colloidal Fe^{+2} .

Classification of natural lake iron has been developed from analyzing solutions of organic iron (ferric citrate and ferric EDTA), inorganically complexed iron (ferric oxyhydroxide, ferric chloride, and ferric ammonium sulfate) and iron present in the free (hydrated) state (ferric perchlorate). Organic iron and free (hydrated) iron reacted the same (i.e., reactive ferric iron) whereas inorganically complexed iron reacted differently (i.e., nonreactive ferric iron). The completeness of the reaction inside the noted

category, i.e., reactive ferric iron for ferric citrate and for ferric perchlorate, was 100%; however, newly made standards containing inorganic iron complexes reacted as nonreactive ferric iron (84-88%) and reactive ferric iron (16-12%). Aged solutions containing inorganic iron complexes were almost completely (96%) nonreactive ferric iron.

An operational classification based upon the reactivity of standard iron solutions was used to compare natural and experimentally-treated waters as to the predominant type of iron found. Reactive ferric iron occurring in the colloidal state is present primarily (not exclusively) as a colloidal Fe^{+3} complex. Nonreactive ferric iron present in the colloidal state is primarily an inorganic complex (e.g., ferric oxyhydroxide). Thus, in addition to three physical categories of iron (Table 1), the iron at four depths was characterized as Fe(III) (reactive and nonreactive), colloidal reactive Fe^{+2} , and dissolved (ionic) Fe^{+2} (Tables 2 and 4).

Water samples for chemical analysis were collected with a modified Hale sampler using acid-washed 300-ml B.O.D. bottles. Duplicate samples were analyzed in the laboratory after minimum exposure to sunlight. Since membrane filtration drastically alters the oxidation state of iron, ferrous iron was determined on unfiltered samples, and only the total iron analyses were performed on aerobically filtered samples. Total iron was determined with bathophenanthroline (BPN) by the method of Golterman and Clymo (1969) and ferrous iron by the acid-free BPN method of Koenings (unpublished). Absorbance of the ferrous-BPN complex was determined at 540 nm on a Klett-Summerson colorimeter using a 4-cm light path length. Total organic carbon was determined by the method of Maciolek (1962) using a dextrose standard curve prepared according to Strickland and Parsons (1972). Color was determined by absorbance of the sample at 410 nm using a Spectronic 20 with a 1.0-cm light path length. Stable chemistry of phosphorus as well as dissolved oxygen and hydrogen sulphide was determined according to Strickland and Parsons (1972). All determinations were made against distilled water blanks treated identically to the water samples. Activity of samples was measured by drying triplicate 5 and 10 ml subsamples on planchets, and counting on a Nuclear-Chicago low background beta counting system.

Results

DISTRIBUTION OF COLOR, ORGANIC MATTER, AND IRON

North Gate Lake undergoes incomplete mixing during the spring and fall overturns. Mixing is accomplished to a depth of 5-6 m, leaving a monimolimnion, which is not oxygenated at any time of the year below 6.5 to 7 m. The 5-m depth is usually anaerobic and contains H_2S while the 2-m depth alternates between anaerobic conditions and very low oxygen concentrations ($0.5 \text{ mg liter}^{-1}$).

The surface layer is oxygenated at all times ($3-5 \text{ mg liter}^{-1}$) but is only 50-70% saturated. Hence conditions exist which grade from continuous anaerobiosis (7-8 m) to constant aerobiosis (surface).

Organic carbon ranged from 13,791 to 25,704 $\mu\text{g liter}^{-1}$. Only 12-18% of the organic carbon of North Gate Lake was in true solution (dialyzable) and 60-85% was colloidal. Colloidal carbon was predominant at all depths (Table 1). Similarly, very little of the lake's color was in solution (3-6%) and the predominant contribution came from colloidal sized particles (68-74%). Thus, the predominant fraction of the lake's organic carbon was in the colloidal size class and this fraction possessed most of the yellow coloring matter. The changes with depth of color and colloidal organic carbon were correlated and both showed minimum concentration in the thermocline (Table 1). However, changes in colloidal iron concentrations were not correlated with either color or organic carbon. This indicates that the size distribution and color of the COM is independent of iron complexation.

Iron forms in the water column can also be identified on the basis of oxidation state and reactivity (Table 2). In the anaerobic monimolimnion, 1,394 $\mu\text{g liter}^{-1}$ of the iron is present as BPN reactive Fe(II) of which 1,394 $\mu\text{g liter}^{-1}$ is dialyzable. This iron is considered to be ionic. Consequently, it appears that reduced iron does not form either a stable inorganic (ferrous gel) colloid or an organic complex with COM in the constantly anaerobic part of the water column above the sediments. However, in the oxygenated surface waters almost all of the iron (226 $\mu\text{g liter}^{-1}$ or 80% of the total iron) is Fe(III). Of this, 209 $\mu\text{g liter}^{-1}$ (92%) is colloidal iron. At 2 m and 5 m, 97-99% of the iron is present as reactive Fe(II). Of this, 53-56% is present as reactive colloidal Fe(II) and 44-47% is dialyzable and hence present as dissolved Fe^{+2} . These depths are oxygenated during spring overturn enabling reduced ionic iron to form colloidal Fe(III) as in the epilimnion. In this low pH, highly colored lake, Fe(II) is not of colloidal size until the iron is oxidized. The colloidal Fe(III) thus formed is subsequently reduced during anaerobiosis in the presence of COM, and other reducing substances (e.g., H_2S) to form colloidal Fe(II). Since this colloidal Fe(II) is also reactive to BPN, it may exist either as a weak inorganic colloid or as a less stable colloidal organic acid complex than the BPN- Fe^{+2} complex.

Fe^{+2} -organic complexes have been suggested as occurring in natural waters (Mortimer 1941), but evidence for their existence is sparse. We have demonstrated the presence of BPN reactive ferrous iron that is not dialyzable in recently anaerobic waters. However, Fe^{+2} does not form complexes with COM in perpetually anaerobic waters. The formation of this nondialyzable Fe^{+2} appears to come from colloidal reactive Fe(III). That is, it is residual colloidal Fe(III) that has been reduced, and like EDTA-Fe^{+2} complex (formed from Fe^{+3} -EDTA) which has a moderate stability if not exposed to oxygen (Jones and Long 1952), it may exist in anaerobic to oxygen-deficient acid waters. Coolidge (1932) recognized dipyridyl reactive iron that was complexed to a protein, so analogs have been demonstrated. Whereas we feel that Fe^{+2} does not form complexes with

Table 1. Size distribution of organic matter, color, and iron in North Gate Lake.

Depth(m)	Particulate			Colloidal			Dialyzable		
	Organic Carbon*	Color+	Iron*	Organic Carbon*	Color+	Iron*	Organic Carbon*	Color+	Iron*
0 (surface)	2,874	44	10	18,943	105	249	3,887	5	23
2 (thermocline)	903	20	7	10,412	61	321	2,481	5	266
5 (hypolimnion)	4,065	30	24	16,857	104	395	2,729	6	358
7.5 (monimolimnion)	5,009	29	50	12,867	84	99	3,722	7	1,394

* $\mu\text{g liter}^{-1}$

+absorbance at 410 nm $\times 10^{+3}$

Table 2. Changes in (A) chemical reactivity of iron (percent of total iron in parentheses), and (B) ferrous iron (percent of BPN reactive ferrous iron in parentheses) in North Gate Lake.

Depth (m)	Iron ($\mu\text{g liter}^{-1}$)					B	
	A						
	Particulate	Non-particulate Ferric	BPN Reactive Ferrous*	Dialyzable BPN Reactive Ferrous*	Total Dialyzable	Colloidal Fe(II)*	Ionic Fe(II)*
0 (surface)	10(4%)	226(80%)	46(16%)	6	23	40(87%)	6 (13%)
2 (thermocline)	7(1%)	0 (0%)	590(99%)	262	266	328(56%)	262 (44%)
5 (hypolimnion)	24(3%)	0 (0%)	757(97%)	355	358	402(53%)	355 (47%)
7.5 (monimolimnion)	50(3%)	99 (6%)	1,394(91%)	1,394	1,394	0 (0%)	1,394(100%)

*Iron reacting with bathophenanthroline without prior acidification of the sample

COM, the complexes are present as residual reduced products of colloidal Fe (III).

Jones and Long (1952) have also shown that organic complexed iron (e.g., ferric citrate, ferric oxalate, and ferric EDTA) are readily reduced by light into a ferrous complex that may exist indefinitely if not exposed to oxidizing conditions. This complexed ferric iron is present as reactive ferric iron (fully dissociated Fe^{+3}). As we will demonstrate, COM reduces the formation of ferric oxyhydroxide (slowly photoreduced) with more ferric iron present as reactive colloidal Fe^{+3} . In this manner, COM may facilitate the photoreduction of iron, which then may exist as a ferrous-organic complex in the presence of a low level of oxygen. The existence of this complex may be lengthened by an acid pH, since at an acid pH the oxidation of iron becomes independent of oxygen concentration (Stumm and Lee 1961).

Dissolved Fe^{+2} is formed either by solution of the ferrous colloid (e.g., removed by H_2S) or it may originate from forms of iron other than colloidal reactive Fe(III). In an experiment described later, $^{59}\text{FeCl}_3$ was added to anaerobic lake water which equilibrated completely with the colloidal fraction but was later recovered as dialyzable $^{59}\text{Fe}^{+2}$ so it is apparent that ionic ferrous iron can be formed from colloidal ferric iron in the presence of organic colloids.

EXPERIMENTAL ANALYSIS OF IRON-ORGANIC MATTER-PHOSPHORUS RELATIONSHIPS

As a check upon the origin of the iron cycle proposed above and to explore the possible formation of iron-organic acid complexes and their relationship to phosphorus, we undertook the following experiments.

Experiment I (anaerobic containers). In this experiment, water samples were collected from the 7.5 m zone with a 2-l Kemmerer sampler. The water was transferred rapidly and with minimum aeration to four acid-washed plastic bags. Each bag contained duplicate sealed sections of dialysis tubing filled with anaerobic distilled water. The water in one bag was then labeled with 1-2 μCi of ^{59}Fe (FeCl_3), a second was labeled with 1-2 μCi of ^{32}P (NaHPO_4). After labeling, bags were sealed and the contents mixed. The remaining two plastic bags prepared in an identical manner but without tracer were used for stable chemical analyses. All four bags were then suspended in the lake at the depth at which water samples were collected (7.5 m) for 5 days.

Experiment II (aerobic containers). Water was collected from 7.5 m as in Experiment I, but in this experiment, was returned to the laboratory and poured into four acid-washed plastic containers. These containers were supplied with duplicate dialysis sacs filled with anaerobic distilled water, as before. Two were labeled and two were retained for stable chemical analysis, as in Experiment I. All four containers were then placed in a dark, constant temperature room and incubated for 5 days at 5°C . During this period all four

containers were aerated by bubbling air from the same source to maintain a constant pH and dissolved oxygen concentration in all containers.

Experiment III (aerobic dialysate). In this experiment we first prepared anaerobic dialysate of 7.5 m water by suspending a series of sealed sections of dialysis tubing filled with anaerobic distilled water at 7.5 m for 5 days. This yielded water equivalent to natural 7.5 m water in all respects except that yellow colloidal organic acids were excluded. This water was transported to the laboratory and treated and labeled in a manner identical to samples in Experiment II. Difference in results between Experiments II and III can be attributed to the influence of organic acids.

Thus, four containers were used in each of three experiments and each experiment was duplicated. One container in each experiment was used to follow iron labeled with ^{59}Fe , one was used to follow ^{32}P label, and two were used for stable chemical analysis of iron and phosphorus fractions after equilibration.

Anaerobic Iron and Phosphorus Distributions in Lake Water Containing COM

Control conditions for these experiments were established by collecting a series of samples from the 7.5 m depth and analyzing them with minimum exposure to the atmosphere. Initial conditions in controls were typical of the monimolimnion and the coordination of iron was similar to that reported above. Only 8% of the Fe(II) was nondialyzable (Table 3); the remainder was BPN reactive ionic ferrous iron. The concentration of BPN reactive Fe(II) was the same in dialyzed samples as in unfiltered samples (Table 4). The small particulate fraction was nonreactive Fe(III) . Phosphorus, like iron, was nearly entirely dialyzable reactive phosphate (85%). The remainder was particulate unreactive phosphorus (Table 3).

After 5 days incubation in the lake, the anaerobic container samples (Experiment I) appear to have equilibrated. The concentration of BPN reactive ferrous iron was nearly the same in the dialysis bags as in the unfiltered samples (Table 3). Activity in the dialysis bags indicates that 76% of the added activity was dialyzable. Clearly, the ferrous iron was in a dissolved Fe^{+2} state, not as an inorganic colloid or as a COM complex. The fractions of colloidal and particulate activity present were similar to the fractions of stable colloidal and particulate iron.

The same relationship of phosphorus fractions was encountered in the anaerobic bag as in the controls. There was no colloidal sized phosphorus, 21% of the phosphorus was particulate, and 79% was dialyzable. The ^{32}P data indicate that most of the particulate pool of phosphorus was not in exchangeable equilibrium with the tracer. Thus, 96% of the added orthophosphate tracer

Table 3. Particulate (A), colloidal (B), and dialyzable (C) fractions of iron and phosphorus of 7.5 m water of North Gate Lake prior to experimentation (control), and the effect of aeration (experiments I and II) and of the presence of colloidal organic matter (experiments II and III).

Experiment		Iron			Phosphorus		
		A	B	C	A	B	C
Control	($\mu\text{g liter}^{-1}$)	80	7	1,055	106	0	605
(natural anaerobic lake water)		(7%)	(1%)	(92%)	(15%)	(0%)	(85%)
I	($\mu\text{g liter}^{-1}$)	70	86	849	74	0	266
(anaerobic lake water)		(7%)	(9%)	(84%)	(22%)	(0%)	(79%)
	(cpm ml^{-1})	25	25	165	4	2	143
		(12%)	(12%)	(76%)	(3%)	(1%)	(96%)
II	($\mu\text{g liter}^{-1}$)	90	949	15	112	107	132
(aerobic lake water)		(9%)	(90%)	(1%)	(32%)	(30%)	(38%)
	(cpm ml^{-1})	41	209	5	45	43	59
		(16%)	(82%)	(2%)	(30%)	(29%)	(40%)
III	($\mu\text{g liter}^{-1}$)	42	1,102	48	0	417	204
(aerobic dialysate of lake water)		(4%)	(93%)	(4%)	(0%)	(67%)	(33%)
	(cpm ml^{-1})	14	346	15	10	116	63
		(4%)	(93%)	(4%)	(5%)	(61%)	(33%)

Table 4. Iron fractions obtained by physical and chemical characterization of natural lake water (control), the effect of aeration (experiments I and II), and the changes due to the presence of colloidal organic matter (experiments II and III).

Experiment	Iron ($\mu\text{g liter}^{-1}$)				
	BPN Reactive Fe(II)*	Free BPN Reactive Fe(II)+	BPN-Non Reactive Fe(II)*	Reactive Fe(III)*	Non- Reactive Fe(III)*
Control (natural anaerobic lake water)	1,055	1,055	8	1	79
I (anaerobic lake water)	850	849	39	49	69
II (aerobic lake water)	138	0	179	346	384
III (aerobic dialysate of lake water)	66	0	0	128	998

*unfiltered sample

+dialyzed sample

remained in the dialyzable pool indicating a detrital form of stable particulate phosphorus. Failure to find activity in the colloidal fraction supports the contention that by themselves colloidal organic acids have no affinity for phosphorus.

It is clear that manipulations in preparation and incubation of samples permitted some oxidation to take place and resulted in formation of larger quantities of BPN unreactive Fe(II) and labile Fe(III), and a slight reduction in the quantity of dialyzable Fe(II) in these samples as compared to controls.

EFFECT OF AERATION ON LAKE WATER CONTAINING COM

Comparison of the aerated container samples (Experiment II) with controls and anaerobic bag samples (Tables 3 and 4) indicates major changes in iron and phosphorus fractions. Colloidal iron increased from 1-9% of the total iron to 90% of total iron and dialyzable iron decreased from 84-92% to 1% (Table 3). Dialyzable BPN reactive ferrous iron decreased from 84-92% of total iron to none. Ferric iron increased from 6-12% to 70% of the total iron (Table 4). Nonreactive Fe(III) represented 53% of this or only 37% of the total iron while ferrous iron represented 13% of the total iron. The radiochemistry data support the results from the stable chemistry. The ^{59}Fe was in a colloidal state in equilibrium with only a minimal pool of dialyzable iron. Colloidal phosphorus increased from that in the anaerobic system from 9% to 30% of total phosphorus, while dialyzable phosphate was reduced from 79% to 38% (Table 3). The corresponding tracer data are similar and show a change from about 1% colloidal inorganic ^{32}P to 29% colloidal inorganic ^{32}P , and a decrease of ionic tracer from 96% to 40%. The generation of colloidal ^{32}P from dialyzable ortho- ^{32}P was exactly the change observed in stable phosphorus and paralleled the change of free ferrous iron to colloidal sized ferric iron.

EFFECTS OF AERATION ON LAKE WATER WITHOUT COM

The dialysate used in Experiment III was identical to undialyzed 7.5 m lake water except that it lacked COM, and all of the iron was in the ionic Fe^{+2} state prior to oxygenation. Following oxygenation 93% of the stable iron was colloidal with 94% as ferric iron. However, unlike aerated lake water containing COM, 89% is nonreactive ferric iron (84% of total iron) while ferrous iron represents 5.5% of the total iron, along with very small amounts of either free or particulate iron (Table 3). The radiochemical distribution closely paralleled the change in stable iron. Just as non-colloidal inorganic ferrous iron formed colloidal iron when oxidized, colloidal phosphorus was formed from non-colloidal phosphate. Only 33% of the reactive phosphate remained in true solution and colloidal reactive phosphate increased to 67%. The ^{32}P data verified this conversion. Thus, like iron, upon oxygenation and in the absence of COM, orthophosphate changed from a dialyzable to a colloidal form.

INFLUENCE OF COM

Differences in size distribution of iron between the 7.5 m water containing the organic colloids after aeration, and the dialyzed 7.5 m water after aeration were small. However, the chemical separation showed that significantly greater quantities of nonreactive ferric iron colloids were present than when the COM was present (Table 4). Nonreactive ferric iron increased from 36% of total iron with COM to 84% of total iron in their absence while ferrous iron dropped from 30% of the total iron to only 6%. Thus, the organic matter reduced both the oxygenation reaction of ferrous iron and the formation of nonreactive ferric iron during aeration.

In Experiment II which contained COM, $31 \mu\text{g liter}^{-1}$ of colloidal reactive phosphate was formed which comprised 13% of total phosphorus, and 19% of soluble reactive phosphorus (SRP) (Table 5). In Experiment III in which colored organics were excluded, $384 \mu\text{g liter}^{-1}$ of colloidal reactive phosphate was found which was over 62% of the total phosphorus and 65% of the SRP. These data demonstrate both the formation of colloidal phosphate from the noncolloidal inorganic pool, and the effect of COM on colloidal phosphate formation.

Table 5. Soluble reactive phosphate (A), dialyzable (free) reactive phosphate, and colloidal reactive phosphate (A-B) in 7.5 m water from North Gate Lake prior to and following oxygenation (experiments I and II), in the presence and absence of COM (experiments II and III). Estimates of SRP for the anaerobic samples were made from unfiltered samples due to the loss of reactive material upon filtration.

Experiment	Phosphorus ($\mu\text{g liter}^{-1}$)		
	SRP (A)	Free Reactive [†] (B)	Colloidal Reactive (A-B)
Control (natural anaerobic lake water)	605*	605	0
I (anaerobic lake water)	262*	266	0
II (aerobic lake water)	163 [†]	132	31
III (aerobic dialysate of lake water)	588 [‡]	204	384

*unfiltered sample.

[†]filtered (0.45 μ) sample.

[‡]dialyzed (4.8 nm) sample.

In anaerobic water a colloid of iron, phosphorus and COM is absent. However, in recently oxygenated waters colloid formation of iron and phosphorus takes place both in the presence and absence of the COM. The components of this colloid appear to be related to the amount of COM, and form of ferric iron present. Where 26-38 mg liter⁻¹ of the COM, and reactive ferric iron were present, 19% of the inorganic phosphate was colloidal after aeration. In the absence of COM, but in the presence of approximately 7 mg liter⁻¹ of dialyzable organic matter, and nonreactive ferric iron, 65% of the inorganic phosphate was colloidal after aeration. The differences in both iron and phosphate distributions indicate that the COM reduces the ability of colloidal ferric iron to react with the phosphate anion, which is consistent with the hypothesized formation of the low fraction of iron-organic acid-phosphate complexes. Thus compared to lakes with low organic matter, more unsorbed phosphate would remain in the water column of stained acid bog lakes during circulation. Along with the latter major fraction, there is the iron-phosphate-organic colloid which may be mobile and may be less subject to sedimentation, but from which the phosphate is less readily desorbed.

Chamberlain and Shapiro (1973) argue that molybdate reactive material present in natural lake waters may be regarded as inorganic phosphate, although not necessarily free phosphate ion. They propose that water soluble fulvic acids forming complexes with phosphate through iron coordination may be the source of the observed differences between reactive phosphorus and free phosphate analyses. It seemed probable that lake water contains such complexed phosphate, which could easily account for these differences. We have demonstrated the formation of similar but colloidal sized adsorption complexes in natural lake waters. Even in the presence of a high concentration of COM, reactive colloidal phosphate was formed from ionic orthophosphate. This colloidal inorganic phosphorus may be the source for the discrepancies found between chemical estimates of soluble reactive phosphate and radiobiological assay of ion-exchange estimates of free orthophosphate ion. The mechanism of colloidal phosphate formation described above (i.e., by oxygenation of natural lake water) supports the observation of Hutchinson (1941) that acid soluble phosphorus appears during and for a short time after lake overturn.

FORMATION OF COLLOIDAL PHOSPHORUS

Aerated samples (experiments II and III) differed markedly from anaerobic samples in that the reactive phosphorus of the dialysate was less than the reactive phosphorus of the filtered samples (Table 5). This indicates that a new reactive phosphate component of colloidal size was formed. This conversion of stable phosphorus is paralleled by the movement of ³²P into the colloidal fraction which suggests that the stable phosphorus and the added ortho-³²P are chemically similar. Since phosphorus shows little tendency to react with organic matter when not in the presence of colloidal ferric iron, this suggests iron-phosphate complexation, and demonstrates that SRP is not equal to free reactive orthophosphate.

Anion exchange chromatography was used to test whether this colloidal reactive phosphate could account for the difference between reactive phosphate and free phosphate analysis. Of the predominantly colloidal phosphate present in dialyzed lake water (experiment III) after aeration, 99.2-99.7% of the SRP found prior to the application of the sample to the resin column was recovered upon elution (Table 6). Since recoveries were nearly complete, and equal to that of the standards, it appears that the iron-reactive phosphate colloid was not ferric phosphate or occluded phosphate, but orthophosphate adsorbed on colloidal ferric oxyhydroxide. That this was orthophosphate was demonstrated by both the synchronous elution position of the reactive component in the elution curve with known orthophosphate, and by the exact agreement between the total phosphorus and the reactive phosphate analyses of the orthophosphate peak. Therefore, the SRP analysis does measure orthophosphate. However, when water from the same depth but containing COM was analyzed, only 67-81% of the SRP was eluted, while all the COM remained on the column. This suggests that the presence of COM was responsible for the incomplete recovery of SRP as orthophosphate.

Thus, SRP was not equal to free reactive phosphate when the water contained a portion of colloid (COM) bound reactive phosphate which was not measurable by ion exchange extraction. This colloidal reactive phosphate represented 19% of SRP, while nonrecovery of SRP as orthophosphate equalled 19 and 33%. Thus, it is conceivable that the colloid (COM) bound reactive phosphate is responsible for the incomplete recovery by ion exchange of SRP as free orthophosphate.

Discussion

Iron in oxygenated and deoxygenated waters differed in both oxidation state and physical size. Constantly anaerobic waters contained ionic ferrous (Fe^{+2}) fully filterable and dialyzable, and not adsorbed to COM. Aerobic waters contained filterable but nondialyzable (colloidal) ferric iron which is only partially reactive as ferric oxyhydroxide. Most of the iron is in the colloidal state as are the organic acids, but the COM is not dependent on iron complexation for its size or its color.

In contrast to perpetually anaerobic waters, those that undergo brief aeration during spring overturn contain ionic Fe^{+2} along with a reactive form of ferrous iron that was not dialyzable. This colloidal BPN reactive iron was either in a complex with the yellow organic acids as Fe^{+2} (which was less stable than the BPN- Fe^{+2} complex) or existed as a weak residual ferrous iron colloid reactive to BPN. From circumstances of its formation, it appears to have been derived from colloidal reactive Fe(III) , since COM reduces formation of colloidal nonreactive ferric iron (i.e., ferric oxyhydroxide), and may be a COM- Fe^{+2} complex.

Table 6. Recovery of soluble reactive phosphorus (A) as ortho-phosphate (B) from distilled, and from experimental lake waters in the presence and absence of colloidal organic matter (experiments II and III) using gradient elution anion exchange chromatography.

Experiment	Elution System	Aeration Time, hr	Elution Volume (range)*	Elution Volume (peak)*	SRP (A) [†]	Ortho-P (B) [†]	Recovery*
Control (distilled water)	acetate	6	23-33	26.5	100.0	99.6	99.6
	formate	48	11-19	16.0	100.0	99.4	99.4
II (aerobic lake water)	acetate	6	16-37	27.0	116.0	94.0	81.0
	formate	48	10-24	18.0	47.0	31.5	67.0
III (aerobic dialysate of lake water)	acetate	6	18-45	26.0	151.0	149.8	99.2
	formate	48	11-19	16.0	38.0	37.9	99.7

*percent

[†]μg

The oxidation of iron and the presence of COM influenced the extent of the reaction of iron with phosphorus. Neither dissolved Fe^{+2} nor orthophosphate in anaerobic waters had any sorption reaction with the colloidal organic acids. Iron [nonreactive Fe(III)]-phosphate colloid formation occurred readily without COM. The truly soluble organic matter (i.e., dialyzable) did not affect the adsorption or desorption of phosphate from the colloid as did COM. Colloidal ferric iron in the presence of COM contained less nonreactive Fe(III) and formed a smaller amount of colloidal reactive phosphate. Thus, colloidal nonreactive ferric iron may be more adsorptive for phosphate than other forms of colloidal ferric iron since its concentration parallels phosphate adsorption. COM may decrease the formation of colloidal phosphate both by reducing the cationic properties of the colloidal nonreactive ferric iron, and more importantly, by reducing the amount of colloidal nonreactive iron formed. Thus, COM definitely modifies the behavior of the dissolved inorganic components of the lake water.

The explanation suggested by Mortimer (1941) for the lack of productivity in acid bog lakes (the formation of phosphate sorbed onto iron-organic complexes) is not supported since phosphate adsorption to iron has been shown to decrease in the presence of COM.

Dialysis indicated that truly soluble complexes (COM-iron-phosphate) were not formed. However, had $0.45\ \mu$ membrane filtration been used to define the dissolved state, the data would have erroneously indicated the formation of soluble ferric iron upon aeration of the anaerobic water. Thus, it is very apparent that filterability ($0.45\ \mu$) is not sufficient to distinguish between dissolved and nondissolved materials. Similarly, filtration combined with reactivity to BPN (acid free) or reactivity to "mixed reagent" is not sufficient to indicate the presence of free or ionic ferrous iron, or free orthophosphate, respectively; but it is suggested that soluble reactive phosphate is equal to orthophosphate except where COM interferes with the analysis. Other anomalies were observed, e.g., that BPN reactive ferrous iron (acid free) does not indicate Fe^{+2} (free ferrous iron) due to the presence of a BPN reactive ferrous iron colloid that can pass through $0.45\ \mu$ filters. This same anomaly was observed for phosphate analysis. In addition, filterable iron in aerobic waters consists of several species of iron, not just nonreactive colloidal Fe(III) , e.g., ferric oxyhydroxide, and since ferric iron was colloidal in the absence of COM, nondialyzability does not, in and of itself, mean metal-organic complexation.

Iron-phosphate-organic acid complexation is much less important in anaerobic water than in aerobic waters where colloidal organics do interact with iron, in the time period of these experiments. COM not only decreases nonreactive iron formation and subsequent orthophosphate adsorption, but forms a small fraction of an iron-reactive phosphate-organic acid colloid. COM-bound phosphate may be similar to a class of organic bound phosphorus (humic phosphorus) suggested to exist by Golterman (1973), and may account for the differences between soluble reactive phosphorus (SRP) and orthophosphate

measurements as indicated by our ion-exchange experiments. Nonrecovery of SRP equalled 19-33% depending on the length of aeration, which suggests a time-dependent phenomenon. However, some of the nonrecovery may be due to phosphate capture by the ion-exchange resin in the presence of colloidal organic acids. The apparent porosity, which determines the size of the ion which can migrate through the gel matrix of the resin to the exchange sites, is about $40 \text{ \AA}$. The colloidal organic acids in our studies cannot pass through $48 \text{ \AA}$ dialysis tubing, but can and do absorb irreversibly to the outside of the resin bead. By this enveloping action, these acids may trap migrating orthophosphate ions in the matrix of the resin resulting in low orthophosphate recoveries.

Finally, the effect of oxygenation on the iron and phosphorus in these experiments may be applicable to those reactions occurring in the lake overturn, since all concentrations of the elements and the elements themselves, were of natural origin. In addition, conditions under which these experiments were performed closely simulated the natural conditions, and the results obtained paralleled those observed in natural lake waters.

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APPENDIX C

MAT-WATER EXCHANGE IN A STRATIFIED BOG LAKE

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Bog lakes with their surrounding mats of Sphagnum are limnologically distinctive because of the physical and biological properties of the shore zone. In bog systems of the type we have studied, the littoral zone is replaced by a vertical wall of Sphagnum and peat extending from above the lake surface to a depth below the thermocline. The vegetation and interstitial water of the mat system is in contact with the open lake water and may interact with the water in a different way than the littoral sediments of non-bog lakes do. This paper describes water exchange phenomena occurring within the mat-water interface zone of a bog lake system, and their significance in relation to the bog lake's circulation, thermal structure, and chemical cycles.

The bog community considered in this report consists of (1) an open lake of highly stained and acid water, (2) a marginal floating mat of Sphagnum and bog shrubs, and (3) a marginal bog forest consisting of black spruce (Picea mariana) and tamarack (Larix laricina). This type of system is a characteristic landscape feature of northeastern United States and Canada. The studied area, North Gate bog, is one of the dozen or more small bog lakes within the University of Notre Dame Environmental Research Reserve in Vilas County, Wisconsin, and Gogebic County, Michigan. North Gate Lake is located within Section 36 of T45N, R42W, Gogebic County. It is approximately 50 m long and 40 m wide. The 7-m contour encloses approximately 2/3 of the surface area. There is an abrupt drop from the edge of the mat to a depth of 6 m, and in most areas the 7-m depth is to be found 3 m or less from the edge of the mat. There are two small depressions slightly more than 8 m deep near the central portion of the basin (Figure 1). Lakes of the Notre Dame Tract area differ very little from similar systems of northern Wisconsin and Michigan except in morphometry. Several of the bogs of the tract have unusual depth for their surface area. Several bog systems of the tract have even more extreme surface-depth ratios than North Gate. Tender bog has a surface area of 0.25 acre and a maximum depth over 9 m; Beaver bog has a depth of 9 m and a surface area of 0.30 acre. These lakes replicate many of the physical, chemical, and biological conditions of North Gate, including a strong tendency toward meromixis in certain years. In North Gate, a stratum between 7 and 8 m does not circulate during the vernal overturn, leaving a small monolimnion and temperature inversion. Physical and biological dispersion of water of the monolimnion was investigated in 1969 by means of a double labeling experiment with rhodamine WT and ³²P. In 1970 we investigated the dispersion of water of the lower hypolimnion using the same two labels (Hooper and Imes, 1973).

Figure 1. Hydrographic map of North Gate Lake showing area treated with ^{32}P in 1971.

During the 1969-1970 labeling experiments we observed water movements within the basin which were not consistent with the three-layer model of meromictic lakes (Hutchinson, 1967) but suggested water intrusions below the thermocline. We hypothesized that these movements were created by density currents arising in the bog mat and passed through a mat-water interface zone into the hypolimnion. In 1971 we undertook a mat labeling experiment to test the hypothesis that mat water penetrated directly into the hypolimnion, thereby bypassing the thermocline zone.

MOVEMENT OF MAT WATER INTO HYPOLIMNION

On May 30, 1971, we labeled approximately one-half of the Sphagnum mat surrounding North Gate Lake with a mixture of ^{32}P as phosphate and the dye rhodamine WT. The Sphagnum from the edge of the mat to the limits of the tree zone was labeled (Figure 1) by sprinkling the mixture over the surface with sprinkling cans. Sprinkling the label on the Sphagnum simulated natural rainfall. We applied a total of 130 mCi of ^{32}P over an area of approximately 215 m². After labeling we measured activity at a central station in the open lake (station B), at 3 mat-water interface stations adjacent to the labeled area (stations A, C, D), and at 2 mat-water interface stations on the side of the lake opposite the labeled area (stations E and F). Interface stations were located in open water immediately adjacent to the edge of the mat. At this location we were able to collect water samples at the 1-, 2-, 3-, and 6-m depths at all stations except station E. Samples were collected with a modification of the Hale water sampler, designed for sampling a thin water stratum. Samples were collected in triplicate, and a 50-ml sample was evaporated to dryness and counted on a low-background Beta counting system. Activity of water samples was measured on May 30, 6 hr after labeling and at 4, 8, and 20 days after labeling (Table 1).

Six hours after labeling only a trace of activity was encountered. This was chiefly in the surface 1-m stratum, although a significant amount of activity was encountered in the 6-m depth in the open water (station B). On June 1 approximately 0.1 cm of rain was recorded on a rain gauge located in the center of the lake. Surface drainage from this rainfall moved a large quantity of the label and stable phosphorus into the open water of the lake (Tables 1 and 2). Movement into the open water of the lake was not confined to the surface but also took place at 2, 3, and 6 m. In the open water (station B) activity was detected only at 6 m. At interface stations, the highest concentration was detected at 2 m at station A (125 cpm), which indicated a large subsurface intrusion. The 6-m intrusion was detected not only at station B but at interface stations C and E, indicating that the label had spread horizontally over a major portion of the lake basin. The activity must have moved horizontally a distance of at least 44 m to reach station E. On June 6, the concentration of activity at 6 m at station B had been dispersed by both horizontal and vertical mixing. It appeared in the water column from 4 to 7.4 m, and had spread horizontally to station F on the unlabeled side of the lake.

Table 1. Water activity at various depths in open lake (station B) and at mat-water interface stations (A, C, D, E, F) appearing after labeling mat with 120 mCi of ^{32}P on May 30, 1970. Mat adjacent to E and D was not labeled. Changes in water activity between 7/12 and 7/20 occurred during period of heavy rainfall. May 30 sample collected 6 hours after labeling. Activity corrected for decay. Mat labeled again on June 24.

Station and Depth (meters)	Total Activity cpm/ml and Dates						
	5/30	6/02	6/06	6/18	7/12	7/16	7/20
B	1	3.8	0	4.3	3.2	14	14
	2	0	3	5	0	1	5
	3	3.7	0	0	0	0	5
	4	3.5	0	2	0	--	--
	5	0	0	3	0	--	--
	6	3.8	45.3	5	0	3	3
	7.5	0	0	6	5	0	0
A	1	3.3	4.0	7.1	4.8	5	52
	2	0	125.1	3.4	3	0	13
	3	0	4.8	3.2	3	0	4
	6	0	0	0	0	0	0
C	1	3.3	12.6	3.8	5	18	5
	2	0	0	3.1	3	0	0
	3	0	0	5.8	0	0	0
	6	0	4.0	0	6	0	5
D	1	0	0	4	5	18	6
	2	0	0	4	5	18	6
	3	0	0	0	0	0	0
	6	0	0	3	5	0	0
E	1	0	0	0	3	30	4
	2	0	0	5.1	5	3	0
	3	0	3.4	0	4	3	0
	6	0	4.9	3.1	4	5	0
F	1	0	0	3.7	5	12	0
	2	0	0	3.7	0	3	0
	3	0	0	0	0	3	0

Table 2. Calculated stable phosphorus inputs into open water (station B) from bog mat and rainfall measured at lake surface following labeling of Sphagnum (May 30) and mat interstitial water (June 24) with ^{32}P . Stable phosphorus input calculated from specific activities of phosphorus fractions collected at three locations in the labeled area.

	5/29 to 6/01	6/02 to 6/06	6/07 to 6/11	6/12 to 6/16	6/17 to 6/21	6/26 to 6/30	7/01 to 7/05	7/06 to 7/10	7/11 to 7/15	7/16 to 7/20
Rainfall (cm)	0.08	0.64	0	0.19	0	0.85	0	0	0.92	0.62
Phosphorus (grams)	0.65	70.3	0	0	0	145.0	0	0	303.0	170.0

Similar intrusions of phosphorus from the mat into the open water were noted after the mat was labeled a second time with ^{32}P on July 24. Input from the second label on June 26 after rainfall occurred chiefly at the surface; however an intrusion into deeper strata occurred during heavy rainfall between July 16 and July 20. Rain on the bog basin brought mat radioactivity into the basin at 1-, 2-, and 3-m depths at station A, and at the 6-m depth at station B. The input continued for several days. By July 20 there was also a substantial input into the 6-m stratum at stations A and C. Since a strong thermocline existed during the entire summer with a temperature gradient between 1 and 3 m in excess of 10°C (Figure 2), these vertical intrusions could not have originated in the open water but must have occurred within the mat-water interface zone.

MECHANISMS OF SUBSURFACE INTRUSION

The observation from the labeling data that the mat-water interface zone was the site of subsurface intrusion suggested the possibility of density flow currents moving through this zone into the deeper water. To investigate this possibility further, we made a series of temperature measurements through a cross section of the mat-water interface zone adjacent to station A on June 3 and June 8, 1973 (Table 3). On June 3, open water temperatures were almost identical to those encountered on June 2, 1971 when we noted subsurface intrusion of tracer at station A. The principal difference in mat water between 1971 and 1973 was that there had been more rain in 1971; water levels were slightly higher and there was slightly more surface water impounded within the mat in certain areas (station C) than in 1973.

Thermal profile data were collected in 1973 (Table 3) during a period in which there was rapid temperature change in the open water of the lake.

The mat temperature profile data demonstrate that the isotherms of the mat interstitial water dip sharply toward the hypolimnion of the lake. Although weather changes brought large temperature changes in the open water, temperatures at a point in the mat 0.6 m from the open water were not significantly different on the two dates in any portion of the profile (Table 2). The mat water is conservative as regards temperature changes because of the insulating qualities of the Sphagnum and the shading effects of the overlying shrubs and vegetation. Temperature gradients between the lake and the mat interstitial water frequently exceeded 10°C during May and June, 1973.

With the assumption that the thermal profile in the interface zone in 1971 was similar to that measured in 1973, it is possible to predict the level in the lake of density flow intrusions in 1971 following mat labeling and inflow of rainwater through the mat. The above assumption would appear to be valid since the open water thermal profile on June 2, 1971 was almost identical to that of June 3, 1973. If it is further assumed that the temperature of the rain falling on the mat in 1971 was the same as the average air temperature during that period (7°C), then the predicted intrusion depth from a density

Figure 2. Spring and summer temperatures in the North Gate Lake basin 1969-1971.

Table 3. Comparison of open water temperatures with temperature profile of adjacent mat interstitial water at station A before (June 3) and after (June 8) weather changes cooling the lake basin.

Depth in Meters	Open Water		Mat-Interstitial Water							
			Distance from open water (meters)							
			0.6		1.5		2.4		3.5	
	6/03	6/08	6/03	6/08	6/03	6/08	6/03	6/08	6/03	6/08
Sfc.	26.6	19.2	17.5	17.5	13.0	13.0	11.8	11.8	8.9	7.4
0.5	17.8	17.3	13.5	13.5			5.5	5.7		
1.0	12.0	13.2								
1.5	7.7	7.7								
2.0	5.5	5.5	5.2	5.4			4.7	4.7		
2.5	5.0	4.9								
3.0	4.4	4.6								
4.0	4.2	4.2	5.4	5.5			5.5	6.0		
6.0	4.3	4.3								
7.0	4.5	4.5								
8.0	4.7	4.7								

flow of water along isotherms through the interface zone would have been between 1.5 and 2 m. The observed intrusion at 2 m therefore appears to fit a model of density flow through the thermal gradient of the interface zone.

INFLUENCE OF MAT WATER INTRUSIONS

The subsurface water intrusions from the mat described above could influence the vertical physical-chemical profiles of the lake by (1) creating weak currents in the thermocline and hypolimnion, and thereby mixing materials in these zones, and by (2) transporting solutes and other materials from surface mat water directly to the hypolimnion. Hutchinson (1941) suggested that currents moving down the slope of the basin of Linsley Pond added carbonates from the basin surface and spread horizontally into the open water of the hypolimnion. He proposed this explanation to account for the steplike vertical change in alkalinity in the basin during the summer. We have examined our thermal and chemical data for 3 summers (1969-1971) for evidence of hypolimnetic mixing arising from density flow, and evidence of transport of chemicals from the mat water to the deeper zones of the lake.

SUBTHERMOCLINE WATER MOVEMENTS

Temperature measurements at approximately 0.6-m depth intervals were made every 4 to 5 days each summer at a central station on the lake (station B). A resistance thermometer was used and was read to the nearest $\pm 0.05^{\circ}\text{C}$. Open water isotherms for the years 1969-1971 show a dichothermic condition each spring (Figure 1). The minimum temperature in the water column differed in the three years. It was lowest in 1969 with a 4°C minimum when there was a period of unusually warm weather immediately following disappearance of the ice. A labeling experiment previously described (Hooper and Imes, 1973) demonstrated that the layer below 7 m did not mix with the upper water during the entire summer.

There was a similar pattern of warming of the 3- to 7-m zone all three years. During the late spring and early summer the hypolimnetic temperature minimum was eliminated by heat inputs from both the surface and bottom of the lake. Divergence of isotherms indicated mixing arising from subthermocline energy input. In most instances, particularly in early spring, there was no displacement of isotherms in the thermocline concurrent with these thermal changes in the hypolimnion, but there appeared to be a strong correlation between subthermocline mixing and prevailing weather conditions. Isotherms in the 3- to 7-m zone were relatively stable during warm, clear weather when epilimnetic temperatures were increasing, e.g., June 30-July 7, 1970; June 3-19, 1969. On the other hand, divergence of isotherms in the 3- to 7-m zone tended to take place during periods of rainy, cold weather, e.g., April 27-30, 1969; May 24-28, 1970; June 21-28, 1970; and May 21-24, 1971 (Figure 2). Thermal changes in the basin of April 27-30, 1969 are of particular

interest. At this time, temperature of the surface water was approximately 9°C but air temperatures were below freezing for three nights—a minimum of -8°C. During the period, there was approximately 2 cm of precipitation, followed by the formation of an ice crust on the surface of the mat and over open water impounded behind the mat. Intrusion of mat water into the 3- to 7-m zone was indicated by the displacement of the 4° isotherm and also by change in dissolved oxygen and pH in this stratum.

TRANSPORT OF CHEMICALS INTO THE HYPOLIMNION

Density flow in the mat-water interface zone provides the mechanism for vertical displacement of dissolved and particulate materials from the mat directly into the hypolimnion without movement through the thermocline. Koenings (1973) provided evidence for such movement by demonstrating the presence of certain labeled organic phosphorus compounds in the interface water of the mat and in the hypolimnion that were absent in epilimnetic water. To further explore the possibility of chemical transport of materials from the mat into the deeper zones of the lake, we measured phosphorus and iron at locations in the mat-water interface zone before and after a period of heavy precipitation on July 27, 1973 (Table 4). The concentration of phosphorus and iron in the vertical profile in the mat could then be compared with the concentrations in various strata of the open lake.

Before the rainfall, the total phosphorus at the interface station 1 (0.6 m from the open water) was at least 10-fold greater than the open water concentrations (Table 2). A rainfall of approximately 0.3 cm on the mat surface brought about a 60% reduction in the phosphorus concentration at the surface and at 0.5 m, and a smaller decrease at the 2-m stratum. At 4 m there was a slight increase in concentration after rainfall which however was within the range of day to day variability at this depth within the lysimeter from which samples were collected. Farther from the open water (station 3) concentrations of total phosphorus were higher and flushing of the mat by the rainfall produced a large decrease at the surface of the interstitial water but small and insignificant changes at other depths. Thus rain appears to have leached the interface phosphorus profile not only at the surface water at station 3 but leachate included materials from the mat adjacent to the open water. Comparable after-rain data for iron are not available, but if iron is leached from the profile as is phosphorus, a large quantity of iron would be removed from the mat interstitial water profile since concentration at the surface was over 1500 ppb. The fate of the leached materials is not indicated from the data at hand, however since increases in the deeper layers of the mat were small, it appeared that the leachate entered the lake water.

Although the concentrations of phosphorus in the open water were in all cases at least 10-fold smaller than in the interface zones of the epilimnion, thermocline and hypolimnion, concentrations of iron and phosphorus in the monimolimnion (7.5 m) were very similar to those of the mat interface (Table 4). Below 7 m and also at station 3, 90-100% of the phosphorus was SRP. The chemical

Table 4. Comparison of concentration of total iron ($\mu\text{g/l}$) and total phosphorus ($\mu\text{g/l}$) of open lake with concentration at two locations in the mat-water interface zone (stations 1 and 3). Phosphorus concentrations at interface stations measured before and after heavy rain (July 27). Total Fe and P fractions include only particulates passing through a $153\text{-}\mu\text{m}$ filter.

Depth in Meters	Open Lake		Interface Zone					
			Station 1 (0.6 M from open water)			Station 3 (2.4 M from open water)		
	Iron	Phosphorus	Total Fe	Total P		Total Fe	Total P	
				Before Rainfall	After Rainfall		Before Rainfall	After Rainfall
Sfc.	267	57	1423	500	212	2383	1123	686
0.5			538	420	193	651	1284	1224
2	607	80	264	807	686	456	1621	1795
4			564	689	767	226	1087	1212
5	766	78						
7		197						
7.5	1400	877						
8		1458						

similarity between the interface zone and the monimolimnion is consistent with the hypothesis of transport of chemicals from the surface of the mat directly into the monimolimnion by density currents.

Phosphorus concentrations of the monimolimnion reached a maximum in late May but decreased slightly during June and July in both 1969 and 1970 (Hooper, 1969; 1970). If the phosphorus concentrations in this stratum are sustained by settling of materials through the thermocline, increases rather than decreases would be expected in June and July since this is the period of high primary production in the photosynthetic water. The observed increases after the vernal overturn support the hypothesis of transport by density currents since this is the season in which mat interstitial water temperatures are low and the thermal gradients within the mat would direct the flow to the bottom of the basin.

Discussion

MAINTENANCE OF DICHOTHERMY

Evidence that phosphorus and iron from the mat zone are transported to the monimolimnion early in the spring suggests that this may be the mechanism for the maintenance of dichothermy in North Gate Lake. In all three years there was heat input from the bottom of the basin after the spring overturn which increased the thickness of the monomolimnion. This occurred at approximately the same time that the concentration of phosphorus was increasing. In 1970, the temperature minimum was eliminated as cooling took place in late summer. However a distinct chemocline and a dichothermic layer between 7 and 8 m was present during the winters of 1969-1970 and 1970-1971, indicating that dichothermy is restored in the fall by density flow from the mat into the basin. Thus dichothermy of the type noted in the Notre Dame tract bog lakes appears to be maintained by density currents from mat interface zones to the bottom water rather than arising from purely morphometric considerations.

MAT-WATER INTERACTIONS

It appears that the cold thermal wedge of mat water can be displaced lakeward by rain and the increase in mat water level may also be displaced shoreward during periods of warm, dry weather when there is high transpiration in the mat and bog forest. This was demonstrated by a simple tracer labeling experiment in the mat in 1968 (Hooper, 1968). Displacement of the thermal bar separating the lake and mat water may also take place when there is a strong wind-driven drift of surface water across the lake, and warm lake water is carried into the mat and mixed with mat water. With mixing, vertical displacement may take place and in this way surface water may be carried vertically to or below the thermocline. This appears to have taken

place after labeling of the mat in 1971 at stations E and F. After a period of strong winds, we detected labeled particulate phosphorus at 3 and 6 m at stations E and F on the downwind side of the lake but not at other interface stations. The mat at these stations had not been labeled but there was high concentration of labeled particulate phosphorus at the surface over the entire lake.

The mat-water interface of stratified bog lakes such as North Gate provides a site for dispersion processes not encountered in non-bog stratified lakes. Nutrient displacement from the littoral zone directly to the deeper water precludes the recycling of these materials in the photosynthetic zone. This appears to be one of many factors limiting the productivity of these lakes.

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