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

Technical Progress Report

FRANK F. HOOPER

December 1972

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School of Natural Resources
Resource Ecology Program

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THE UNIVERSITY OF MICHIGAN

SCHOOL OF NATURAL RESOURCES
Resource Ecology Program

Technical Progress Report

NUTRIENT CYCLING AND PRODUCTIVITY OF DYSTROPHIC LAKE-BOG SYSTEMS (PART A)

Frank F. Hooper

ORA Project 016440

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TABLE OF CONTENTS

	Page
SCOPE OF WORK	1
APPENDIX I. ORGANIC PHOSPHORUS COMPOUNDS OF A NORTHERN MICHIGAN BOG, BOG-LAKE SYSTEM	3
Identification of Dissolved Organic Phosphorus Compounds	5
Methods	7
Labeling with ^{32}P and treatment with lime	9
Phosphorus compounds and ion exchange chromatography	11
Results	11
Gradient elution patterns	13
Discussion	19
Literature Cited	20
APPENDIX II. PRELIMINARY REPORT OF THE ROLE OF <u>SPHAGNUM</u> IN THE CYCLING OF PHOSPHORUS IN A BOG-LAKE SYSTEM	23
Introduction	25
Methods	25
Measurement of phosphorus of mat interstitial water	25
Labeling of bog mat	27
Background radioactivity data prior to labeling	29
Sampling of the bog system after labeling	29
Biological sampling	29
Weather data	30
Results	30
Stable phosphorus of mat interstitial water	30
Activity fractions of mat interstitial water	34
Lake-bog-mat exchange	36
Effect of lime treatment upon distribution of labels	41
Fate of labels in mat	43
Discussion	45
Marginal circulation and the phosphorus cycle	45
Climatic effects on bog metabolism	47
Summary	47
Literature Cited	48
APPENDIX III. IONIC STATE AND COORDINATION OF IRON IN BOG LAKES	49
Methods	52
Results	56
Discussion	66
Literature Cited	69

SCOPE OF WORK

December 1, 1971 - November 30, 1972

During the 1971-72 year, some work continued on Subproject B (Phosphorus Cycling), but most of our work was concerned with Subproject D (Iron Cycling) of the original proposal. Our efforts during the past year were grouped into the six items given below.

1. Studies on Bog Organic Phosphorus Residues. In the spring of 1972, Mr. J. P. Koenings continued work on his organic phosphorus residue samples, and completed his Master's dissertation entitled "Differential Distribution of Phosphorus Compounds in a Dystrophic Bog Lake Ecosystem." This work was subsequently presented as a paper at the meeting of the Michigan Academy of Science in Lansing. The paper has been refereed and accepted for publication. It will appear in an early issue of the Michigan Academician. This paper is appended to this report (Appendix I). This report incorporates some of the data presented in the last progress report, but it includes analyses not available at that time.

Koenings plans to continue work on the problem of the cycling of organic phosphorus residues in North Gate Lake as a doctoral dissertation problem. He is now working with Professor I. A. Bernstein of the Department of Biochemistry perfecting procedures for more complete characterization of these organic residues, and techniques which will facilitate their analyses at more frequent intervals. Further work on this problem is proposed for the 1973-74 year.

2. Continued Analysis of Data from Earlier Phosphorus Labeling Experiments. In the past year, we have continued work on the analysis of the mat labeling experiment carried on during the summer of 1971. A report summarizing progress to date is appended (Appendix II). Yet to be studied are data from the open lake, e.g., phytoplankton, zooplankton, Chaoborus, and the mud minnow Umbra. These data are in various stages of analysis. At the present time, two student assistants are employed part time in this work.

3. Construction of a Predictive Model of Phosphorus Flow. Some effort has gone into assembling the necessary data input for a compartment model of phosphorus flow in the North Gate system. Professor Richard Patterson, systems analyst in the School of Natural Resources, has provided a theoretical outline for such a model. The objective of such a model is to be able to make predictions as to the quantity of phosphorus added to the food chain in various years when one is provided with a forecast of certain seasonal weather variables. The proposal for further input and development of this model is included in the proposal for 1973-74.

4. Energetics of the Mud Minnow Umbra. Edward Brady, a graduate student in the Department of Zoology, has started a Ph.D. thesis program on the energetics of the principal fish species present in the bog lake system of the Notre Dame tract. Mr. Brady's studies on energetics utilize data collected in earlier labeling experiments to quantify rates of material flow in the simple zooplankton-Chaoborus-Umbra food chain. In addition to an energetics study, Mr. Brady is attempting to manipulate Umbra density levels in one of the small bog lakes of the Notre Dame tract to determine its influence upon prey populations.

5. Construction of a "Bag Apparatus" for Conducting Localized Labeling Experiments with Tracers in North Gate Bog. During the past summer, we wished to undertake localized labeling experiments with ^{59}Fe within the entire water column for the purpose of determining the extent and distribution of the various biologically active pools of iron in the bog system. To do this we constructed a plastic cylinder 36 in. in diameter using metal ring supports which was supported by a surface float and extended from the surface of the lake to the maximum depth. The apparatus is equipped with a device for injection of a sample into the water column, and a collecting system whereby samples can be drawn off from a series of depths without disturbance to the water column. These operations are conducted from the surface of the lake.

We utilized this system in a preliminary labeling experiment with ^{59}Fe . In this case, the label was introduced at the surface and we followed the movement of various forms of the labeled Fe as the tracer moved down in the water column. These data are presently being analyzed.

6. Analysis of Forms of Stable Iron in North Gate Bog System. From consideration of previous studies of iron and its distribution in bog lakes, it was apparent that methodology currently in use for the collection and analysis of samples for the various forms of iron produced in bog waters was completely unsatisfactory, and that faulty methodology has led to some errors in the interpretation and analysis of iron data from other lakes. It was therefore necessary to design and construct an in situ filtration device which would permit a more accurate assessment of the chemical and physical states of iron in various strata of the lake basin which would avoid errors arising from surface filtration and handling of samples.

Data collected utilizing the procedures which we devised clearly indicate large errors in the earlier data. Mr. Koenings has provided a manuscript summarizing use of this apparatus and demonstrating these differences in interpretation of iron distribution which arise from methodology. This manuscript is appended (Appendix III). It will be submitted to Limnology and Oceanography for publication after additional editing and informal review by other specialists.

APPENDIX I

ORGANIC PHOSPHORUS COMPOUNDS OF A NORTHERN MICHIGAN BOG, BOG-LAKE SYSTEM

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(To appear in Michigan Academician, March Issue)

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ORGANIC PHOSPHORUS COMPOUNDS OF A NORTHERN MICHIGAN BOG, BOG-LAKE SYSTEM*

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Within natural and artificially enriched eutrophic lakes, high phosphorus content is ordinarily associated with high productivity and a large biomass of plants and animals (Edmondson, 1969). It appears that certain Michigan bog lakes however do not follow this pattern. North Gate Lake, a one-acre bog lake located in Gogebic County, Michigan (T 45 N, R 42 W), is a lake of low productivity but with an average phosphorus concentration of between 80 and 90 $\mu\text{g}/\text{l}$. This is nearly fourfold greater than the average of 479 neighboring Wisconsin and Michigan lakes (23 $\mu\text{g}/\text{l}$) (Juday and Birge, 1931). The interstitial water of the bog mat has a concentration of dissolved organic phosphorus in excess of 300 $\mu\text{g}/\text{l}$. Hence this lake is something of a paradox and does not display the coupling usually observed in freshwater lakes between nutrient level and trophic relationships. The purpose of this paper is to describe the chemical nature and distribution of the organic phosphorus compounds in the North Gate bog, bog-lake system and to present data from a ^{32}P labeling experiment which bear upon the dynamics and cycling of these compounds and upon the paradoxical relationship between nutrient level and productivity.

Identification of Dissolved Organic Phosphorus Compounds

Despite a wealth of data on the gross quantity of dissolved organic phosphorus (DOP) residues in marine and freshwater systems, there have been only fragmentary and isolated efforts to identify specific compounds. More important, little effort has been directed toward linking observed organic fractions with the metabolic activities of bacterial and plant cells from which these materials must arise. Other than the suggestion that DOP represents the more refractory compounds that decompose slowly (Ketchum, 1962; Grill and Richards, 1964) and that it is liberated by bacterial cells, there are few guides as to its role in biochemical cycles of either marine or freshwater systems. Phillips (1964) separated the DOP of seawater that had been equilibrated with ^{32}P into six fractions. Three were identified as nucleotides or polynucleotides on the basis of adsorption on charcoal. Three other chromatographic peaks were not adsorbed and were believed to represent phosphorylated hydrocarbons.

*Research carried out under U.S. Atomic Energy Commission Contract AT(11-1)-1771.

Since plant and bacterial cells are known to accumulate large quantities of polyphosphates, the question arises whether or not these fractions exist free in lake waters, since they would be liberated by the lysis of cellular material. Solórzano and Strickland (1968) and Armstrong and Tibbetts (1968) found only small quantities of polyphosphates in unpolluted coastal waters. This suggests that polyphosphates do not accumulate to any extent in unpolluted situations and that they are absorbed by algal cells even in the presence of bacteria. This is supported by Clesceri and Lee (1965) who found that the alga Chlorella pyrenoidosa would utilize both pyrophosphate and tripolyphosphate to support growth. Comparable growth levels were achieved by the alga in the unialgal (bacteria present) as in the pure (bacteria absent) cultures supplied separately with each of the two polyphosphates. However, it is well-known that they accumulate when there are deficiencies of nutrients other than phosphorus (Harold, 1966). Hence under adverse environmental conditions, they might occur in the tropholytic waters and their presence may be an indication of limiting micronutrients.

In our first attempt to isolate and to characterize the organic phosphorus compounds in North Gate Lake (Hooper, 1969), we found that between 0.3 and 4.2% of the total phosphorus in the aphotic zone was adsorbed by charcoal and presumably was nucleic acid. This fraction, however, was not present in the euphotic zone. In 1969 between 1.7 and 12.3% of the total phosphorus was labile inorganic phosphorus, presumably polyphosphate or pyrophosphate. This fraction was present at all depths. However, between 35 and 47% of the total phosphorus, i.e., DOP, remained unidentified. Our observations in 1969 indicated that organic substances exhibiting properties of nucleic acids were present in the aphotic zone but not in the surface waters.

In 1970 further characterization of this fraction was performed. In addition to being an organic phosphorus-containing compound capable of being adsorbed to charcoal, the compounds reacted positively to the orcinol test for ribose sugar and showed maximum absorption in the UV at 260 nm. Both of the latter two properties are also characteristic of hydrolyzed ribonucleic acid and/or free ribonucleotides. In summary, the characterization data indicated the presence of compounds exhibiting the following properties: (1) a ribose sugar, (2) maximum absorption at 260 nm, (3) the presence of phosphorus, and (4) reactivity to acid molybdate only after the digestion procedure characteristic of organic compounds.

Lehninger (1970) states that proteins, due to the presence of three amino acids, do show absorption in the ultraviolet. However, maximum absorption occurs at 280 nm. Pyrimidine and purine bases exhibit maximum absorption at 260 nm which is used as a characteristic test for compounds containing these bases. Some overlap does occur but the fact that the purine bases show nearly two orders of magnitude greater absorptive capacity than proteins does permit the use of ultraviolet absorption methods (at 260 nm) for the determination of nucleotide phosphorus. Holm-Hansen (1968) cited the data of Kissane and Robins

(1958), who used quantitative ultraviolet absorption values of DNA (deoxyribonucleic acid) to validate a fluorometric procedure for the measurement of DNA, to substantiate in turn his proposed procedure to measure DNA in the ocean. Therefore, this method (i.e., optical density in the ultraviolet at 260 nm) in conjunction with ion exchange chromatography separation of the inorganic and the organic acids containing phosphorus was used for the determination of the hydrolysis products of the ribonucleic acids, as well as the related free mono-, di-, and triphosphorylated nucleotides present in the waters of North Gate Lake as part of dissolved organic phosphorus.

Methods

Filtered samples were concentrated by flash evaporation in 1969, however, this procedure proved both time consuming and difficult. In 1970, we therefore perfected an alternate procedure for concentrating the organic residues on an ion exchange resin. This proved more efficient and more reliable. For these analyses we collected 600-ml water samples in clean polyethylene bottles from three locations in the lake: (1) the center of the lake at a depth of 1 m, (2) the center of the lake at a depth of 7 m, and (3) at a depth of 0.2 m in the bog mat at the southeast corner of the lake, 5.5 m from the edge of the open water (Fig. 1).

The samples were immediately frozen and transported back to the laboratory (an interval of approximately one week) where they were unfrozen and applied to a 2 x 15 cm column of Dowex 1 x 8 anion exchange resin, 100-200 mesh formate form. The effluent and the subsequent water washes of the resin were colorless, contained no phosphate, and did not absorb light at 260 nm, as compared to a water blank. A gradient elution was set up between 0.01 N and 0.5 N formic acid and allowed to flow at a rate of 0.5 ml/min. Measured fractions were collected on a Buchler fraction collector. Fractions were checked for absorbance at 260 nm against an appropriate formic acid blank. Phosphorus levels were analyzed according to the classification scheme given by Strickland and Parsons (1965), with orthophosphorus being determined according to the method of Stephens (1963). Selected fractions were assayed for ribose content, using the orcinol method (Ashwell, 1957) and UV absorption spectra were determined on a Beckman DB-G scanning spectrophotometer. Fractions were assayed for labile inorganic phosphorus by digesting the sample in 1 N HCl, boiling for 10 min, and assaying for orthophosphate (Vogler, 1966). Selected fractions were digested with perchloric acid to determine total phosphorus content according to Strickland and Parsons (1965).

Due to the limited size of the fractions (10 ml) only one determination of each parameter was possible, however, extreme care was taken to avoid contamination of the samples, and blanks as well as phosphate standards were run

NORTH GATE BOG
survey data 1969
(depth in meters)

Fig. 1. Depth contours and the position of the surrounding *Sphagnum* mat of North Gate Lake.

with each series of samples. Some hydrolysis of the polyphosphates may have occurred in the acidic eluting solution (lowest pH = 2.08), however, since the method routinely used in the determination of polyphosphate (1 N H₂SO₄, 100° C for 10 min) is much more severe, the amount of hydrolysis should have been minimal.

LABELING WITH ³²P AND TREATMENT WITH LIME

On June 5, 1971, we treated the mat surrounding the open water with 200 mCi of ³²P in the form of orthophosphate. This tracer was mixed with a nonradioactive tracer, the inert organic dye rhodamine WT. The mixture was injected into the interstitial water of the Sphagnum mat. Soon after treatment both tracers appeared in the open water of the lake. Rainfall flushed the labels from the mat into the lake basin. Upon reaching the lake water, the ³²P soon appeared in the plankton and the remainder of the biota.

On July 6, 1970 and July 22, 1971, surface water of the lake was treated with agricultural lime. The lime was mixed into a slurry and spread over the lake surface. To insure good distribution, the surface water was then mixed with an outboard motor. In the process of mixing, a section of the mat adjacent to the open water was flooded with the treated lake water. This treatment brought about an increase in pH of the surface water and adjacent mat water from 4.7 to approximately 8.0. It also caused a significant increase in SUP (soluble unreactive phosphorus), and in particulate phosphorus levels in the epilimnion (0.9 m) (Fig. 2). This phenomenon was previously noted by Waters (1957), when he added lime to the epilimnion of Starvation Lake. He postulated the origin of these materials to be the interstitial water of the surrounding Sphagnum mat. By labeling the phosphorus compounds produced in the mat and by then monitoring the influx of phosphorus resulting from the addition of lime the origin of the phosphorus as well as the nature of the compounds released could be ascertained. Identification of such inputs to the lake is important in understanding the cycling of phosphorus in this system since naturally-occurring inputs of labeled phosphorus from the mat had been previously observed and may provide a sporadic source of phosphorus for the epilimnetic biota during summer stagnation. Underlying the design of this experiment, therefore, was the belief that by assaying the radioactivity in various peaks in the gradient elution pattern before and after labeling with ³²P, we would be able to identify organic phosphorus compounds produced by the biota after the time the label was added, which were subsequently released into the mat and/or lake water; and by comparison of the gradient elution pattern before and after liming, we would be able to study the effect of the lime upon the character and distribution of organic phosphorus compounds in the mat and lake water.

Fig. 2. Concentration of stable phosphorus fractions at selected depths during 1970.

PHOSPHORUS COMPOUNDS AND ION EXCHANGE CHROMATOGRAPHY

Phosphorus compounds such as phosphoric acid, polyphosphoric acid, nucleotides, and nucleic acid can be adsorbed on an anion exchange resin along with the colored organic matter of the bog water (e.g., fulvic and humic acids). The latter produced a banding pattern on the resin column, which was not removed by the gradient elution with formic acid. However, the acid gradient removed and separated the inorganic and organic phosphorus compounds from the resin. Cohn (1957) showed that elution with increasing normality of acid or salt can successfully separate the various phosphorus compounds into peaks which have definite reproducible positional relationships to each other.

The monophosphate nucleotides appear first in the elution patterns followed by the diphosphate nucleotides and then by the triphosphate nucleotides (Hurlbert, *et al.*, 1954). Inorganic phosphorus compounds also appear in order of their phosphorus content with ortho- appearing first followed by the pyro-, tri-, tetrameta-, and trimetaphosphate ions (Inczeedy, 1966; Samuelson, 1963). Inorganic phosphorus (ortho-) always appears in the organic elution pattern within the monophosphate nucleotide section (Hurlbert, *et al.*, 1954). Generally then, the amount of phosphorus per compound increases with increasing acidity along the elution pattern. However, this holds true only when the inorganic and the organic elution patterns are considered separately. When the two patterns are considered together there is considerable overlap in phosphorus content per compound. Free ribose nucleosides, which also absorb in the 250-260 nm range do not absorb to the resin and would have been detected in the pre-elution wash, but were not present in these samples.

Results

Lake water analyzed in the above manner showed several different ribose and phosphate-containing compounds which absorbed in the 250-260 nm wavelength region (Table I). Such properties are characteristic of ribose nucleic acids. It was not determined whether these compounds were ribose nucleotides or polymers.

Mat water and lake water from a depth of 1 m contained smaller amounts of RNA than did the 7 m water. All samples had a clearly defined orthophosphate peak and a second inorganic peak that corresponded in order of elution to polymers of orthophosphate (Beaukenkamp, *et al.*, 1954). Since earlier work showed that water from this lake contains a labile inorganic phosphate, similar in properties to polyphosphate (Hooper, 1969), this peak is described in Table II as a polyphosphate.

There were sharp differences between samples from the three locations in the amount and character of the 260 nm absorbing materials. This may indicate

Table I. Characterization of nucleic acid fractions from water collected August 12, 1970.

Depth	Sample Number	RNA		Ribose, $\mu\text{mol/ml}$	Phosphorus, $\mu\text{mol/ml}$
		Peak absorption (nm)	$\mu\text{mol/ml}$ (using extinction coefficient of $14,000 \text{ cm}^{-1}\text{M}^{-1}$)		
7 m	9	258	12.0	20.0	---
	10	258	7.0	16.0	1.8
	15	broad	4.7	5.4	1.5
	25	broad	1.7	1.4	1.2
1 m	17	broad	6.4	6.5	1.2
	18	260	10.2	6.5	1.2
Mat	14	broad	1.1	1.6	0.1
	26	broad	2.1	2.4	0.3

Table II. Phosphorus fractions isolated from North Gate bog-bog lake system, August 12, 1970.

Sample	RNA*		Orthophosphate, $\mu\text{g/l}$	Polyphosphate, $\mu\text{g/l}$
	$\mu\text{mol/l}$	$\mu\text{g P/l}$		
Lake water, 7 m	1.56	48.3	22.7	13.0
Lake water, 1 m	0.52	16.1	6.6	4.3
Mat water	0.40	12.4	32.8	5.0

*Based on UV absorption, using an average extinction coefficient of $14,000 \text{ cm}^{-1}\text{M}^{-1}$. P content assumes molar equivalence between phosphate content and base content.

differences of origin of the RNA fractions or differences in state of degradation. Peaks in the 250-260 nm range in some cases were broad and lacked prominent shoulders, indicating that the material was not a single nucleotide but contained polymers. The lack of agreement in stoichiometry between UV absorption, ribose, and phosphorus leaves some uncertainties. These differences arose because in some cases we were below the desirable range of accuracy in a given procedure.

The larger quantity of RNA observed at 7 m than at 1 m may arise from the greater degradability of nucleic acids in light (Armstrong, Williams, and Strickland, 1966). However, larger quantities of RNA might be expected at 7 m from autolysis of cells settling into this layer. Since water exchange takes place between the mat and the lake, the lower RNA content of the mat water compared to 7 m and 1 m water suggests a limnetic origin of much of the RNA. Differences in character of the RNA fractions, however, were apparent. These differences appeared in the gradient elution patterns of these three locations discussed below.

GRADIENT ELUTION PATTERNS

The above schedule of lime treatments and labeling made possible the comparison of gradient elution patterns from samples collected on August 12, 1970, before labeling, with the pattern on July 9, 1971, 2 weeks after labeling but before treatment with lime (July 22, 1971). The ^{32}P was in the orthophosphate form when injected into the mat, and it appeared in the elution patterns in fractions 32-37%, which correspond to the ortho form of inorganic phosphorus identified in 1970 at all stations (22-35%).

By using this inorganic peak (orthophosphate) as the marker for interpretation of peaks on the elution curve, and by comparison of fractions in corresponding percentages of the total eluted volume it is possible to match the activity pattern in 1971 with the identified residues in peaks from the 1970 data (Figs. 3-5).

The inorganic orthophosphate peak is small in the epilimnion and in the mat (Figs. 3 and 4), indicating that this fraction had been assimilated in these locations, but it is high in the hypolimnion because of regeneration of detrital phosphorus under anaerobic conditions. The first activity peak (35) of the mat water contains most of the inorganic phosphorus present in the sample. The second peak (70) and the third (82) are organic compounds. Hence the potential supply of phosphorus compounds to the epilimnion from the mat consists of both orthophosphate and organic phosphorus compounds.

The peaks that appear in the epilimnion are the inorganic peak (32) and a second organic peak in the 50-53 fractions. Peaks corresponding to the organic phosphorus fractions in the mat water (70, 82) are entirely missing.

Fig. 3. Activity of fractions eluted from surface water of North Gate Lake after mat labeling in 1971, compared with inorganic and organic phosphorus components eluted from the surface water in 1970. Numbers on graphs indicate percentage of total elution volume at peaks.

Fig. 4. Activity of fractions eluted from mat water of North Gate bog after mat labeling in 1971, compared with inorganic and organic phosphorus components eluted from mat water in 1970. Numbers indicate percentage of total elution volume at peaks.

Fig. 5. Activity of fractions eluted from 6.5-7.0 m water of North Gate Lake after mat labeling in 1971, compared with inorganic and organic phosphorus components eluted from 6.5-7.0 m water in 1970. Numbers on graphs indicate percentage of total elution volume at peaks.

Either plankton organisms of the epilimnion have removed these fractions which have been supplied by the bog mat and converted into the organic activity peak at 50, or these fractions have never entered the open water.

It is clear, however, that the 70 and 83 fractions can move into the epilimnion under certain conditions. The elution curve of epilimnetic water 1 week after addition of lime (Fig. 6) contains the peaks of activity in the range found in the mat waters (78-90). These activity peaks did not appear in the July elution curve for the epilimnion (Fig. 3). Thus these organic fractions are not limnetic in origin and must be compounds produced in the mat after the July labeling, but which were liberated from the mat under conditions of high pH that existed after addition of lime. Supporting evidence for the observed movement of phosphorus compounds from the mat to the water following liming comes from chemical analysis of stable phosphorus made after lime treatment (Fig. 2). After treatment there was a large increase in the soluble unreactive phosphorus (dissolved organic phosphorus) component immediately after treatment.

Activity peaks in the hypolimnion (6.5-7.0 m) are somewhat more difficult to interpret. Peaks in this stratum could represent compounds produced from inorganic label entering either the mat or the epilimnion. Peaks were observed at 35, 55, and 75%, and corresponding peaks are to be found on the characterization curve (Fig. 5). The fraction at 55% must be a labeled organic compound because inorganic residues were not found in this region of the characterization curve. It very likely is identical to the labeled compounds of peak 50-53 in the epilimnion (Fig. 3). If identical, it must be lost to the hypolimnion rapidly since this labeled fraction was abundant in the hypolimnion about 2 weeks after labeling. The activity peak in fraction 35 could be either the inorganic phosphorus found in the characterization patterns in fractions 24 to 35, or the organic fraction at 35.

There were many unlabeled organic and inorganic fractions in the hypolimnetic water. This is to be expected since bacteria of this stratum carry on the mineralization of compounds of limnetic origin descending from the upper water as well as fractions arising from the bog mat. The latter case appears to be illustrated by the large organic peaks in fractions 20-30 in the characterization curve of the hypolimnion (Fig. 5) corresponding to a smaller organic peak (25-35) in the mat water sample (Fig. 4). The large peak in the hypolimnion may represent an accumulation of this compound. Peak 83 of the hypolimnion (Fig. 5) appears to be an accumulation of polyphosphate arising from either bacteria or autolysis of algae. It is of interest to note that this fraction was not labeled in the samples collected 2 weeks after treatment with ^{32}P although the polyphosphate residues were also present in the characterization curve in the spring of 1971. Hence it would appear that the polyphosphate is not produced in the epilimnion and lost to the hypolimnion as in the case of fraction 55, but must arise either from in situ production or from sedimentation and regeneration at some other time of the year.

Fig. 6. Activity of fraction eluted from surface water of North Gate Lake after treatment with lime in 1971.

Discussion

Compounds produced in the bog mat appear to be hydrolysis products of ribonucleic acids as well as free mono-, di-, and triphosphate nucleotides. The ultraviolet (260 nm) absorbing materials appearing late in the elution pattern (peaks 78-80%) (Figs. 3 and 6) are nucleotides richer in phosphate than early appearing materials (Hurlbert, *et al.*, 1954). These compounds exist in the interstitial water of the mat but can be flushed into the open lake water. An increase in pH appears to have increased the mobility of these polyphosphorylated compounds. Under normal circumstances the phytoplankton and bacteria of the epilimnion utilized these mat nucleotides (Fig. 3). However the adverse physiological effects of the large pH change (from 4.7 to 8.0), and ion shock (calcium rose from 1.6 ppm to 10.4 ppm, and alkalinity from 0.39 ppm to 64.6 ppm) upon the biota (Waters, 1957; Ruttner, 1953) may have permitted the observed accumulation (Fig. 6). The hypothesis that organic phosphorus compounds can be directly utilized by algae was first reported by Chu (1946), and later verified by Harvey (1953). Moreover, Provasoli (1958), McLaughlin (1958), and Fogg and Miller (1958) showed that algae could utilize mononucleotides to support growth.

The organic phosphorus compounds produced within the epilimnion of the lake appear earlier in the elution pattern (50-55) (Fig. 3), therefore contain less phosphorus (Hurlbert, *et al.*, 1954) and would appear to be diphosphate nucleotides (e.g., ADP, etc.). Kuenzler (1970) showed that several species of algae are capable of extracellular release of large amounts of organic phosphorus as dissolved organic phosphorus (DOP). The composition of the DOP in sea water has been characterized as containing nucleic acid (Holm-Hansen, *et al.*, 1968). Moreover, Armstrong and Tibbitts (1968) demonstrated the presence of an organic polyphosphate in sea water, whose polyphosphate group (released after photochemical combustion) exhibited a hydrolysis rate similar to pyrophosphate, i.e., an organic polyphosphate similar in properties to a diphosphate nucleotide like ADP. Iwamura (1962) confirmed the presence of the free mono-, di-, and triphosphate nucleotides in intact algal cells. Thus we feel that the large organic phosphorus reservoir on the mat interstitial waters may be cycled through the biota of the epilimnion and utilized in the growth of the phytoplankton with subsequent production of DOP either through autolysis or extracellular release.

While the inorganic phosphorus of the epilimnion and the mat waters appears to be chiefly orthophosphate, the hypolimnion contains an inorganic pool consisting of both ortho- and polyphosphate phosphorus. Based on its position in the gradient elution pattern (83%) (Fig. 5), the latter compound may contain three to four phosphate groups. Our failure to find an accumulation of labeled polyphosphates in the hypolimnion indicates that these compounds arise from *in situ* autolysis of algae or bacteria rather than from materials produced in the euphotic zone.

Literature Cited

- Armstrong, F.A.J., and S. Tibbitts. 1968. Photochemical combustion of organic matter in sea water, for nitrogen, phosphorus, and carbon determination. J. Mar. Biol. Assoc. U.K., 48:143-152.
- Armstrong, F.A.J., P. M. Williams, and J.D.H. Strickland. 1966. Photo-oxidation of organic matter in sea water by ultraviolet radiation, analytical and other applications. Nature, 211:481-483.
- Ashwell, G. 1957. Colorimetric analysis of sugars. In: Methods in enzymology, Vol. 3 (Colowick, S. P., and N. O. Kaplan, eds.). Academic Press, New York.
- Beukenkamp, J., W. Rieman, III, and S. Lindenbaum. 1954. Behavior of the condensed phosphates in anion-exchange chromatography. Anal. Chem., 26:505-512.
- Chu, S. P. 1946. Utilization of organic phosphorus by phytoplankton. J. Mar. Biol. Assoc. U.K., 26:285-295.
- Clesceri, M. L. and G. F. Lee. 1965. Hydrolysis of condensed phosphates. I: Non-sterile environment. Int. J. Air and Water Poll., 9(11):723-742.
- Cohn, W. E. 1957. Chromatography of nucleic acid derivatives. In: Ion exchangers in organic and biochemistry (Calmon, C., and T.R.E. Kressman, eds.). Interscience, New York, 350 pp.
- Edmondson, W. T. 1969. Eutrophication in North America. In: Eutrophication: causes, consequence, correctives. Nat. Acad. Sci., Washington, p. 124-149.
- Fogg, G. E., and J.D.A. Miller. 1958. The effect of organic substances on the growth of the freshwater alga Monodus subteraneus. Verhandl. Intern. Ver. Limnol., 13:892-895.
- Grill, E. V., and F. A. Richards. 1964. Nutrient regeneration from phytoplankton decomposing in seawater. J. Mar. Res., 22:51-69.
- Harold, F. M. 1966. Inorganic polyphosphates in biology: structure, metabolism, and function. Bacteriol. Rev., 30:772-794.
- Harvey, H. W. 1953. Note on the absorption of organic phosphorus compounds by Nitzschia closterium in the dark. J. Mar. Biol. Assoc. U.K., 31: 475-476.

Literature Cited (Continued)

- Holm-Hansen, O., J. D. Strickland, and P. M. Williams. 1966. A detailed analysis of biologically important substances in a profile off southern California. *Limnol. Oceanogr.* 11(2):548-561.
- Holm-Hansen, O., W. H. Sutcliffe, Jr., and J. Sharp. 1968. Measurement of deoxyribonucleic acid in the ocean and its ecological significance. *Limnol. Oceanogr.*, 13(3):507-514.
- Hooper, F. F. 1969. Nutrient cycling and productivity of dystrophic lake-bog systems. Tech. Prog. Rep., submitted to A.E.C., November, 1969.
- Hurlbert, R. B., H. Schonitz, A. F. Brumm, and V. R. Potter. 1954. Nucleotide metabolism. II. Chromatographic separation of acid-soluble nucleotides. *J. Biol. Chem.*, 209(1):23-39.
- Inczedy, J. 1966. Analytical applications of ion exchangers. Pergamon Press, Inc., New York.
- Iwamura, T. 1962. Nucleotides and nucleic acids. *In: Physiology and biochemistry of algae* (Lewin, R. A., ed.). Academic Press, New York, pp. 231-238.
- Juday, C., and E. A. Birge. 1931. Second report on the phosphorus content of Wisconsin lake waters. *Trans. Wis. Acad. Sci., Arts, Lett.*, 26: 353-382.
- Ketchum, B. H. 1962. Regeneration of nutrients by zooplankton. *Internat. Council for Explor. of the Sea. Rapports et Procès. Verbaux des Reunions*, 153:142-147.
- Kuenzler, E. J. 1970. Dissolved organic phosphorus excretion by marine phytoplankton. *J. of Phycology*, 6(1):7-13.
- Lehninger, A. L. 1970. *Biochemistry*. Worth Publishers Inc., New York, 833 pp.
- McLaughlin, J. J. A. 1958. Euryhaline chrysomonads: nutrition and toxigenesis of Prymnesium parvum, with notes on Isochrysis galbana and Monochrysis lutheri. *J. Protoz.*, 5:75-81.
- Phillips, J. E. 1964. The ecological role of phosphorus in waters with special reference to microorganisms. *In: Principles and applications in aquatic microbiology* (Heukelekian, H., and N. C. Dendero, eds.). J. Wiley and Sons, New York, pp. 61-81.

Literature Cited (Concluded)

- Provasoli, L. 1958. Nutrition and ecology of protozoa and algae. *Ann. Rev. Microbiol.*, 12:279-308.
- Ruttner, F. 1953. Fundamentals of limnology. Univ. Toronto Press, Toronto.
- Samuelson, O. 1963. Ion exchange separations in analytical chemistry. John Wiley and Sons, New York.
- Sol6rzano, L. and J.D.H. Strickland. 1968. Polyphosphate in seawater. *Limnol. Oceanogr.*, 13:515-518.
- Stephens, K. 1963. Determination of low phosphate concentrations in lake and marine waters. *Limnol. Oceanogr.*, 8(3):361-362.
- Strickland, J.D.H., and T. R. Parsons. 1965. A manual of sea water analysis. Fish. Res. Bd. Canada Bull., 125:37-53.
- Vogler, P. 1966. Zur analytik der phosphorverbindungen in Gew6ssern. *Limnologica.*, 4(2):437-444.
- Waters, T. F. 1957. The effects of lime application to acid bog lakes in northern Michigan. *Trans. Amer. Fish. Soc.*, 86:329-344.

APPENDIX II

PRELIMINARY REPORT OF THE ROLE OF SPHAGNUM
IN THE CYCLING OF PHOSPHORUS IN A BOG-LAKE SYSTEM

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Introduction

In our analysis of the North Gate bog-lake system, we have previously described the upward movement of hypolimnetic phosphorus during the summer and its horizontal dispersion into the epilimnion and its biota, and into the surrounding bog mat. It has also been demonstrated that the translocation of phosphorus from the lower waters must be related to inclement weather phenomena and that such inputs are aperiodic. A logical extension of the notion of phosphorus flow from the epilimnion into the mat as observed in this and other studies (Coffin, *et al.*, 1949) is that there should be a return flow of phosphorus from the mat system to the lake water. The purpose of the mat labeling experiment described in this report was to explore the return flow, and (a) to determine the rates of exchange between the water and mat systems, and (b) to define the chemical characteristics of the phosphorus compounds taking part in this interchange.

Methods

MEASUREMENT OF PHOSPHORUS OF MAT INTERSTITIAL WATER

To measure the stable phosphorus and ^{32}P of the interstitial water, we sampled the mat water at four locations in 1970 and 1971. We collected 1-liter samples for analysis of stable phosphorus in the surface layer of the mat (0 to 0.5 m) by inserting a pipette to the proper depth and sucking mat water into an evacuated flask. For subsurface samples we employed the mat sampler previously described (Hooper, 1970). Briefly, this sampler consists of a section of heavy plastic pipe sealed at one end. Three inches above the seal there are small windows in the plastic covered externally by plastic screening. During penetration of the mat, these windows are sealed by an inflated balloon on the inside of the tube. The balloon is deflated when the sampler is in place at the proper depth, thus allowing the pipe to fill with water from the desired depth.

Mat water consists of a mixture of a wide variety of particulate and dissolved organic compounds. In sampling for analysis of various phosphorus components, it was necessary to set an upper limit as to the size of particulates to be investigated since there are many large plant fragments in the interstitial water. We chose to study only those particulates smaller than 153 μ . This size was selected because particles smaller than 153 μ appeared to be somewhat mobile within the mat, whereas larger particles are held within the interstices of the Sphagnum. Fractions investigated therefore are as follows: (a) particulate unreactive (PUP)—particles filterable through 153 μ nytex netting but retained by 0.45 μ millipore filter, (b) soluble unreactive (SUP)—materials filterable through 0.45 μ filter but not reacting with molybdate in the orthophosphate procedure without digestion, and (c) soluble reactive (SRP)—phosphorus passing through 0.45 μ filter but reacting with molybdate under the conditions of orthophosphate procedure.

In certain of the sampling locations (chiefly in the sections of the mat distant from the open lake) we were unable to collect and filter a sufficient quantity of water to complete an analysis, and data are incomplete. This sampling problem arises in late summer as the mat dries out and the water table falls. It was necessary to filter samples as soon as possible after removal from the mat to prevent biological changes in the phosphorus fractions. In most instances, samples were filtered within 1 hr after collection. However in the case of samples collected in 1970 at a distance of 8 m from the open water, there may have been some conversion of soluble reactive phosphorus (SRP) to soluble unreactive phosphorus (SUP) by bacteria since approximately 2 hr were required for filtration of these samples.

Chemical procedures used in the analysis of phosphorus components follow closely outlines given by Strickland and Parsons (1965). Water ^{32}P activity was measured by evaporating samples to dryness on a 2-in. planchet and counting on a low background beta system. In most instances, 10-ml samples were used but in some instances it was necessary to evaporate 50 ml of water. For measurement of total phosphorus activity, we plated a 5-ml aliquot of a solution that had been digested with perchloric acid and used for analysis of total stable phosphorus. To identify activity associated with the three stable phosphorus components it was necessary to employ some separation technique which would differentiate reactive from unreactive ^{32}P activity. To do this, we extracted the molybdate blue complex produced in the orthophosphate procedure with hexanol using the technique outlined by Stephens (1963). This extract was then dried on a planchet and counted to give an estimate of orthophosphate activity. Subtracting this activity from that obtained by evaporation of a 0.45 μ filtered sample gives the activity associated with the soluble unreactive phosphorus component (SUP).

For the mat labeling experiment, we utilized the following procedures for collection and analysis of mat interstitial water. We selected three sites for transects across the section of the mat to be treated, from the open water

to the tree zone, and designated these Transects I, II, and III (Fig. 1). Transect I crossed a section of the mat adjacent to an upland area and was close to the primitive shoreline of the basin before it was filled with peat and vegetation. Transect III was at the opposite side of the lake and was at the center of the lowland area representing the primitive lake basin (Griffing, 1970). Transect II was intermediate between these two sites, and represented a site at which an intermediate influence of upland drainage might be anticipated. For controls, we established three stations on the untreated side of the lake (Transects IV, V, and VI; Fig. 1).

To minimize radioactive contamination during sampling, we constructed wooden walkways across the transect area. Adjacent to each of the three walkways we established three sampling stations. On each transect, Station A was entirely within the treated zone, but was approximately 1 m from the open lake; Station B was 2 m from the open water; and Station C was 3 m from the open lake. At each of these stations, a section of perforated plastic pipe was inserted into the Sphagnum. Openings in the pipe were approximately 3 mm in diameter. The pipes were put in place at each station before applications of the isotope, and were then sealed with a rubber stopper. The sections of pipe served as sampling reservoirs for collection of interstitial mat water. The 3-mm perforations partially filtered the water; thus samples were relatively clear and free of debris. Openings were sufficiently large, however, to permit free flow of mat water through the mat. The mobility of mat water through the sampling reservoirs was verified by a labeling trial using rhodamine as a tracer. Samples for radioactivity measurements were removed from the reservoir with 10-ml disposable plastic syringes. In the neck of each syringe we inserted a small piece of nytex with 150 μ diameter openings. This filtered the water as it was drawn into the syringe. Syringes were then transported to the laboratory for further filtration, evaporation, and counting.

LABELING OF BOG MAT

The section of the bog mat labeled was the area adjacent to the northeast half of the lake. We labeled only sections of the mat covered by Sphagnum and other low-growing herbaceous species and avoided labeling sections with high-growing shrubs and trees. This made a very irregular pattern to the labeled area. The area labeled was marked off by rope and mapped (Fig. 1). On May 30, 1971, we made an application of tracers which consisted of 200 gm of rhodamine WT and 120 mCi of ^{32}P mixed with 50 gal of surface lake water. This mixture was sprinkled over the mat surface with 2-gal sprinkling cans. To secure a degree of uniformity in labeling, we allocated quantities of the mixture to treated areas in proportion to the surface area. By sprinkling the labels onto the surface of the mat, large quantities were absorbed by Sphagnum and there was slow penetration into the interstitial water. Sampling the lake water immediately following this application indicated that there had

Figure 1. Map of North Gate Lake, showing section labeled with ^{32}P and rhodamine, mat sampling transect, and mat-water interface sampling locations.

been little movement of either ^{32}P or rhodamine into the open lake water. A second application was made on June 24. On this day we used 190 mCi and we added the labels directly to the interstitial water in the following manner. Holes were punched through the mat to the water table at approximately 1-ft intervals. We then poured approximately 1/2 liter of the rhodamine- ^{32}P mixture into each of these holes. Labeling was completed in a 6-hr period.

BACKGROUND RADIOACTIVITY DATA PRIOR TO LABELING

Since the bog system had been labeled on two previous occasions, it was necessary to monitor carefully background radiation levels in the water and in the biota. Between May 13 and 30 we took careful monitors of the activity levels in plankton, Chaoborus, fish, and in the various water fractions of the lake and the mat. Activity background levels were approximately the same as the previous year before treatment and, as before, large quantities of rhodamine WT persisted in the monimolimnion.

SAMPLING OF THE BOG SYSTEM AFTER LABELING

Following labeling, sampling of the system was carried out at 4-day intervals. Data gathered consisted of the following: physical, chemical, and temperature profile at open water station (Station B) at 0.5-m intervals; oxygen, pH, and rhodamine concentration at surface, 1, 2, 3, 4, 5, 6, 7, and 8 m depths. A vertical profile of water activity was obtained at Station B by sampling at the surface and at depths of 1, 2, 6, and 7 1/2 m. Activity was measured on two fractions of each water sample; these were, total activity obtained by evaporating 10 ml of the sample on the planchet, and the soluble fraction obtained by filtering water (10 ml) through a 0.45 μ membrane. Three replicates were collected at all locations for water activity measurements and rhodamine determinations. Routine counting time for all samples was 10 minutes; however 30-min counts were necessary for low activities. In addition to sampling at Station B (open water), we collected data on water activity and rhodamine concentration at five mat-water interface stations adjacent to the mat (A, C, D, E, and F) (Fig. 1). Stations A, C, and D were at the edge of the mat adjacent to mat sampling Transects I, II, and III, respectively. Stations E and F were on the control side of the lake adjacent to control mat sampling Transects V and VI, respectively. Activity at these stations was measured at 4-day intervals at depths of 1, 2, 3, and 6 m. Data from interface stations served to identify local movements of the tracers from the mat into the lake.

BIOLOGICAL SAMPLING

Zooplankton and phytoplankton samples were collected from the surface and at a depth of 2 m at Station B and also adjacent to the five interface stations

(A, C, D, E, and F). Samples consisted of the catch from 10 l of lake water filtered first through 153 μ nytex and then through 10 μ nytex. We used the catch from the 153 μ netting as a measure of zooplankton and the catch from the 10 μ netting for phytoplankton. Net catches were transferred to a 0.45 μ membrane; the membrane was then digested, dried, and counted. Two collections of the phantom midge Chaoborus were made every 4th day at 10 p.m. and 3 a.m. Catches were made at the five marginal sampling stations and at Station B. Five replicates were collected at Station B. Collections were made by making a vertical haul with a closing net from a depth of 3 m to the surface. These samples were filtered on to a 0.45 μ filter paper, dried, and counted. Collections of the mud minnow Umbra were also made at 4-day intervals. These fish were collected in minnow traps placed at the five marginal stations and at Station B. A sample of trunk muscles was removed from one fish from each of the five stations; this sample was digested and counted. In addition, the intestinal tract was removed from the sample fish from each of the five collection stations. The contents were weighed, food items identified, and activity was measured on a small aliquot.

WEATHER DATA

Daily weather observations of wind direction, temperature, and cloud cover were routinely made at the lake. In addition, we set out rain gauges, which consisted of plastic funnels 7 1/2 in. in diameter; these funnels were set up on a floating raft at the center of the lake. The volume of rainfall accumulating in these devices was recorded daily. Thus for each period of precipitation we have a volume measured in the center of the lake (Fig. 2). To compare with these records we obtained the Forest Service weather records for the station at Watersmeet, Michigan, located approximately 20 miles to the southeast. These observations include maximum and minimum temperature, time and amount of precipitation, and average cloud cover (Fig. 2).

Results

STABLE PHOSPHORUS OF MAT INTERSTITIAL WATER

In 1970 we first made measurements of stable phosphorus fractions of the mat; some of these results are presented in Fig. 3. In 1971 we measured stable phosphorus fraction samples at the sampling reservoirs of Transects I, II, and III (Fig. 4). The 1970 measurements of total phosphorus included all particles filterable through the netting of the sampler which was of a coarser mesh than used in 1972. This did not appear to increase the phosphorus content substantially over collections in 1971 made with the 153 μ mesh netting. Thus the larger particulate matter of the interstitial water seems to be of low phosphorus content.

Figure 2. Weather data, 1971. Air temperature and cloud cover from records of U.S. Forest Service, Watersmeet, Michigan. Precipitation from rain gauge located on float in center of North Gate Lake.

STABLE PHOSPHORUS OF MAT

INTERSTITIAL WATER AT VARIOUS DEPTHS

(TRANSECT VI)

□ SOLUBLE REACTIVE (SRP)

■ SOLUBLE UNREACTIVE (SUP)

Figure 3. Stable phosphorus at various depths in bog mat and at various distances from lake-mat interface, July 25-August 8, 1970.

STABLE PHOSPHORUS OF MAT

INTERSTITIAL WATER (1971)

(0-0.1 METER DEPTH)

- ☐ SOLUBLE REACTIVE (SRP)
- ☒ SOLUBLE UNREACTIVE (SUP)
- ☒ PARTICULATE- $>0.45\mu$, $<153\mu$ (PUP)

Figure 4. Stable phosphorus of interstitial water of mat transects, August 5, 1971.

The phosphorus content of the interstitial water increased with increasing distance from the lake-mat interface (Figs. 3, 4). At a site 14.6 m from the lake, the soluble phosphorus component alone was in excess of 1000 ppb at the lake-mat interface (Fig. 3). The relative abundance of the three phosphorus components also changed with distance. At the mat-water interface, a major fraction of phosphorus consisted of SRP, as in the open lake. The PUP fraction increased with increasing distance from the edge of the mat (Fig. 2). SUP was present adjacent to the open water and at intermediate stations (up to 8 m from the water) but was absent at greater distances (Figs. 1, 2). The SUP fraction extended farther shoreward in some transects than others and was variable in quantity. It also decreased with increasing sample depth below the surface (Fig. 3). As the SUP component decreased there was an increase in the PUP and SRP fractions in the profiles. At depths greater than 0.4 m in 1970 all of the soluble phosphorus component was SRP at a distance of 4 m and beyond from the lake (Fig. 3). The SUP component not only decreased with increasing depth within the mat, but in the deeper layers of the mat it appeared only at stations adjacent to the open water. This distribution of SRP and SUP in the mat is similar to that in the open water in that SUP was present in the aerated zone, whereas SRP was absent in aerated water but abundant in anaerobic zones.

ACTIVITY FRACTIONS OF MAT INTERSTITIAL WATER

To further identify the form of the activity after entering the mat interstitial water, we assayed the activity in the hexanol extracts, SRP, and PUP fractions of the mat water on each of the three transects on June 19 and August 5. On June 19, fractions were measured at Station A; on August 5, activity fractions were measured at Stations A, B, and C (Table I). Labeled fractions were roughly in the same proportions as the corresponding stable fraction. In most samples, the PUP fraction made up the major percentage of the activity (55 to 92%). The SUP made up 7 to 13% of total activity on June 19. However on August 5 on Transects I and II, it made up a larger fraction of the total activity (30 to 100%). Very little of the activity was in the hexanol extract (SRP). In many instances this fraction was undetectable or comprised less than 2% of total activity. It was most abundant at stations farthest from the open water (Station C) on all transects, as was stable SRP. On August 5 at Stations B and C of Transect II, particulate activity was absent, indicating that the label had passed through the biota and been excreted as SUP or SRP. On August 5 on Transect III the quantity of SUP did not differ significantly between Stations A, B, and C but there was a significantly larger quantity of PUP activity at Station C than at Stations A and B. This indicates that uniformity had been achieved by movement of the soluble phases through the sections of the mat. Particulates however appeared to be less mobile.

Table I. Activity fractions of mat interstitial water after labeling with ^{32}P , 1972. Percentages are of total activity at station. Activity values corrected for decay.

Date and Station	Hexanol extract		Soluble unreactive		Particulate $>0.45 < 153 \mu$	
	Mean pci/ml	Percentage	Mean pci/ml	Percentage	Mean pci/ml	Percentage
Transect I						
June 19						
A	0.06	0.10	5.45	13.0	55.8	86.9
Aug. 5						
A	*		2.2	45	2.7	55
B	1.0		0		**	
C	0.59		8.1		**	
Transect II						
June 19						
A	*		4.4	7.0	58.3	92.9
Aug. 5						
A	*		2.36	41	3.5	59
B	*		1.2	100	0.0	0
C	8.7	70	3.7	30	0.0	0
Transect III						
June 19						
A	1.6	0.3	40.0	7.1	521	92.6
Aug. 5						
A	0.83	1.8	11.3	25.0	33.0	73
B	*		12.1	28.9	29.8	71.1
C	1.1	1.0	10.1	9.0	101.5	90

*Count not significantly above background.

**Fraction not collected (site with insufficient interstitial water).

The activity data are in general consistent with the distribution pattern observed for the stable phosphorus components of the interstitial water. The specific activity data (Table II) indicate that the activity entered large pools of the particulate phosphorus and dissolved organic phosphorus adjacent to the lake water on Transects I and II but not III. At greater distances from the mat-water interface, particulate, and SUP activity was replaced to some extent by SRP activity.

Table II. Specific activity (pci/ml) of mat interstitial water, August 5, 1971.

Fraction	Transect	Station A	Station B	Station C
Soluble Reactive	I	a	5.5	6.3
	II	b	b	8.1
	III	267	b	5.0
Soluble Unreactive	I	73	a	a
	II	78	20	a
	III	26	b	a
Particulate	I	20	---	---
	II	28	0.0	0.0
	III	733	289	117

^aFraction not present

^bActivity not above background

LAKE-BOG-MAT EXCHANGE

Sampling of the open water station (B), three mat-water interface stations (A, C, and D) and two control stations (E and F) at 4-day intervals enabled us to follow inputs of labeled phosphorus from the mat into the lake water. Little activity appeared in the open water immediately after the first labeling (May 30). Traces of soluble material were detected adjacent to the mat-water interface (Stations A and C) and a small amount of activity was detected in the open water station (Station B). A cold front passage on May 30 brought falling temperatures and light rain (Fig. 2). This precipitation carried activity into the lake (Fig. 5). On June 2 there was a large pool of soluble activity at interface stations A and C as well as a large concentration at 6 m at Station B. Input during the period May 30 to June 2 was recorded at

Figure 5. Activity of open water (Station B) and at mat-water interface stations (A, C, D, E, F) after mat labeling experiments in 1971.

interface stations A and C but not at D. A concentration appeared in the bottom water at Stations B and E. The appearance of the local concentrations of activity at the 6 m depth at Station B and the smaller amounts at Stations C and E indicate that activity penetrated to the upper level of the monimolimnion and spread over this surface. The large differences between the interface stations and the open water on June 2 indicate that mixing in certain parts of the basin was limited. Samples of June 6 (Fig. 5) showed mixing of activity within the water mass. Activity recorded at Station B on June 2 had now spread throughout the bottom water. Within the epilimnion, activity appeared for the first time at interface Stations D and F. There was little further leaching of activity into the basin from this treatment. After June 6 the total quantity detected in most profiles was lower but there was less heterogeneity in distribution within the basin. Precipitation on June 7 did not appear to leach additional activity from the mat but brought about additional mixing and eliminated traces of activity in deeper strata at some of the marginal stations.

Although there was considerable vertical and horizontal heterogeneity in the basin through the June 6-22 sampling period, the total quantity of activity in the water column was not significantly different on the five sampling dates either at the open water station or for the pooled data for the marginal stations. Thus the small quantity of SUP leached into the lake between May 30 and June 2 seems to have persisted unchanged for a period of three weeks. In fact, it may have persisted until July 12 as the small dissolved fraction present after entrance of the particulate activity following the second tracer application.

Application of the activity to the interstitial water of the mat on June 24 brought about an entirely different pattern of activity input to the open water. Two days after this treatment (June 26) particulate activity appeared at all stations. The largest inputs were at Station B and at the interface stations on the labeled side of the lake (Fig. 5). After labeling, but before the June 26 sampling date, a heavy rain fell on the mat surface. Much of this occurred on June 24 immediately after the application of the tracer. This rain caused flooding of the mat and flushed much of the introduced activity into the lake.

Adding the labels directly to the water table labeled a different fraction than was labeled by sprinkling labels on the Sphagnum. On June 26, from 75 to 90% of the activity was filterable by a 0.45μ membrane and was not removed from particulates by washing membranes with $0.01 \text{ N H}_2\text{SO}_4$. This indicated that the activity was taken up by microorganisms upon injection into the interstitial water. On June 30 the particulate activity leached from the mat had spread over the entire lake surface.

Between June 30 and July 12, activity patterns remained much the same at Station B and at the other interface stations on the labeled side of the lake

(Stations D and C). There was a slight but statistically significant increase in the particulate activity at Station D over that at Stations A, B, and C. Of greater interest, however, is the appearance of soluble activity both at surface and subsurface depths between June 30 and July 16 at control Stations E and F. These stations are located downwind from the treated area and receive large quantities of surface drift water when there are strong westerly and northwesterly winds. The soluble activity was first recorded at 6 m at Station E on June 30, but appeared throughout the water column at Stations E and F on July 8. The latter samples were collected after strong westerly winds and an observed drift of surface water into the mat-water interface zone on the east side of the lake.

A strong surface flow such as this brings about mixing of the warmer surface water with the cold anaerobic water of the mat. This intermixing then permits labeled particles to sink below the strong thermal barriers in the open water of the lake and enter the hypolimnion. In the process of mixing with the cold anaerobic water at the mat-water interface, particulate phosphorus appears to be solubilized and thus enters the hypolimnion as SUP. Thus physical mixing at the mat-water interface zone appears to be a mechanism whereby epilimnetic phosphorus is moved into the hypolimnion as SUP and does not require the sinking of particulate matter through the concentration zone and the density barrier of the thermocline (Fig. 6). Similar penetration of SUP to the 3- and 6-m depth at Station E were observed immediately after the first labeling (June 2) and between June 14-18. Density currents arising from inputs of cold mat water at the interface zone may not always penetrate to the lower hypolimnion but may at times be injected into the thermocline or hypolimnion at intermediate depths. This has been indicated by thermal and chemical data in other years.

Changes in the form of activity in the water column between July 8 and July 12 at Stations E and F also provide data on the rate of conversion of SUP to PUP. On July 8, interface stations E and F had approximately 10 cpm of SUP at the surface and 4 to 5 cpm at 2 and 3 m. On July 12, the surface activity at Station E had increased and was much the same as at Station B and other interface stations. However this increase reflects nothing more than a movement of surface water into the interface zone. The remaining part of the water column at Station E showed the same distribution of activity in the water column as on July 8; however on July 12 all of the activity was in particulate form. Similar changes occurred at 2 and 3 m at Station F. These changes within the mat-water interface environment indicate that there was conversion of SUP to SRP and subsequent uptake in particulate form within a 4-day period. Although there was a rapid transformation of the drift particulate material in the interface area on the downwind side of the lake, there is little indication of similar transformation elsewhere in the system. There were no statistically significant differences at the 0-, 2- and 3-m depths in the quantity of either particulate or soluble components at the open water site (Station B) and at other interface sites (A, C, D) during the period

Figure 6. Marginal circulation scheme as visualized for a west-to-east transect across the lake (interface Stations D to F).

between June 26 and July 16, and a significant quantity of activity did not appear in deeper strata. Thus particulate activity carried into the lake after the second labeling on June 26 was not mineralized or lost by sedimentation but remained in the surface water for a period in excess of 14 days. This finding suggests remarkable chemical stability and resistance to biological breakdown under the environmental conditions present in systems of this type.

The comparatively high climatic stability during the period between July 8 and July 12 (Fig. 2) was interrupted by the passage of a strong frontal system on July 12 after the time of sampling. Wind velocities up to 55 mph were recorded and there was heavy precipitation (0.72 cm). Rain and windy weather continued with falling temperatures for approximately 4 days. An additional 0.3 cm of precipitation was recorded. This period of unsettled weather with heavy rain and wind had a decided input influence upon the distribution of labels within the system. These were as follows: (1) There was a large input of SUP from the labeled area adjacent to the upland (Station A) in the surface, 2- and 3-m samples (Fig. 5). This increase also appeared at the open water area (Station B) at the surface and at 6 m. These data also indicate that the marginal mat input frequently penetrates to the upper surface of the monimolimnion. (2) Data of July 16 indicate that vertical mixing during this period (Fig. 7) effectively dispersed and reduced the persistent concentration of particulate activity in the marginal interface locations (Stations C, D, E, F). Unstable weather continued between July 16 and 22, and there appeared to be a continued input of SUP from the mat at Stations A and C. This led to a buildup in the open water at the surface and at 2 and 3 m (Fig. 5). During the period from July 12 to 16, the particulate fraction previously introduced into the mat disappeared at all locations except Station F.

EFFECT OF LIME TREATMENT UPON DISTRIBUTION OF LABELS

Treatment of the lake with agricultural lime on July 22 again brought changes in the distribution of activity and confirmed observations from previous treatments that lime brings about a release of both soluble and particulate phosphorus from the mat system. On July 30, a large input was recorded from the labeled side of the lake (chiefly Station A). This input appeared to be both PUP and SUP. At the open water station a large quantity of PUP appeared but the SUP fraction was only slightly greater than recorded on July 20. Between July 23 and July 30 there was an additional 0.22 cm of rain. The greater input at Station A compared with other interface stations indicates that the phosphorus released by lime was flushed into the lake from areas where there was hydrologic gradient after precipitation. The increase in SUP after addition of the lime appears to be in situ conversion of phosphorus within the interface zone. This was indicated at interface Station D, a section of the interface zone which normally receives very little input from the mat. On July 20 this station had only SUP, but after lime treatment had only PUP. Thus it would appear that lime caused microbiological breakdown of SUP which in turn

Figure 7. Temperature and rhodamine WT in North Gate Lake in 1971.

was followed by release of SRP and uptake into particulates.

FATE OF LABELS IN MAT

On May 30, samples from the mat sampling reservoirs collected approximately 4 hr after application showed high activity levels at all stations except B and C of Transects I and II (Fig. 8). At B and C, activity was only slightly above background, indicating that little of the tracer had moved into the reservoirs and a lack of uniformity in labeling at these locations. The day following treatment (May 1) there was a sharp decrease in activity at all stations on Transect III and at Station A on Transects I and II. After May 1, the rate of loss of activity at the A station of each transect and at all stations of Transect III was considerably less. By June 9-13, the loss rate curves become nearly asymptotic with the X axis, indicating equilibration with phosphorus of the mat system. Superimposed upon these curves, however, are fluctuations which appear to represent advection of labeled water through the mat. For example, the June 13 activity levels on Transect III were all significantly higher than the levels on June 9. This was true also for Stations B and C of Transect I and II, and indicate widespread water movements within the mat.

Activity curves indicate that the three transects responded quite differently following treatment as regards retention and movement of activity. These differences appeared to be largely the result of differences in the slopes of phreatic surfaces following rain on various sections of the mat watershed. Inputs following labeling at interface Station A (Fig. 5) as well as data from the transect itself (Fig. 8) indicated that there was rapid flushing of activity after each rain into the lake on Transect I where the slope was greatest due to the adjacent upland. Rapid flushing was also apparent on Transect II (Fig. 8) and is also reflected in the inputs to the lake measured at interface Station C (Fig. 5). There was, however, little or no flow to the lake from the sections of Transect III nearest the lake following precipitation on the watershed. This could be predicted since this station is near the center of the primitive lake basin and rains would have little influence on the hydrologic gradient at this point. Thus there was relatively high retention following each application of tracer.

Retention by the mat differed markedly between the first and second treatments. On Transect II there was no indication of an activity input following the second labeling. On June 24, 1 day after treatment, activity levels were below levels encountered on June 21 at all three stations. This pattern also appeared at Station C of Transect I and Station C of Transect III. Only the A stations of Transects I and II showed significant increases after the second labeling. There was an input at Station A of Transect III, but the amount was much smaller than that observed after the first labeling. Activity levels fell at a slower rate at these two stations following the second labeling than

Figure 8. Activity of interstitial water from collection reservoirs after labeling mat with ^{32}P on May 30 and June 24, 1971.

after the initial treatment. The activity level at Station A of Transect III was 350 cpm 24 hr after the second treatment, compared to the earlier value of 22,394. At Station D of Transect I, levels were only 15 cpm after the second treatment, compared to 140 after the first labeling. There was a slow decrease at both of these stations until July 3, followed by a more rapid fall in activity until July 11. Between July 11 and 15, activity levels increased at not only these two stations, but also at Stations A of Transect II and B of Transect III. These increases indicate advection of activity in the mat and coincided with the input of SUP observed in the open lake (Station B) and at interface Station A on July 16 (Fig. 5). Continued advection of activity in the mat was indicated by the increase in activity at Station A of Transect II between July 19 and 29. This may have been activity mobilized by the action of lime.

The two treatment methods resulted in major qualitative as well as quantitative differences in the phosphorus retained within the mat as well as that which entered the lake water. There was much greater retention in the first treatment when the ^{32}P was applied directly to the surface of the Sphagnum. In this case there was slow leaching of SUP into the interstitial water. The rate of leaching was low on Transect III because of the lack of horizontal flow through the mat. The effect of the input from the second treatment was not observed on Transect II, and was minor on Transect I because the tracer was applied directly to the phreatic surface and then was flushed into the lake by the 0.32 cm rainfall which followed application of the label on June 24. Direct application of the isotope to the phreatic surface did not permit adsorption and uptake by Sphagnum and equilibration with the organic phosphorus compounds arising from Sphagnum. Hence the labeled phosphorus did not appear as organic leachings but was taken up by microbiota of the interstitial water or was discharged into the lake as particulates or entered as SRP and was taken up by phytoplankton.

Discussion

MARGINAL CIRCULATION AND THE PHOSPHORUS CYCLE

The marginal circulation scheme proposed above (Fig. 6) offers a pathway whereby both autochthonous seston and allochthonous debris can penetrate through the strong thermal barrier present in the lake during the spring and summer period. This type of circulation is made possible in bog systems because of storage of cold water in the mat (often at least 10°C colder than the water of the epilimnion). Earlier labeling experiments have indicated that during the growing season most of the phosphorus present in the seston of the epilimnion passes by way of sedimentation into the thermocline (concentration zone) and does not penetrate into the hypolimnion (Hooper, 1969, 1970, 1971). The

marginal circulation pattern permits a limited amount of sedimentation of epilimnetic debris into the hypolimnion directly, but it is perhaps more significant in the cycling of phosphorus debris and leachates from the bog mat system.

Phosphorus entering the bog-bog lake system by way of runoff appears to first be converted into SRP in sections of the mat distal from the open water (Fig. 3). Areas of the mat high in SRP are areas that periodically are dried and reflooded; thus there are opportunities for aerobic breakdown of organic phosphorus debris. Rain and flooding of the mat permit movement of SRP toward the lake basin. In transit to the lake surface this phosphorus is probably recycled many times via the biota from SRP to PUP to SUP and again to SRP before arriving at the mat-water interface. Adjacent to the lake, the Sphagnum mat is continually wet. There is greater microbiological activity so that SRP is never present (Figs. 3, 4). The allochthonous input at the interface consists of both SUP and particulates produced by the plant systems of the mat. Peripheral circulation permits breakdown of these stable compounds arising from the mat within the hypolimnion.

Data from the two labeling experiments also indicate that there is considerable in situ conversion of particulates to SUP throughout the water column of the interface zone. The marginal circulation scheme proposed above also appears to explain the appearance of organic compounds characteristic of the mat within the hypolimnetic water without appearance in the epilimnion (Koenings and Hooper, this report).

The morphometry of the lake basin reflects this peripheral sedimentation pattern. At the east corner of the lake is an extensive flat shelf at the 6-m depth, indicating deposition of sediments on the downwind extremities. On the other hand, on the west extremity from the mat surface there is an almost vertical drop of 26 ft to the maximum depth. Sedimentation of materials upon the shelf followed by regeneration of solutes and transport by density currents moving over the shelf may lead to their concentration in the monimolimnion (below 6.5 and 8 m), and thus may be the underlying cause for the persistent meromixis.

Regeneration of orthophosphate is high within the monimolimnion because of the higher pH and greater concentration of calcium and magnesium. Partial circulation of this stratum at the time of the spring overturn each year transports SRP upward in the water column and permits upwelling later in the summer into the trophogenic zone (Hooper, 1971). Thus the marginal circulation pattern brings about the transfer of mat phosphorus into the monimolimnion and thereby increases the stores of phosphorus available for the spring overturn and upwelling during the summer. It is clear from our earlier reports as well as from the published reports of Coffin, et al. (1949), that during upwellings as well as during the spring overturns a large fraction of the regenerated SRP is returned to the mat system. Thus there is, in fact, a circulation

regeneration cycle between the mat and the lake basin. However the long residence time in the bottom water makes this cycle inefficient in providing phosphorus for the trophogenic water compared to the littoral zone of stratified lakes.

CLIMATIC EFFECTS ON BOG METABOLISM

The above data on distribution of mat labels and their persistence in the circulation of the systems tend to reinforce previous data which emphasize the major role of weather phenomena in regulation of production in this bog system. Earlier reports have emphasized the role of weather in the upwelling of phosphorus residues accumulating in the deeper water and their biological effects upon the system. In the 1970 experiment in which the bottom water was labeled and in which we followed the loss of the total quantity of ^{32}P activity added to the basin we demonstrated that total quantity of activity measured in the water fell during warming trends and during periods of stable weather. On the other hand, much of the phosphorus that had disappeared from the water reappeared when there were water movements after periods of heavy rains and when there were falling temperatures.

Present data indicate that weather phenomena (1) regulate the input of mat phosphorus resources into the basin, and (2) regulate their residence times. Residues from the mat persist with little loss or transformation until periods of strong water movements which bring about their displacement and regeneration.

Summary

An area of Sphagnum adjacent to the open water of a bog lake was labeled with ^{32}P two times during the summer of 1971. In the first labeling experiment, the isotope was applied directly to the Sphagnum surface. This produced a leachate consisting entirely of soluble unreactive phosphorus which was flushed into the lake when rains created a hydrologic gradient of flow within the mat. This leachate not only penetrated the surface water, but also entered the hypolimnion. The second label, which was injected directly into the interstitial water of the mat, produced a leachate which was an entirely particulate unreactive phosphorus. This was also flushed into the lake by the action of the rain and persisted in the surface water with little or no loss or transformation for a period in excess of 3 weeks. There was a third input into the lake of the tracer but consisting of both SUP and PUP following a heavy rain approximately 4 weeks after the second treatment. Both of these label fractions penetrated to the lower parts of the hypolimnion by sinking as a cold current in the mat-lake interface zone.

Literature Cited

- Coffin, C. C., F. R. Hayes, L. H. Jodrey, and S. G. Whiteway. 1949. Exchange of materials in a lake as studied by the addition of radioactive phosphorus. *Can. J. Res.*, 27:207-222.
- Griffing, T. G. 1970. Nutrient cycling and productivity of dystrophic lake-bog systems. Technical Progress Report, submitted to the Atomic Energy Commission.
- Hooper, F. F. 1969. Nutrient cycling and productivity of dystrophic lake-bog systems. Technical Progress Report, submitted to the Atomic Energy Commission.
- Hooper, F. F. 1970. Nutrient cycling and productivity of dystrophic lake-bog systems. Technical Progress Report, submitted to the Atomic Energy Commission.
- Hooper, F. F. 1971. Nutrient cycling and productivity of dystrophic lake-bog systems. Technical Progress Report, submitted to the Atomic Energy Commission.
- Strickland, J.D.H., and T. R. Parsons. 1965. A manual of sea water analysis. *Fish. Res. Bd. Canada, Bull.* 125:
- Stephens, K. 1963. Determination of low phosphate concentrations in lake and marine waters. *Limnol. Oceanogr.*, 8(3):361-362.

APPENDIX III

IONIC STATE AND COORDINATION OF IRON IN BOG LAKES

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IONIC STATE AND COORDINATION OF IRON IN BOG LAKES

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Because of their high content of particulate and dissolved organic matter, low pH and extremes of Eh, bog lakes present a complex system for determination of the physical and chemical state of iron. Sampling difficulties and analytical problems have created some uncertainties in our understanding of the ionic state and type of coordination which exists at specific depths in the water column. The purpose of this report is to clarify some of these uncertainties and to more precisely define physical and chemical states of iron in these environments.

Inorganic iron is considered to 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). In natural waters, the most common species of ferric iron is ferric hydroxide, and the most common species of ferrous iron is the simple ferrous ion (Hem and Cropper, 1959). Since ferric hydroxide has a very low solubility, dissolved ferric iron exists in solution as $\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})^{++}$, and Fe^{+++} . In addition, ferric iron has a strong tendency to form organic as well as inorganic complexes, which increase its solubility (Hem and Cropper, 1959; Stumm and Lee, 1960). Ionic ferrous iron has weaker complexing properties and forms few complexes; however, its solubility, under favorable pH and Eh conditions, is much greater than ferric iron (Hem and Cropper, 1959; Stumm and Lee, 1960). Judging from solubility relationships, Cooper (1937) believed that in sea water (pH 6-9) dissolved iron exists predominantly in the Fe^{++} and the $\text{Fe}(\text{OH})^{++}$ state and in other natural waters he believed it to be in equilibrium with $\text{Fe}(\text{OH})_3$.

These relationships have been delineated by solubility studies under well-defined conditions of Eh and pH in laboratory systems. The lack of a truly specific reagent for ferrous iron prevented the uncoupling of the ferrous-ferric iron equilibrium in natural waters. With the introduction of bathophenanthroline (BPN) as a specific reagent for ionic ferrous iron, this problem was alleviated. However sampling procedures and the necessity for preservation with subsequent digestion of samples, permitted the measurement of only total ferrous and total iron; ferric iron was obtained by subtraction. The form of the dissolved ferrous and ferric iron in solution remained undetermined, although it is now known that ferrous iron can exist in some form in both oxygenated and deoxygenated waters (McMahon, 1969). The later phenomenon was observed in the early studies of Hutchinson (1941), but was attributed to poor techniques.

In dystrophic acid bog lakes, which are highly colored, conditions exist which alter the normal solubility relationships of iron. Low dissolved oxygen levels, high CO_2 , low pH's, and the presence of reduced organic and inorganic compounds all favor formation of ferrous iron. Decomposing organic residues (including "humic acid") readily reduce ferric iron to ferrous iron or form ferrous-organic complexes (Bloomfield, 1952). Both of these changes increase the solubility of iron (Stumm and Lee, 1960). Shapiro (1966a) demonstrated the reducing power of colored organic acids in the presence of ferric iron in natural waters and Hem (1960) demonstrated the reducing power of tannic acid toward ferric iron in artificial laboratory waters. Finally, sulfur (-II) compounds (HS^- , cysteine) as well as a variety of organic substances can reduce ferric iron (Stumm and Morgan, 1970). The solubility of iron in the ferrous state, therefore, should be enhanced in a highly colored acid (pH 4-5) bog lake water. Shapiro (1964) showed the increasing ability of organic acids to hold oxidized iron in solution at pH levels greater than 5 as peptized sols of $\text{Fe}(\text{OH})_3$. Because of the presence of only ferric iron in his systems, he rejected the previously suggested mechanism for increasing iron and manganese solubility (Hem, 1960; Rawson, 1963), namely, iron being brought into solution by reduction to the divalent state with or without subsequent complex formation. However, both mechanisms may be operating in different strata in the lower pH condition found in bog lakes.

Low pH decreases the ionization of organic acids, enables the rate of oxidation of iron to become independent of pH, and greatly increases the solubilities of all ionic forms of iron. These effects, combined with low oxygen tensions and a highly reducing environment, should permit large amounts of ionic iron to be present without complexation. Under these conditions, complexation of ferrous and ferric iron by organic acids may be altered from those previously reported.

In the summer of 1972, I attempted to separate and characterize the iron compounds present in the epilimnion and in the hypolimnion of an acid bog system (North Gate Lake) and also attempted to assess the effects of reduced organic compounds on the forms of iron. A new sampling method, classification scheme, and reaction scheme were devised to enable the measurement of the forms of dissolved ferrous and ferric iron without interferences. Finally, the naturally existing physical state of iron at various depths within the basin was determined in an in situ radiochemical study on a column of lake water contained in a polyethylene cylinder.

Methods

The forms of iron actually present in natural waters has never been determined because of the presence of both oxidation states simultaneously and

because of the presence of organic matter capable of forming masking complexes with both Fe(II) and Fe(III). This was partially overcome by the introduction of organic reagent bathophenanthroline which forms a highly specific red complex with the Fe(II) ion even in the presence of Fe(III) (Shapiro, 1966a; Gosh, *et al.*, 1967; O'Connor, *et al.*, 1965; Lee and Stumm, 1960). Because Fe(II) is so unstable in the presence of oxygen, preservation of samples was necessary. Preservation with acid has had the side effects of reducing Fe(III) to Fe(II), releasing iron from organic complexes and dissolving inorganic ferrous compounds (McMahon, 1967). Also, sunlight interfered with the determination of ferrous iron by photochemical reduction of Fe(III) (McMahon, 1967) as did the presence of particulate iron and bacteria. McMahon (1969) used anaerobic *in situ* filtration (utilizing individual 10 ml samples) to reduce the errors in Fe(II) determinations due to particulate iron, bacteria, and dissolved oxygen. The presence of Fe(II) compounds in the oxygenated waters of the epilimnion was ascertained, yet the addition of a preservative (acid) and the brief exposure of the samples to light while transferring the sample to the storage container were still necessary.

To circumvent the necessity of adding acid to the samples, a containerized, anaerobic, *in situ* filtration system was devised which could yield 200 ml of a 0.45 membrane filtered sample (Fig. 1). Individual airtight, light-proof containers were used at each depth sample. The samples were filled with inert helium (99.05%) and isobutane (0.95%) at the laboratory and transferred in a closed, slightly-pressurized condition (by stopcocks) to the lake. When a depth was sampled, the appropriate closed container was attached to the 0.45-membrane filtering apparatus and to the filtering line. The sampling apparatus was submerged into the water and the stopcocks opened. The slight pressure of the gas removed any air in the lines leading to the membrane and to the vacuum pump. The container was then lowered to depth and filled with sampled water by vacuum filtration. The stopcocks were closed while the apparatus was submerged to prevent the introduction of oxygen. The sampler was returned to the laboratory, still under reduced pressure, and a gas-filled balloon was fitted to an upper stopcock. The stopcock was then opened, depressurizing the sampler. This allowed the sample to remain anaerobic during the removal of aliquots from the bottom of the chamber.

I performed four analyses on duplicate 25-ml aliquots from a series of depths. Analysis 2 gave a measure of total soluble ferrous iron, and analysis 4 gave a measure of total soluble iron (Golterman, 1969). In addition, two further analyses which only had significance when applied to a sample without acid preservative, were applied to the series. Analysis 1 consisted of the addition of bathophenanthroline (BPN) directly to a sample (pH 4-5) followed by the regular color development and hexanol extraction procedure (Golterman, 1969). This measured ionic ferrous iron. Analysis 3 consisted of adding a reducing agent (hydroxylamine hydrochloride) to the sample, then adding BPN, followed by the color development and extraction procedures. This measures total dissolved inorganic iron.

Fig. 1. In situ filtration system used to collect 0.45 μ membrane anaerobically filtered samples from four strata of North Gate Lake.

Since acid dissolves insoluble ferrous salts (O'Conner, et al., 1965) and releases Fe(II) from organic complexes (McMahon, 1969) and reacts similarly toward Fe(III) (Shapiro, 1966a, 1967b) the addition of BPN without acid should measure the ionic forms of iron in natural waters. Therefore this system of taking unaltered samples allowed the application of a characterization scheme which could delineate between ionic and complex forms of iron II and III. The term complex describes iron both inorganic and organic not in the ionic Fe^{+2} or the Fe^{+3} state.

In summary the characterization scheme was as follows:

- analysis 1 = dissolved inorganic ionic ferrous iron
- analysis 2 = dissolved total ferrous iron
- analysis 3 = dissolved total inorganic iron
- analysis 4 = dissolved total iron

Results from these four analyses were used as follows in calculation of other fractions:

- analysis 1 = ionic ferrous iron
- analysis 2 - analysis 1 = complex ferrous iron
- analysis 3 - analysis 1 = inorganic dissolved ferric iron (result A)
- analysis 4 - analysis 2 = total dissolved ferric iron (result B)
- result B - result A = complex ferric iron

A series of samples was taken on July 19 and again on July 21 using in situ anaerobic filtration at four different depths in the center of the lake: 0-2 cm (surface), 2 m, 5 m, and 7.5 m. Subsequent to this, an additional series of samples was taken on July 24 and again on August 24 using normal collecting procedures. These samples were transported back to the laboratory with minimal exposure to light or atmospheric oxygen and were then filtered through 0.45 membranes in the presence of oxygen. Finally, solutions of a known concentration of iron were prepared utilizing ferrous ammonium sulfate, ferric ammonium sulfate, and ferric chloride.

The above characterization scheme was first applied to the standards to determine if the method could successfully differentiate between the various forms of iron. If the chemical form of the standards matched what was obtained from the proposed classification with minimal contamination of the other categories, it would support the application of the system to the compounds in natural waters. The scheme was then applied to the anaerobically in situ filtered samples. The distribution of iron species in the lake with depth was therefore determined without any interferences that might upset the naturally existing ferrous-ferric iron equilibria. Finally, the method was applied to the aerobically filtered samples to decipher the effect of oxygen on the iron in the samples, and to delineate the appearance of any oxidation products of iron.

An in situ radiochemical experiment using Fe^{59} was performed to determine the physical state of iron in natural waters. Fe^{59} , as FeCl_3 , was added to the surface of a column of lake water contained in a polyethylene tube, and its movement with depth and time was recorded. A polyethylene bag 3 ft in diameter and 8 m deep was constructed with sampling tubes at every meter. These allowed samples to be taken from the center of the bag at every meter without disturbing the water column. Vacuum bottles were used to draw samples to the surface through attached tygon tubing. The volume of water equal to that of the tubing was discarded prior to sampling. The samples were taken back to the laboratory, filtered, and the total activity and soluble activity obtained by drying 5 ml duplicate subsamples on previously monitored planchets, and then measuring the activity on a low background Beta counting system. In addition, dialysis bags were placed in the bag at 1-m intervals prior to the addition of the label. These were sampled (in triplicate) after 5 days for activity by using 5-ml hypodermic syringes, and then evaporating the samples on monitored planchets. The physical state of the iron was determined by comparing the activity in the total, soluble total, and dialyzable fractions.

Results

Ferrous ammonium sulfate, prepared as a ferrous iron standard, gave a reaction as ionic ferrous iron in the characterization scheme (Table I). Small amounts of $(\text{Fe}^{+2})_c$ was the only other form of iron appearing in the standard (Table II). Results, therefore, were as expected. A solution of ferric ammonium sulfate which went into solution slowly appeared chiefly as a complexed ferric iron compound (82%) and almost completely as Fe(III) (99%). (Tables I and II.) Finally, a ferric chloride solution gave a reaction as an inorganic complexed ferric iron (85%) and nearly 90% as Fe(III) (Tables I and II). The ability of ferric chloride to form undissociated inorganic complexes is well known (Cooper, 1937; Stumm and Lee, 1960; Rabinowich and Stockmayer, 1942); therefore its behavior is as expected. The characterization scheme yielded results consistent with the predicted chemical behavior of the compounds dissolved in water with an average contamination of unpredicted categories of 1.8%. Thus it was felt that the scheme could be used operationally to classify the iron compounds found in North Gate Lake.

The application of the characterization scheme to the anaerobically in situ filtered samples yielded forms of iron that reacted as both Fe(II) and Fe(III) (Table III). The chemical state of iron present in the lake varied considerably in form and amount with depth (Table IV). However, the levels of dissolved iron obtained are in the range of the highest values for unfiltered samples determined in 11 of 74 northeast Wisconsin lakes studied by Juday, et al., (1938).

Table I. The quantity of iron recovered from known solutions using four analysis procedures.

Standard	Dissolved iron (ppb)				Total iron added
	Inorganic ferrous	Total ferrous	Total inorganic	Total iron	
Ferrous ammonium sulfate	200.0	202.9	195.5	200.0	200.0
Ferric ammonium sulfate	0.0	2.4	48.4	400.0	400.0
Ferric chloride	15.1	43.4	30.2	400.0	400.0

Table II. The distribution of iron in the known solutions obtained from the characterization analysis by calculation.

Standard	Dissolved iron (ppb)				Total iron added
	Fe ⁺²	(Fe ⁺²) _c	Fe ⁺³	(Fe ⁺³) _c	
Ferrous ammonium sulfate	200.0	2.9	0.0	0.0	200.0
Ferric ammonium sulfate	0.0	2.4	48.4	349.2	400.0
Ferric chloride	15.1	28.3	15.1	341.5	400.0

Table III. The quantity of iron recovered from four strata of North Gate Bog using the four analysis procedures on anaerobically in situ filtered samples.

Depth	Dissolved iron (ppb)			Total iron
	Inorganic ferrous	Total ferrous	Total inorganic	
Surface	73	119	193	265
2 m	426	462	509	594
5 m	656	659	698	699
7.5 m	1199	1203	1197	1282

Table IV. The distribution of ionic and coordinated iron in the four strata of North Gate Bog obtained by calculation from the characterization analysis on anaerobically in situ filtered water.

Depth	Dissolved iron (ppb)				Total
	Fe ⁺²	(Fe ⁺²) _c	Fe ⁺³	(Fe ⁺³) _c	
Surface	73	46	119	26	265
2 m	426	36	84	49	594
5 m	656	4	8	32	699
7.5 m	1199	4	0	79	1282

Total iron increased from 265 $\mu\text{g}/\ell$ (ppb) at the surface to 1282 ppb in the hypolimnion. Ionic ferrous iron increased at the same depths from 73 ppb (28% of total iron) to 1199 ppb or 94% of total iron. As you would expect from these data, oxygen was absent below 2 m which enabled the iron to be present almost exclusively as ionic ferrous iron. Ionic ferric iron is present in significant quantities only at the upper two depths, and at the surface is present at a level of 119 ppb or 45% of the total iron at this depth. Of extreme interest is the lack of any complexed forms of Fe(III) at any depth sampled. These values range from 26 ppb to 79 ppb but represent only less than 10% of the total iron. At the surface of the epilimnion, 55% of the iron is in the ferric state compared to 6% of the iron in this state in the hypolimnion. In addition, ionic iron (including both Fe(II) and Fe(III)) rose in the lake from 73% in the epilimnion to 95% in the hypolimnion. This leaves only from 27% to 5% as the amount of inorganic or organic complexed iron in these two strata. These results are in apparent contradiction to the complexed nature of colored organic acids and Fe(III) in pH 6-8 oxygenated waters as discussed by Shapiro (1964) and Ghassemi and Christman (1968). However, in these low pH (4-5) surface waters the ionic forms of Fe(III) are preferred (Hem and Cropper, 1959), and because of the protonation of ionized groups on the acids, complexation is reduced (Shapiro, 1964). In these waters then, environmental conditions exist which allow iron to remain chemically in an ionic state. However, as will be seen from the radiochemical data, this is not a free ionic Fe(III) state.

Since most of the iron in highly colored bog lakes is filterable (Shapiro, 1966b), the fraction analyzed in this study should include most of the iron present. The predominant forms of iron in the bog waters are forms reacting as ionic Fe(II) and Fe(III). In the hypolimnion, the reduced organic matter and H_2S seem to have the ability to reduce but not complex iron. The predominant form of Fe(II) indicated by solubility equations for this pH range is ionic Fe(II) as found in my measurements. The upper surface waters appear to have enough dissolved oxygen (4-5 ppm) to oxidize Fe(II) to Fe(III) since 55% of the iron is in this state. If there are any protected organic Fe(II) complexes being formed they must be weak. This is the only stratum with significant amounts of ionic ferric iron (45%), although at 2 m 14.5% of the total iron is in this form. This may be formed in part by ligand exchange by the hydroxyl ion (OH^-), introduced by precipitation, with organic acids (Stumm and Morgan, 1970). However, it appears that the downward diffusing atmospheric oxygen and the oxygen produced by the phytoplankton layer at 2 m provide the necessary oxygen to perform the continual oxidation of Fe(II) diffusing upward from the hypolimnion.

To further test the ability of the reaction scheme to differentiate operationally between the forms of iron described, aerobically filtered samples were characterized by the same reaction series (Table V). Ionic and coordination categories were again obtained by subtraction (Table VI). Exposure to oxygen (5-10 min) in the samples results in a considerable shift in iron

Table V. The quantity of iron recovered from four strata of North Gate Bog using the four analysis procedures on aerobically filtered samples.

Depth	Dissolved iron (ppb)			Total iron
	Inorganic ferrous	Total ferrous	Total inorganic	
Surface	47	65	140	298
2 m	188	205	428	614
5 m	366	491	526	676
7.5 m	610	837	889	1153

Table VI. The distribution of ionic and coordinated iron in the four strata of North Gate Bog obtained by calculation from the characterization analysis on aerobically filtered samples.

Depth	Dissolved iron (ppb)				Total
	Fe ⁺²	(Fe ⁺²) _c	Fe ⁺³	(Fe ⁺³) _c	
Surface	47	19	93	140	298
2 m	188	18	241	168	614
5 m	366	126	160	24	676
7.5 m	610	228	279	37	1153

species between categories (Fig. 2). In the oxygenated waters of the epilimnion exposure to oxygen caused ionic Fe(II) to decrease by 26% (26 ppb), complexed Fe(II) to decrease by 41% (27 ppb), and ionic Fe(III) to decrease by 13% (27 ppb). These decreases were accompanied by a 114 ppb increase in complexed Fe(III), probably as $\text{Fe}(\text{OH})_3$. In the partially oxygenated water at 2 m aeration brought about a 238 ppb decrease in ionic Fe(II) which was balanced by a 157 ppb increase in ionic Fe(III) and a 117 ppb increase in complexed Fe(III), again as $\text{Fe}(\text{OH})_3$. There was also a 18 ppb decrease in complex Fe(II). In the deeper anaerobic waters, for example at 7.5 m, 590 ppb (41%) of ionic Fe(II) was lost by aeration. However, approximately 224 ppb appeared as Fe(II) complex and 279 ppb as ionic Fe(III). The former compound could be either an inorganic acid-soluble species as reported in oxygenated epilimnetic waters by McMahon (1969), or an organic complex. This change could be caused by an increase in pH arising from a loss of H_2S and CO_2 during filtration which in turn permitted organic compounds to dissociate and complex with iron. Hence exposure to dissolved oxygen removed ionic Fe(II) at all depths, but the greatest decrease was in completely or partially deoxygenated waters (Fig. 3). A decrease in ionic Fe(II) due to oxygenation was coupled with an increase in Fe(III) at all depths (Fig. 4). Oxygen not only increased the total amount of Fe(III) at all depths, but produced significant increases in the amount of complexed Fe(III) ($\text{Fe}(\text{OH})_3$) at depths which had Fe(III) in anaerobic samples (Fig. 5). Thus, iron shifted from one form to another by inorganic oxidation reactions and the expected products from these reactions were monitored by the characterization scheme that was used.

The results were in agreement with the observed changes of ferrous iron in recently oxygenated hypolimnetic waters during the overturn period. McMahon (1969) found that Fe(II) decreased in the hypolimnion and in the epilimnion following spring and autumnal overturn, but the total iron concentration in the epilimnion showed no change. Similar results were found in our samples. In the aerobic samples Fe(II) values declined and Fe(III) values increased without change in total iron concentrations (Figs. 3 and 4). McMahon believed that iron must be lost as $\text{Fe}(\text{OH})_3$ during analysis, i.e., retained by the filter. My samples taken after filtration showed that upon aeration Fe(II) is indeed changed to Fe(III) and $\text{Fe}(\text{OH})_3$ (Fig. 5), and with time would probably have formed precipitates of $\text{Fe}(\text{OH})_3$. These results reveal additional information that supports the oxidative effect of lake overturn on the iron held in anaerobic hypolimnetic waters.

By adding Fe^{59} to the surface waters of the lake, contained as a column in a polyethylene bag, and after five days monitoring the activity in the total, 0.45 μ membrane filtrate and dialyzable fraction, the physical nature of naturally occurring iron was determined. In the surface waters 92% of the total activity remained filterable but only 15.5% of the filterable activity was dialyzable. At 2 m, 83% of the total activity was filterable but 63% of the filterable activity was dialyzable. The dialyzable fractions show the same trend, increasing with depth as ionic ferrous iron (28% and 72% of the

Figure 2. (a) The distribution of iron compounds with depth in North Gate Lake. (b) The distribution of iron compounds with depth showing the chemical (oxidative) changes induced by aerobic filtration in North Gate Lake.

Fig. 3. The distribution of ionic Fe(II) iron with depth, compared to total iron, showing the change induced by the introduction of oxygen following aerobic filtration.

Fig. 4. The distribution of Fe(III) iron with depth, compared to total iron, showing the change caused by the introduction of oxygen following aerobic filtration.

Fig. 5. The distribution of Fe(III) ionic reactive iron with depth, compared to total Fe(III), showing the change caused by the introduction of oxygen following aerobic filtration.

total iron, respectively), again indicating the greater ionization of the iron with depth. The colored organics were filterable but were not dialyzable, i.e., the filtrate was tea-colored compared to the slight cloudy appearance of the dialyzate. Griffing (1969) showed that the dialysis membranes used were impermeable to the organic molecules responsible for the brown color, but did not retard the passage of smaller inorganic and organic constituents. Since the exclusion limit of the dialysis bags is at a molecular weight of 12,000 (manufacturer's specification), the molecular weight of most of the highly colored organics is greater than 12,000. This is the molecular size fraction that contains most of the filterable iron activity (84.5%) in the natural surface waters of this bog lake.

Working on highly stained bog lakes in Alaska, Barsdate (1970) found that 80% of the cobalt and 68% of the manganese was retained by dialysis bags. He believed that this was due to the complexation or association of the tracers with highly dispersed organic colloids rather than an inorganic colloid phenomenon such as sorption of ferric hydroxide. Gjessing (1967) working with gel filtration (Sephedex) found that the molecular weight of 64% of the organic matter studied was greater than 5,000, with 31% of this greater than 20,000. In addition, these higher molecular weight organics contained the most color. Shapiro (1967a) also using Sephedex established the molecular weight of the organic matter in certain Minnesota lakes at around 10,000. It is interesting to note that of a total of 22 lakes studied, two were soft, highly colored lakes. It was these two lakes that contained almost exclusively the higher molecular weight organics. In addition, it was these higher weight organics that held the most iron, indicating that the higher molecular weight organics were more effective than the low in complexing with iron. Further, there was an indication that these higher weight organics which held the most iron and had the most color contained the strongest organic acids. Therefore the iron in the oxygenated surface waters appears to be associated with the higher molecular weight (12,000), highly colored, acidic organic acids.

Discussion

The forms of iron found in natural water were characterized according to their differential chemical reactivity to BPN before and after different digestion procedures. With the added information of the size class of the iron from radiochemical data, the predominant physical and chemical form of iron in the epilimnion and the hypolimnion can be deduced.

In previous studies, dissolved ionic ferrous iron has either been measured with nonspecific methods or its presence has been assumed because the prevailing environmental conditions in the lake (pH, Eh) are favorable for its formation. It has never been operationally measured because of errors created

by sampling, filtering, and preservation. Although the acid used in preservation prevents the oxidation of Fe(II), it dissolves inorganic ferrous compounds and releases Fe(II) from organic complexes. Thus, only total ferrous iron is measurable. The sampling method used with in situ filtration alleviates these errors, and when coupled with the above reaction scheme separates the various forms of iron, and enables ionic ferrous iron to be operationally measured.

High concentrations of reduced organics may hold Fe(II) in complexes like those of tannic acid (Hem, 1960). This may take place when the decomposing leachate solubilizes iron while moving through soil (Bloomfield, 1952). However, in a dilute, low pH solution, as in the bog, Fe(II) becomes or remains ionic because of the weak ability of Fe(II) to form complexes. The anaerobic nature of the hypolimnion and the low dissolved oxygen concentration in the epilimnion, combine with the presence of a high amount of reducing substances, both organic and inorganic, and the low pH to provide an environment in which the Fe(II) is preferred. The presence of ionic Fe(II) without complexation as the predominant form of iron in the deoxygenated waters of the bog, is confirmed by the sampling methods and reaction scheme used in this study.

Unlike Fe(II), Fe(III) forms strong inorganic and organic complexes which make the iron less susceptible to further oxidation and precipitation (Stumm and Lee, 1960). However, the labile nature exhibited by iron upon aerobic filtration indicates the lack of a strongly complexed iron. By molecular size classification with Fe⁵⁹ the epilimnetic iron appears to be associated with the high molecular weight organics (84.5% of soluble activity), but by reactivity upon chemical analysis, the iron appears to be ionic inorganic Fe(III) 45% and complexed Fe(III) 10%. This indicates both an uncomplexed or weakly complexed form, and a strongly complexed form. Shapiro (1967b) found the same differential reactivity of the iron towards thiocyanate in Cedar Bog Lake. These results obtained from this study on the in situ physical and chemical states of iron in pH 4-5 oxygenated surface water, can be explained in terms of loose iron III-colored organic associations that allow the iron to react as ionic ferric iron. This is probably a loose charge-to-charge interaction between un-ionized or weakly ionized organic acids, and Fe(III) produced by the oxidation of Fe(II) diffusing upward from the hypolimnion.

By acting as the fundamental control variable for inorganic surface phenomenon (Healy, 1971) as well as by determining the physical and chemical states of iron in natural waters (Hem and Cropper, 1959) and by controlling the degree of ionization and molecular size of dissolved organic acids (Ghassemi and Christman, 1968), pH controls the relative roles of iron and organic matter in oxygenated water. In the lower pH waters (4-5), the central particle may be the weakly ionized, high molecular weight compact organic acid with its surrounding ionic Fe(III). In the higher pH waters (7-10), the central particle may be colloidal iron held in solution by the surrounding highly charged (polyelectrolytic) organic acids. This hypothetical model accounts

for the increasing association of iron to color above and below the zero point of charge of hydrated iron oxides (pH 6-8) by taking into account the changes occurring in the preferred physical and chemical states of both the iron and the colored organic acids with a change in pH.

Literature Cited

- Barsdate, Robert J. 1970. Transition metal binding by large molecules in high latitude waters. In: Organic Matter in Natural Waters. Hood, Donald W. (ed.). Institute of Marine Science Occasional Publication No. 1. 625 pp.
- Bloomfield, C. 1952. Translocation of iron in podzol formation. *Nature*, 170:540.
- Cooper, L. H. N. 1937. Some conditions governing the solubility of iron. *Proc. Roy. Soc. (London) (B)*, 124:299-307.
- Ghassemi, Massod and R. F. Christman. 1968. Properties of the yellow organic acids of natural waters. *Limnol. Oceanog.* 13(4):583-597.
- Gjessing, E. T. 1967. Humic substances in natural water: Method for separation and characterization. In: Chemical Environment in the Aquatic Habitat. Golterman, H. L. and R. S. Clymo (eds.) I. B. P. Symposium. 322 pp.
- Golterman, H. L. 1969. Methods for chemical analysis of fresh waters. Blackwell Scientific Publications, Oxford, England. 166 pp.
- Gosh, M. M., J. T. O'Conner, and R. S. Engelbrecht. 1967. Bathophenanthroline method for the Determination of ferrous iron. *J. Amer. Water Works Assoc.*, 59(7):897-905.
- Griffing, Thomas G. 1969. Nutrient cycling and productivity of dystrophic lake-bog systems. Technical Progress Report, submitted to the Atomic Energy Commission. 1969.
- Healy, Thomas W. 1971. Selective adsorption of organics on inorganic surfaces. In: Organic Compounds in Aquatic Environments. Faust, Samuel J. and Joseph V. Hunter (eds.). Marcel Dekker, Inc., New York. 638 pp.
- Hem, J. D. and W. H. Cropper. 1959. Survey of ferrous-ferric chemical equilibria and redox potentials. *U.S. Geol. Surv., Water Supply Papers*. 1459-A, pp. 1-31.
- Hem, J. D. 1960. Complexes of ferrous iron with tannic acid. *U.S. Geol. Surv., Water Supply Papers*. 1459-D, pp. 75-94.

Literature Cited (Continued)

- Hutchinson, G. E. 1941. Limnological studies in Connecticut IV. The mechanism of intermediary metabolism in stratified lakes. *Ecol. Monogr.* 11:21-60.
- Hutchinson, G. E. 1957. A Treatise on Limnology, v. 1. John Wiley and Sons, New York. 1015 pp.
- Juday, C., E. A. Birge, and V. W. Meloche. 1938. Mineral content of the lake waters of northwestern Wisconsin. *Trans. Wisc. Acad. Sci. Arts Lett.*, 31:223-276.
- Lee, Fred G. and Werner Stumm. 1960. Determination of ferrous iron on the presence of ferric iron with bathophenanthroline. *J. Amer. Water Works Assoc.*, 52(12):1567-1573.
- McMahon, J. W. 1967. The influence of light and acid on the measurement of ferrous iron in lake water. *Limnol. Oceanog.*, 12:437-442.
- McMahon, J. W. 1969. The annual and diurnal variation in the vertical distribution of acid-soluble ferrous and total iron in a small dimictic lake. *Limnol. Oceanog.*, 14(3):357-367.
- O'Conner, John T., Kusug Komolrit, and Richard S. Engelbrecht. 1965. Evaluation of the orthophenanthroline method for ferrous-iron determination. *J. Amer. Water Works Assoc.*, 57(7):926-934.
- Rabinowich, E., and W.H.J. Stockmayer. 1942. Association of ferric ions with chloride, bromide, and hydroxyl ions (A spectroscopic study). *J. Amer. Chem. Soc.*, 64:335-347.
- Rawson, J. 1963. Solution of manganese dioxide by tannic acid. *U.S. Geol. Surv., Profess. Paper*, No. 475-C.
- Shapiro, J. 1964. Effect of yellow organic acids on iron and other metals in water. *J. Amer. Water Works Assoc.*, 56:1062-1082.
- Shapiro, J. 1966a. On the measurement of ferrous iron in natural waters. *Limnol. Oceanog.*, 11:293-298.
- Shapiro, J. 1966b. The relation of humic color to iron in natural waters. *Verh. Internat. Verein. Limnol.*, 16:477-484.

Literature Cited (Concluded)

- Shapiro, J. 1967a. Yellow organic acids of lake water: Differences in their composition and behavior. In: Chemical Environment in the Aquatic Habitat. Golterman, H. L. and R. S. Clymo (eds.). I.B.P. Symposium. 322 pp.
- Shapiro, J. 1967b. Iron available to algae. In: Chemical Environment in the Aquatic Habitat. Golterman, H. L. and R. S. Clymo (eds.). I.B.P. Symposium. 322 pp.
- Stumm, W. and G. F. Lee. 1960. The chemistry of aqueous iron. Schweizerische Zeitschrift fur Hydrologie, 22:295-319.
- Stumm, W. and J. J. Morgan. 1970. Aquatic Chemistry. John Wiley and Sons, New York. 583 pp.