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Technical Progress Report

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

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SCOPE OF WORK: DECEMBER 1, 1968 - NOVEMBER 30, 1969

The major effort during the past year was the winter labeling experiment undertaken at North Gate Lake on March 15. The outdoor field data gathering season began at this time and continued through September 1. We succeeded in labeling bottom water with ^{32}P and rhodamine WT and then gathered data showing the fate of these labels during the following months. An exceptionally calm and warm spring resulted in an incomplete spring circulation, and the labeled water remained undisturbed at the bottom of the lake. We had anticipated only partial mixing of the labeled stratum; however, we did not believe that the labeled layer would remain undisturbed.

Failure of the lake to circulate provided a unique opportunity for experimental studies and for analysis of the metabolic processes taking place within the hypolimnion. To study the fate of the labeled compounds in the photosynthetic zone, we pumped the labeled water to the surface, passed it through an experimental flow-through system containing a biological community, and then returned the labeled water to the hypolimnion. Some of the results of these experimental studies together with an analysis of the physical changes taking place during the spring overturn and an analysis of changes in the distribution of various phosphorus components following the labeling are reported in the preliminary draft of a manuscript included within Appendix A of this report.

In the course of our analysis of the phosphorus changes, ^{32}P activity appeared at the surface without addition of any activity to the water of intermediate depths. This unexpected displacement of activity into the surface water was of great interest and required an immediate diversion of effort and manpower. It was soon discovered that the diurnal migrations of the phantom midge Chaoborus were carrying activity into the surface water where it was promptly incorporated into the phytoplankton and zooplankton. Results of these studies are included in a second preliminary manuscript (Appendix B of this report). Because of time limitations in preparation of this report, several major segments of our data have not been analyzed. These are (1) data dealing with distribution of ^{32}P label within bottom sediments, (2) quantitative data on the biological composition of the in situ box system, and (3) data relating to the effect of calcium upon the uptake of labeled phosphorus from the bottom water after passage through the in situ box system discussed below. Preliminary data suggest that calcium rather strongly modifies the uptake phenomena. These results will be reported in the next progress report.

A joint program with the personnel of the Department of Genetics and Cell Biology, University of Minnesota, aimed at identification of the organic and inorganic phosphorus compounds in North Gate Lake water was initiated during

the past year. Mrs. Kay Terry of this laboratory developed chemical techniques appropriate for measurements on our water samples. Her preliminary results are also reported in Appendix A. These results however must be considered preliminary and further studies using the new techniques are planned for the coming year. The objective here is to determine whether or not these compounds are indicators of nutrient deficiencies or other biochemical processes taking place within the lake system.

PUBLICATIONS DURING 1969
RESULTING FROM AEC SUPPORT

Sohacki, Leonard P., Robert C. Ball, and Frank F. Hooper. 1969. Some ecological changes in ponds from sodium arsenite and copper sulfate. Papers of Mich. Acad. of Sci. Arts, and Letters (Michigan Academician). Vol. 1:149-162. Contract AT (11-1)-655.

Bahr, Thomas G., Robert C. Ball, and Frank F. Hooper. 1969. Arsenic ⁷⁴ metabolism in an aquatic ecosystem. Papers of Mich. Acad. of Sci. Arts, and Letters (Michigan Academician). Vol. 1:163:174. Contract AT (11-1)-655.

APPENDIX A

FURTHER STUDIES ON PHOSPHORUS TRANSFORMATIONS AND CYCLING
IN A⁻ BOG, BOG-LAKE ECOSYSTEM

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INTRODUCTION

An understanding of the factors regulating production in bog lakes is a necessary step in developing guidelines for the preservation and management of these aquatic resources. This paper presents the results of experiments designed to analyze quantitatively critical phases in the movement and transformation of phosphorus within a small bog, bog-lake system in northern Michigan. I will attempt to integrate these and previous findings into a model of the overall cycle of the phosphorus in this ecosystem.

An earlier paper has described the gross features of the phosphorus cycle of North Gate Lake, a small bog-lake in Gogebic County, Michigan (T45 north, R42 west, section 32) (Hooper and Brady, 1968). In that paper I discussed allochthonous sources of phosphorus entering this lake and suggested that: (1) large phosphorus resources exist within the mat system which could be made available to the aquatic biota of the lake depending upon the water exchange between the lake and water entrained in the bog mat; (2) there is a relatively large input of phosphorus from rain and from surface debris entering at the lake surface; (3) among aquatic systems these lakes offer a paradox because they exhibit exceedingly low production in face of abundant phosphorus resources. It would appear from these studies that the key processes regulating use of these phosphorus stores must be found either in (1) exchange of phosphorus between the mat and the lake, and/or (2) factors which regulate chemical transformations of phosphorus from the organic to inorganic state within the lake itself.

The salient features of the phosphorus cycles of eutrophic and oligotrophic lakes are well known (Hutchinson, 1957). Allochthonous input from ground or surface drainage furnishes phosphorus utilized for production of photosynthetic organisms in the trophogenic waters. Regeneration by bacteria of the phosphorus from organic debris in the water of the epilimnion and in the sediments of the littoral zone provides a mechanism for reuse as long as wind-driven currents circulate materials within this zone. At depths greater than the thermocline however, regeneration products are stored until the autumnal or vernal circulation periods when they are reintroduced into the trophogenic waters. The dynamics of phosphorus changes within the stagnant zones of the lakes are obscure because there may be simultaneous (a) regeneration of materials from bottom sediments, and (b) addition of materials from above in the form of plankton descending into the strata. In any case, there is a build-up of phosphorus in these waters during the summer and winter stagnation periods. This increase may be in the form of orthophosphate (P_i) or other inorganic or organic phosphorus compounds.

The North Gate bog system differs from the above model for non-bog lakes in several important aspects: (1) The allochthonous phosphorus input from surface drainage is chemically altered during passage through an extensive Sphagnum mat. The mat system removes alkali and alkaline earth cations and adds decay products from the mat and bog forest. The mat water entering the lake contains orthophosphate and also dissolved organic phosphorus compounds. The mat water of North Gate bog contained from 336 to 1077 $\mu\text{g/l}$ total phosphorus; of this, 262 to 313 $\mu\text{g/l}$ was filterable through a 0.45 μ membrane, and 44 to 240 $\mu\text{g/l}$ reacted with ammonium molybdate without digestion (Hooper and Brady, 1968). (2) During midsummer, with low precipitation and with high transpiration by the bog mat and forest, the flow of water is away from the lake. This precludes renewal by allochthonous sources (ibid, 1968). (3) The bog lake lacks a littoral zone (Figure 1A). This greatly reduces regeneration of nutrients within the trophogenic waters and increases the sediments entering the profundal zone. (4) The small ratio of surface area to mean depth of North Gate Lake, its protection from wind by the bog forest, and its high color all tend to restrict the heat input to the upper 1 to 2 m, and minimize vertical transport by wind-driven currents. This high degree of physical isolation of the bottom water arising from the lack of vertical mixing led to an incomplete vernal overturn during the spring of 1969 when the weather was exceptionally calm and warm at the time the ice melted from the lake.

These unique features of bog lakes bring about simplifications in the phosphorus cycle and permit a more precise analysis than is possible for stratified eutrophic lakes. Major simplification arises from the lack of feedback of regenerated materials from the littoral sediments. The cycle is complicated however, by the losses and gains arising from exchanges between the mat and open water.

EXPERIMENTAL DESIGN

Features of the phosphorus cycle investigated in this study are: (1) the nature and fate of phosphorus compounds stored in the hypolimnion and the extent of their utilization when reintroduced into trophogenic waters, (2) the extent of phosphorus losses or exchange with the profundal sediments, (3) the flow of phosphorus compounds into the hypolimnium, and (4) the transformation of particulate and of organic phosphorus to orthophosphate.

To monitor phosphorus changes within the hypolimnion and to follow the fate of compounds stored in this zone during winter stagnation, we labeled the 7-m stratum of water with ^{32}P and with rhodamine WT during winter stagnation (March 15). Labels were applied to this stratum in the following manner. We pumped 50 gal of water from the 7-m layer into a 50-gal steel drum located on the surface of the ice. The intake hose used to pump the water was designed so that movement of the entering water was horizontal and not vertical. Tests

Figure 1A. Hydrographic map, North Gate Lake, showing injection sites and sampling stations.

of the apparatus with a dye marker indicated that the horizontal flow was confined to a stratum approximately 0.1 m thick. We added 11 g of rhodamine WT and 176 millicuries of ^{32}P to the drum and mixed the contents thoroughly. This mixture was injected back into the 7-m layer in the following manner. We drilled 50 holes through the ice within the projection of the 7-m contour at the surface. The holes were evenly spaced on a 5-m grid network. We injected 1 gal of the double labeled mixture into each of the 50 holes by pouring it into the tube used to collect the 7-m water, and then flushing the tube with 3 gal of unlabeled lake water. We found that this procedure effectively dispersed the label horizontally from the injection site. The flushing effectively removed all traces of the label from the injection tube so that it did not contaminate upper layers when the tube was removed from the lake.

One week following labeling we undertook intensive sampling of the lake to determine the extent of horizontal dispersion under the ice. We then allowed 4 weeks for the phosphorus label to equilibrate with the phosphorus pools within the hypolimnium. A second set of samples collected after this 4-week period and before the ice disappeared from the lake, enabled an assessment of the equilibrium state. After disappearance of the ice (April 17) samples were collected at regular intervals to determine the movement of the labels after the spring circulation arising from biological and physical transport. In addition to the above data which focused upon following the labels in space and in time, we undertook in situ experiments at the lake surface whereby we were able to study experimentally the incorporation of the hypolimnetic phosphorus compounds into the biological systems at the lake surface.

DISPERSION AND EQUILIBRATION OF LABELS FOLLOWING INJECTION

Observations of horizontal and vertical dispersion of the labels were made 1, 3, 4, 5, and 28 days after injection. Horizontal dispersion studies were made by determining the concentration at five of the injection sites (Stations A, B, C, D, E) and at three sites 7.1 m distant from the injection holes (Stations F, G, H). We measured the rhodamine concentration of three samples collected at each depth and at each time on a Turner flurometer. At three stations at which the mixture was injected (Stations A, B, C), we also measured the concentrations of soluble reactive phosphorus (SRP) particulate phosphorus (PUP), and total phosphorus. For each analysis we collected three 100 ml samples in polyethylene bottles. We used a modification of the Hale water sampler (Welch, 1948) to collect water samples. In this modification, the reservoir bottle encloses the sample bottle. A single polyethylene bottle was used as a sample bottle. The remaining space within the reservoir bottle was approximately five times the volume of the sample bottle. Thus a complete flushing of the sample bottle took place before samples were collected. The advantage of the modified Hale water sampler in this investigation was that it made it possible to sample a relatively thin vertical stratum (6 in.) and

also avoided contamination arising from transfer of labeled materials from one container to another at the lake surface. Before use, we tested the apparatus carefully in the laboratory. The single possible source of error is that the bubbles leaving the sampler at a given depth may displace the label vertically, and thus contaminate an upper stratum. To minimize such contamination, the sequence of collecting samples was always from the surface to the bottom.

Procedures used in analysis of soluble reactive phosphorus (SRP), particulate unreactive phosphorus (PUP), and soluble unreactive phosphorus (SUP) are those given by Strickland and Parsons (1965). In some instances the PUP fraction was measured directly by digesting a millipore filter paper containing the solids in the same way the SUP component was digested. In other instances, the PUP component was measured by subtracting the SUP from the value obtained by digesting unfiltered lake water (total phosphorus). We measured the activity associated with the various phosphorus fractions by pipetting 5 ml of the solution read in the colorimeter onto a planchet, evaporating to dryness, and counting the residue.

We were unable to study dispersion of rhodamine WT with the precision we anticipated. Water concentrations were approximately 20% of expected values (Table IA). It was later determined that the large fraction of the rhodamine-³²P mixture had settled below the 7-m stratum. Values significantly above zero on the flurometer occurred chiefly at the 7-m stratum after the initial labeling in March (Table IA). To increase the possibility of following water movements at the time of the overturn, we made a second injection on April 14. At this time we added 100 g of rhodamine WT to the 7-m stratum through 20 holes spaced regularly within the 7-m contour, as in the previous case.

There was a gradual increase in the concentrations of rhodamine at the injection site stations between March 17 and 20 (Table IA); however, values remained low. There was a loss of rhodamine (perhaps due to dilution by surface water) between March 20 and April 13. On April 13 only two of the stations (A and B) had measurable concentrations.

The stations at which rhodamine was not injected had measurable concentrations at 7-m but not at other depths on March 20 (Table IA). This finding, together with observed increases at the injection stations, indicates horizontal water movements after injection within the 7-m stratum. The only evidences that the isotope mixture moved above the site of injection were two samples with significant rhodamine concentrations at Station D at 6.3 m on March 17 and 18. This may have arisen from a variation in injection procedure at this station.

After the second injection (April 14), rhodamine concentrations were considerably higher in the 7-m stratum. There was great variation from station to station. Readings made under the ice on April 16 provided little indication of vertical movement. The small concentrations found at 6 and 3 m may well have been contamination.

TABLE IA. Rhodamine WT concentration (parts per billion) in North Gate Lake after injection of rhodamine-³²P mixture into 7-m stratum on March 16, 1969 and after a second injection of rhodamine on April 14. (Mean of three samples at each station)

Depth	Station	March				April				May					
		17	18	19	20	13	16	20	24	26	29	2	5	9	12
7M	A	0.0	0.5	0.5	1.0	0.5	2.0								
	B	0.5	0.8	0.5	0.5	0.5	4.0	18.5	8.2	14.5	11.0	22.5	17.5	24.0	12.5
	C	0.0	0.5	0.5	1.5	0.0	10.0								
	D	0.5	0.5	0.5	1.5	0.0	8.0								
	E	0.5	1.0	0.5	1.0	0.0	4.0								
	F	-	-	-	0.5										
	G	-	-	-	0.5										
6.3M	A	0.0	0.0	0.0	-										
	B	0.0	0.0	0.0	-										
	C	0.0	0.0	0.0	-										
	D	0.5	0.3	0.0	-										
	E	0.0	0.0	0.0	-										
	F	-	-	-	0.0										
	G	-	-	-	0.0										
6M	A	0.0	0.0	0.0	-	0.0	0.0								
	B	0.0	0.0	0.0	-	0.0	0.0	0.0	0.3	0.5	0.3	0.8	0.3	0.8	0.5
	C	0.0	0.0	0.0	-	0.0	0.5								
	D	0.0	0.0	0.0	-	0.0	0.0								
	E	0.0	0.0	0.0	-	0.0	0.3								
	F	-	-	-	-										
	G	-	-	-	0.0										
3M	A				0.0	0.0	0.3								
	B				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C				0.0	0.0	0.3								
	D				0.0	0.0	0.0								
	E				0.0	0.0	0.0								
	F				0.0										
	G				0.0										
Sfc	A				0.0	0.0	0.0								
	B				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C				0.0	0.0	0.0								
	D				0.0	0.0	0.0								
	E				0.0	0.0	0.0								
	F				0.0										
	G				0.0										

After the disappearance of the ice all readings were made at Station B which was centrally located within the basin (Figure 1A). It is clear that the overturn brought little or no activity to the 3-m and surface strata. Above 7 m, significant concentrations did not appear until April 24 when a significant reading was found at the 6-m depth. Succeeding measurements at 6 and 7 m (Station B) were all significant. After April 20 there was considerable variation in readings at 6 and 7 m. Fluctuations were correlated between the 6- and 7-m layers. Minima occurred on April 24, 29, and May 12. Maxima were noted on April 26, May 2, and 9. These variations appear to be related to water movements in the upper layers of the chemocline arising from cold, dense water entering from the mat and settling as a density current into this stratum.

Significant differences appeared between three sampling stations (A,G,C) in all forms of stable phosphorus and in the activity associated with each form at 7 and 6.7 m. Station A was significantly higher in soluble activity, total phosphorus and total activity at 7 m on March 17, and in soluble phosphorus and total activity on March 19. Significantly higher values at this station were also encountered in soluble reactive phosphorus, soluble activity, and total activity in the 6.7-m layer on March 17. These data together with findings of significantly lower levels at Station C (6.7-m stratum) on March 19, indicate a lack of uniformity in horizontal distribution of phosphorus components under the ice.

Extremely high variations occurred in the particulate activity fractions. (Stations A and C, 7 m, on March 17; Station A, 7 m, on March 19; Station B, 7 m, on March 19 and 20). Activity of samples ranged up to 15,674 pc/ml. Closer examination of these samples revealed that they contained the phantom midge *Chaoborus*. At this time one or two larvae per sample gave the high activity. By March 20 the activity of these larvae had decreased somewhat but it is clear that isotopic equilibrium had not been established. Variations of this sort were not noted in samples taken under the ice on April 13. It therefore appears that isotopic exchange had taken place by this time while these larvae were in residence at the 6- and 7-m levels.

Only the 6.7- and 7-m strata were adequately labeled with ^{32}P by these injections. Significant activities occurred sporadically at the 6-m level. These readings in most instances were in particulate phosphorus. On March 20 data were obtained through the entire water column; the only significant activity was in the 7-m layer. Thus it appears that labeled material did not move above the 6.0-m level.

On March 20 samples were collected at Station F, a station at which injection was not made and which was located approximately 7 m from the nearest injection site (Station B). Filtered water samples collected at this station were compared with samples collected at Station B the same day. The activity at this station did not differ significantly from the activity at Station B at any depth. At the 7-m depth, activity at Station F exceeded that at Station B; however, the difference was on the borderline of significance. These

data tend to confirm rhodamine measurements which indicated that there was considerable horizontal movement of the label under the ice, and that by March 20 there was essentially uniform horizontal distribution of the label. These observations agree with findings of Likens and Hasler (1962) who performed a similar experiment using radiosodium. Tub Lake, the northwestern Wisconsin bog lake studied by Likens and Hasler, has the same surface area and nearly identical maximum depth as North Gate Lake. In the Tub Lake experiment, there was an initial vertical movement of the tracer toward the surface and a horizontal movement from the single injection site. The density of our tracer solution appears to have been better matched to the water density at the injection level than in the Wisconsin study since there appears to have been no vertical movement and horizontal movement was confined to the injection layer. Since we found essentially the same concentration at Station F as at Station B within 4 days, small quantities of the labels must have arrived at Station F before this date. Horizontal movement of approximately 20 m within 24 hr found by Likens and Hasler is not inconsistent with our observations. It would appear that there were few if any biological losses to surrounding strata from the horizontally flowing current since there were no measurable differences in transport between the conservative and nonconservative labels.

The existence of horizontal currents in ice-covered lakes has been previously demonstrated. The origin of these currents is not known and is a subject of considerable speculation. Heat from the bottom sediments and external changes in temperature and pressure have been offered as explanation by Likens and Hasler. In North Gate Lake at the time of the observed horizontal movement, the lake was dichothermal and possessed a well-defined temperature inversion from 7 m to the bottom. Thus there is clear evidence of bathylymnetic heating in this lake. Hutchinson (1957) discusses possible mechanisms of bathylymnetic heating in small lakes under the ice. He suggests that such heating may arise from density currents running down the slope of the basin towards its deepest point. It is suggested that such density currents may arise from heating of water in the shallower regions by solar radiation, thereby initiating a flow down the basin slope to greater depths. Density would then be increased by dissolved substances moving from the mud into this current. Upon arriving at the lake bottom, such a flow could give rise to horizontal currents.

In the present instance, the existence of such currents would explain both the horizontal movements of the labels and also the temperature and chemical inversion at 7 m. Further support for this hypothesis comes from data presented below which suggest that currents of this type also arise during early spring from radiational cooling of water of the surrounding mat followed by its movement down the lake basin as a density current.

VERNAL CIRCULATION

It is clear the lake was dichothermic when the label was injected on March 17 (Figure 2A). On this date there was a layer of uniform temperature (3.95°C) between 4.5 and 6 m. Temperature was slightly higher (4.02°C) at 7 m and increased to 4.22°C at 8 m. The presence of a weak chemocline was indicated by the increase in dissolved calcium from 0.94 ppm at 6 m to 1.00 ppm at 7.5 m.

Several sets of data were collected between April 13 and April 16 before disappearance of the ice cover but after considerable melting and warming had taken place. With warm spring weather and air temperatures above 20°C the ice surface was covered with slush and melt-water and there was a band of open water approximately 2 ft wide on the north side of the lake adjacent to the bog mat. The mat had thawed and was covered with melt-water. The ice itself was honeycombed and was not sufficiently solid for full support. To take samples from the middle of the lake, it was necessary to skid a boat with personnel and equipment across the soft ice.

Data collected on April 14 and 15 indicate changes in dissolved oxygen, temperature, and pH at all levels from readings taken in March. Temperatures had decreased approximately 0.5° at the 6- and 7-m depths, and traces of oxygen were found at these levels. The pH had increased at 3- and 7-m levels but was the same as on March 17 at 6 m. Increases in dissolved oxygen and changes in pH and temperature also appear to have been greater at 3 and 7 m than at 6 m (Figure 2A).

The above differential changes in water quality at 3 and 7 m as compared to 6-m levels are difficult to explain using conventional models of turbulent exchange and suggest that horizontally operative mechanisms inject surface water at selected depths within the lake basin. With the observed vertical temperature gradient under the ice, one would have to hypothesize vertical density currents moving along the slope of the basin which attained higher density by incorporating solids from the lake mud.

Observations on April 20 (three days after disappearance of the ice) indicated that there was a rapid warming of the water from 4 m to the surface, and smaller increases at greater depths. A minimum of the temperature curve was at 5 m (3.9°C). Dissolved oxygen was again absent at 6 and 7 m. The pH was approximately the same at the surface, 1, and 3 m, but there had been further increases at 6 and 8 m (Figure 2A). The temperature minimum at 5 m indicates that little if any turbulent mixing extended beyond this depth. Observed increases in temperature, in pH, and disappearance of oxygen at 6 and 7 m, however, can only be due to some mixing process within the bathylimnion, since there was no indication of the vertical dispersion of the two tracers from this stratum. Such a mixing process must have been limited vertically and must have arisen either from profile-bound currents of the sort mentioned above or from internal waves or some other vertical oscillations within the epilimnion.

Figure 2A. Isopleths of pH, dissolved oxygen, and temperature in North Gate Lake during the spring of 1969.

During the week of April 20 air temperatures remained high and a warming of the epilimnion continued bringing about intensification of the vertical temperature gradient. However, following the influx of a cold air mass on April 28 and 29, there was a drop in air temperature, formation of a thin ice sheet on the bog mat, a fall in surface water temperature, and considerable vertical mixing in the upper 2 m. Of greater interest, however, is the decrease in temperature below the zone of vertical mixing (3 m) and the fall in pH at this depth and at 6 and 7 m (Figure 2A). Concurrent with these changes there was a sizable decrease in rhodamine concentrations at 6 and 7 m. These changes together with small increases in dissolved oxygen suggest injection of mat water into the horizontal strata below the level of wind-driven turbulence. From May 2 through May 5, warming of the epilimnion was resumed. Air temperatures ranged between 20 and 24°C, and wind velocities were low. On May 9 however, a second cold air mass appeared and temperatures again temporarily fell below freezing. The surface water temperature fell over 4° and there appears to have been a second injection of oxygen and water of low temperature and pH below the thermocline. After May 12 the warming trend was resumed and thermal and chemical stability seemed to have been restored throughout the water column. There appears to have been no further injections of mat water below the thermocline. By this time the amount of water flooding the mat had decreased and the mat and its entrained water must have stored considerable heat.

PHOSPHORUS CHANGES FOLLOWING LABELING

The level of SRP remained essentially unchanged between March 17 and April 20 in the 7-m stratum. Induced water movements during the vernal overturn however, brought about an increase in soluble activity, PUP, and in particulate activity within this stratum (Figures 3A and 4A). The May 7 data, however, indicate a large increase in SRP at 7 m. This increase was not accompanied by an increase in dissolved radioactivity. Hence this phosphorus could not have come from the labeled strata but must have been carried down from some other point within the lake basin. Vertical movement through the water column does not seem likely since the 6-m stratum did not show a similar increase. These data appear also to fit the hypothesis of currents descending along the basin slope and carrying dissolved solutes. The increase in SRP at 3 m on May 7 was approximately the same as at 7 m. A smaller increase was detected at the surface. These increases in SRP at the surface and at 3 m were more likely of allochthonous origin than from regeneration since plankton of the upper layers was labeled at that time, and there was no measurable increase in soluble activity. Since the mat water is rich in SRP, these data are in accord with the hypothesis of injection of mat water into the water mass at selected depths.

Figure 3A. The stable phosphorus components in North Gate Lake in 1969.

Figure 4A. ^{32}P activity in North Gate Lake in 1969.

SRP disappeared from the surface in late May but persisted at 3 m until mid-July. The 6-m level did not show the changes noted in early May at other levels (believed to be due to density current input), but did show a steady decline in SRP between March and August. This decline in SRP was accompanied by an increase in SUP both at this level and at 3 m.

The level of PUP remained relatively stable at 3, 6, and 7 m from disappearance of the ice until early August. The maximum in PUP was at 3 m and it decreased steadily from this depth to the bottom. Circulation under the ice preceding the vernal overturn increased the level of PUP but brought about a decrease in SRP at the surface and 3 m. These somewhat complimentary changes suggest that biological transformation of phosphorus begins with the onset of water movements under the ice and with the input of oxygen at these strata, rather than with an increase in light intensity. The only large increases in PUP which might be related to photosynthetic activity were at the surface immediately after the overturn and a second peak at the surface recorded on July 21. The former may represent the uptake by heterotrophic organisms, but the peak on July 21 appears to be related to a peak in phytoplankton in the surface water. The decline in PUP at the surface in April does not seem to be reflected in an increase in other²-phosphorus components at this level but it may be related to the later increase in SRP at the 3-m level.

The highest level of SUP was at the surface in samples collected at the surface of the ice 4 days before the ice break-up. There was much accumulated debris including dead Chaoborus in the stratum. Hence it was not surprising to find a high level of SUP and an absence of SRP if this layer contained an abundance of decomposer organisms. At the overturn SUP appears to have been carried down to the 3-m level. After mixing within the epilimnion SUP appears to have remained relatively constant at the surface until early August. The latter increase may represent material released from the high level of particulate phosphorus noted in this stratum in late July.

After the initial increase in SUP noted above at 3 m, it appears to have remained relatively stable at this depth throughout June, but there appears to have been large increases in late July and August corresponding to the disappearance of oxygen. Similarly there seems to have been large increases in SRP at 6 m in early July and in SUP for the first time at 7 m on July 21. This is the only occasion of concurrent increases in any phosphorus component at all levels below the surface. It would appear that a sedimentation-regeneration process was involved. This may represent biological regeneration of phosphorus components from the particulate matter noted at the surface in late July. This input of SUP into the 7-m level appears to have been regenerated and converted into soluble components rapidly. It had declined by early August and by mid-August it had disappeared from the 7-m stratum. This decline was accompanied by an increase in SRP. Changes at the 6-m level were similar to those at 7 m.

All statistically significant activity readings in March were at the 6- and 7-m strata. Significant activity measurements were found immediately under the ice on April 13, and both soluble and particulate activity were recorded until May 19. Particulate activity appeared in the 3-m stratum on May 7; it was again present on May 21, and small amounts were recorded on June 5. Transport of labeled phosphorus from the bottom water to the surface appears to have been brought about by vertical migration of the phantom midge Chaoborus. Details of phosphorus transport by Chaoborus are included in Appendix B (Imes, 1969). Phosphorus carried into the trophogenic waters by Chaoborus appears to have entered the phytoplankton and food chains within the epilimnion. Particulate phosphorus activity was detected in the 7-m stratum under the ice immediately after labeling (in Chaoborus) and on April 20 soon after the ice had disappeared. The latter activity may have come from labeled solids carried into the open water by the density currents flowing along the bottom at the time of the disappearance of the ice. On the next sampling date (May 7) solid activity was not detected, which indicated that this material had settled or had been released as dissolved activity. Traces of particulate activity persisted at the 6-m level until June 5.

To provide further information on the physical state of the labeled dissolved phosphorus, we dialyzed water from the 7-m stratum against distilled water. On March 17 we put 1 liter of distilled water in dialysis tubing and suspended the tubing at 7 m. The tubing was removed on March 20 and we compared activity measurements inside the tubing with the activity of water collected at the same depth. The mean water activity inside the tubing was 3.0 pc/ml, while the outside water was 3.6 pc/ml. This difference in activity, however, was not statistically significant and may represent the small amount of particulate activity present. Similar experiments with 7 m water run in the laboratory on May 15 with filtered water showed that all of the dissolved activity moved through a dialysis membrane. On May 15 we also analyzed SRP in the distilled water compartment and in the dialysis bag at 48-hr intervals. Changes in SRP closely followed changes in activity. This indicated that the isotopic equilibrium had been established among all soluble components. The absence of SUP at the 7-m stratum during winter stagnation and up until July 21 is surprising since it was abundant at all levels in 1968 (Hooper and Brady, 1968).

Between April 20 and June 26 there was a steady decline in soluble activity at 7 m. Similar declines in SRP occurred between April 7 and July 21 at 7 m and between April 20 and July 21 at 6 m (Figures 3A and 4A). During this period, there was not a comparable increase in particulate phosphorus at either of these depths. It appears that SRP was selectively lost from this portion of the lake basin. The rate of change of ^{32}P and SRP are approximately the same. Specific activity did not change; hence, there had been no influx of stable phosphorus from the upper strata. Failure to find any corresponding increase in particulate phosphorus indicates that the decrease was not the result of in situ biological uptake. Losses to the mud surface could occur if there was vertical turbulent diffusion at this depth.

Except for the possibility of horizontal transport by profile-bound currents noted above during early May, there would not appear to be any physical mechanism for removing phosphorus from these layers. The only known transport mechanism operating within this portion of the lake basin appears to be the diurnal vertical migrations of Chaoborus. Transport of ^{32}P by Chaoborus will be treated in greater detail by Imes (Appendix B).

IN SITU EXPERIMENTS ON PHOSPHORUS TRANSFORMATION WITHIN THE EPIMLIMNION

Failure of the labeled bottom water to be brought to the surface during the vernal overturn provided an opportunity for experimental investigation of the use of the labeled compounds by the biota of the epilimnion. The experimental apparatus used has previously been described in detail (Hooper and Brady, 1968). Briefly, it consists of a clear plastic box made up of four compartments. Water enters the box at one end and is pumped out of the other end by an air lift. The rate of water flow through the system can be carefully controlled by the air supplied. In the present experiment, the box was attached to a large Styrofoam float and anchored at the center of North Gate Lake (Figures 5a and 6a). Water entering the box from the 7-m stratum passes through a plastic tube. In the first compartment of the box the labeled bottom water was aerated by a flow of compressed air. It then passed through a filter panel containing four 2-in. diameter filters of 20 μ mesh Nytex. The two center compartments of the box contained algae and periphyton which developed from an inoculation made from the flora of the surface water on May 5. Passing from the third to the fourth compartment of the box, the flow moved through a second series of 20 μ filters. From the fourth compartment the water was pumped out of the box by the air lift into a second plastic tube which returned the labeled water to the lower part of the thermocline (4.0 - 4.5 m). The two center compartments of the box contained a series of glass slides which served as periphyton substrates that could be removed for counting and for activity measurements.

The air which aerated the in-flowing water and also operated the air-lift mechanism was supplied by a small aquarium pump powered by current from a 12-v battery. Once the apparatus was set up in early May, it was operated almost continuously throughout the summer. Some failures occurred early in the summer when a motor generator power source was used; however we were able to obtain continuous 24-hr a day operation with a battery powered system. Once put into operation, it was only necessary to change the batteries and Nytex filters every 24 hr. We regulated the outflow so that the rate of water movement through the system was approximately 50 ml/min.

After inoculation there was rapid growth of both periphyton and phytoplankton within the two center compartments. Within two weeks, substrates were covered with a dense growth of periphyton and the water was quite turbid with phytoplankton.

Figure 5A. In situ flow-through system in operation in North Gate Lake.

Figure 6A. Location of flow-through system in North Gate Lake.

Activity and stable phosphorus measurements were made at frequent intervals on the box system once it was put into operation. The specific activity of the particulate and soluble components of the outflow became constant after approximately two weeks, indicating that isotopic equilibrium had been established with the small inoculant taken from the surface water. The box was operated with temporary interruptions but without additions of external sources of phosphorus for an additional 4-week period before the experiments described below were initiated. Since no other sources of stable phosphorus were introduced into the system, all experiments were conducted under equilibrium conditions between the biological system at the surface and the labeled phosphorus components entering from the 7-m stratum.

BIOLOGICAL UPTAKE EXPERIMENTS

On July 3 and July 10 we measured phosphorus components of the affluent and effluent passing through the box system to study biological transformations of phosphorus. On each of the dates a morning set of samples was collected at 7 a.m. and an afternoon set at 2 p.m. The affluent water was sampled by inserting a tube into the in-flow and filling a 2-liter Erlenmeyer flask by suction. The outflow from the fourth compartment was sampled using the same device. For analysis of stable and radioactive phosphorus components, 100 ml aliquots were drawn from the 2-liter sample. Samples were filtered within 20 min after being collected; hence there was little opportunity for biological breakdown.

We analyzed three replicates of filtered and unfiltered samples for total phosphorus and SRP. After stable phosphorus levels were read in a colorimeter, 50 ml of the solution was evaporated to dryness and counted. Thus activity measurements were made on samples identical to those used for stable phosphorus determinations. Measurements on filtered and unfiltered samples enabled us to calculate the stable and radioactive phosphorus of particulate matter.

All four measurements of stable phosphorus uptake by the system show that total phosphorus content of the effluent was less than that of the affluent. Storage within the system varied from 10% on the morning run of July 3 to a maximum of 32% on the afternoon run on July 3. There were major shifts in the relative concentration of various components moving through the box (Table IIA). From 60 to 86% of the SRP was removed. Removal of the SRP components was in all cases accompanied by an increase in SUP and in PUP.

There was very little soluble unreactive phosphorus in the affluent water (3-7 $\mu\text{g/l}$). Extractions of the affluent with hexanol removed all activity. Significant counts were not obtained by evaporation of 50 ml of the water phase. Thus it would appear that the soluble organic fraction was unlabeled.

TABLE IIA. Changes in concentrations of phosphorus components of ^{32}P labeled water from 7-m stratum during passage through an in situ flow system suspended in North Gate Lake. Stable phosphorus reported as mg/l; ^{32}P reported as pc/l. Soluble components filterable through 0.45 μ membrane.

Date and Phosphorus Component	Mean Concentration		Change			
	Affluent Water	Effluent Water	Increase		Decrease	
			Concen- tration	Percentage of Affluent	Concen- tration	Percentage of Affluent
July 3, 1969 (Morning)						
Total Soluble	(90)	56			34	38
Soluble Reactive (SRP)	87	35			52	60
Soluble Unreactive (SUP)	3	21	18	600		
Soluble Activity	89	10			79	89
Particulate Unreactive (PUP)	24	47	23	96		
Particulate Activity	24	31	7	29		
July 3, 1969 (Afternoon)						
Total Soluble	103	56			47	45
Soluble Reactive	100	35			65	65
Soluble Unreactive	3	21	18	600		
Soluble Activity	76	1			75	99
Particulate Unreactive	10	21	11	110		
Particulate Activity	36	42	7	19		
July 10, 1969 (Morning)						
Total Soluble	100	46			54	54
Soluble Reactive	93	16			77	83
Soluble Unreactive	7	30	23	328		
Soluble Activity	38	3			35	92
Particulate Unreactive	42	69	27	64		
Particulate Activity	17	49	32	188		
July 10, 1969 (Afternoon)						
Total Soluble	107	45			62	57
Soluble Reactive	109	15			94	86
Soluble Unreactive	4	30	28	700		
Soluble Activity	39	7			32	82
Particulate Unreactive	18	65	47	261		
Particulate Activity	8	22	14	175		

In all four experiments, there was a greater percentage uptake of soluble activity than uptake of the soluble stable phosphorus components. Soluble activity uptake ranged between 82 and 99%, while corresponding uptake of stable components was from 38 to 57%.

It is of interest to explore possible interpretations of the observed differential uptake of labeled and unlabeled phosphorus. Most profitable method of analysis is perhaps to make some predictions as to expected changes in the ratio of ^{32}P to SRP (P_i). Let us assume that (1) isotopic equilibrium was established between ^{32}P and all exchangeable forms of phosphorus within the bottom water and also within the surface system; (2) that all organic fractions within the bottom water were not exchangeable and therefore were not labeled; (3) that none of the organic and/or particulate phosphorus passing through the box system was transformed into P_i ; and (4) that in SRP measurements there was no hydrolysis of nonexchangeable compounds into P_i . With the above assumptions we can make the following predictions as to the ratio of activity to P_i during passage through the box:

$^{32}\text{P}/\text{P}_i$ of affluents should equal $^{32}\text{P}/(\text{P}_i + \text{P}_o)$ of the effluents,

where ^{32}P equals total soluble phosphorus activity; P_i equals SRP; and P_o is the fraction of soluble organic phosphorus in the effluent that was produced from the P_i of the affluent (P_o should be in equilibrium with labeled organic fractions of ^{32}P activity within the effluent). It is clear that the above relationship is not consistent with any existing data from the box experiments. For example, on July 3 (a.m.) the ratio of ^{32}P to P_i was 1.00. However, the ratio of $^{32}\text{P}/(\text{P}_i + \text{P}_o)$ was $10/(35 + 17)$ or 0.19. Therefore we must reject the above model and consider alternatives to the above assumptions which might produce a decrease in the ratio of labeled to unlabeled inorganic phosphorus.

If there was a large pool of unexchanged phosphorus within the organisms, assumption 1 would not be valid and the ratio would decrease. This however does not appear to be the case since the original inoculation of unlabeled plankton was small and we had allowed approximately four weeks for equilibration. The ratio of soluble and particulate activity to stable phosphorus remained relatively constant after the first week and if exchange with a large unlabeled pool was taking place the observed diurnal and other variation in the ratio would not be expected. Evidence for assumption 2 appears to be strong because all activity was dialyzable and was removed by hexanol extraction. If assumption 3 is not valid and unlabeled particulate and/or soluble organic components were converted to P_i during passage through the system, a smaller ratio would appear in the effluent. Unlabeled organics and membrane-filterable particulates might be expected to be present since the label was introduced in late winter after many decomposition products may have accumulated. An invalid fourth assumption might also give a decreased ratio if the measured inorganic phosphorus in the affluent consisted not only of ortho-

phosphate (P_i) but also labile and unlabeled fractions hydrolyzed by addition of the acid molybdate reagent. The possibility of errors of this sort was recently discussed by Rigler (1968). The possibility of the existence of labile nonexchangeable fractions in lake water certainly cannot be dismissed. Further study of this alternative is needed.

IDENTIFICATION OF PHOSPHORUS COMPONENTS

In an attempt to further identify the organic and inorganic phosphorus compounds in North Gate Lake, we collected a replicate series of samples from the surface, 3-m, 6-m, and 7-m depths on May 19 and August 6. Two 16-oz polyethylene bottles were filled from each depth. Samples were then acidified to a pH of 1.8 with hydrochloric acid and were shipped to the laboratory of the Department of Genetics and Cell Biology at the University of Minnesota, St. Paul, Minnesota. Analysis of these samples was undertaken by Mrs. K. Terry, biochemist in this laboratory.

Many of the conventional methods used in the analysis of polyphosphate and other inorganic phosphorus constituents of living cells could not be used successfully on these samples. It was necessary to concentrate samples from 500 ml to 12 ml with a flash evaporator. Concentration produced substances that interfered with the reduction of the molybdate complex in the Fiske-Subborow procedure. The interfering materials were not eliminated by extraction of isobutanol. However, by first precipitating samples with $CaCl_2$, redissolving in acid, and proceeding with analysis according to the Ames modification of the Fiske-Subborow procedure, good results were obtained.

The following procedure was used. Charcoal was added to the sample for adsorption of labile organic compounds. The charcoal was eluted with an alcohol-ammonia mixture. This eluate was then ashed and then analyzed for inorganic phosphorus in the usual manner. The volume of the filtrate was reduced from 550 ml to 15 ml by evaporation and was made 1-N with HCl. An aliquot of this solution was ashed for analysis of total inorganic phosphorus. Aliquots of ash samples were redissolved and extracted with isobutanol to again concentrate inorganic phosphorus. Color was developed in the butanol extract by the addition of molybdate and ascorbic acid.

A second aliquot was taken from the original filtrate and hydrolyzed at 100°C for 10 min. The sample was then precipitated with $CaCl_2$, redissolved in acid, and analyzed for orthophosphate according to the Ames method. This procedure was repeated without hydrolysis on a third aliquot to give orthophosphate.

The fraction eluted from charcoal should include nucleic acid. The labile inorganic fraction appeared to be chiefly polyphosphate and/or propphosphate

since none of the phosphorus could be recovered by precipitation with barium chloride at pH 2.5, and there was no coprecipitation with albumin in 5% TCA. The latter procedures would have isolated long chain polyphosphate. It did not appear that poorly ionized phosphate salts were present since with the Ames procedure hydrolysis is carried out at 55°C for 20 min.

Analyses of samples collected August 6 using the above procedure (Table IIIA) indicated a large fraction of orthophosphate at all three depths (approximately 50%) and a large fraction of unidentified soluble organic matter (35-51%). Significant quantities of labile inorganic and labile organic compounds were found at 6 m. There were indications that these compounds were present in greater quantities at 7 m; however, percentages could not be calculated because of contamination of the aliquot used in the orthophosphate measurements. At 3 m and at the surface, measurable but small quantities of labile inorganic phosphorus were found. At 3 m and the surface, orthophosphate was much higher than found in routine analyses using the Strickland and Parsons procedure. Since samples were transported with only acid as a preservative there may have been some conversion of labile inorganic phosphorus to orthophosphate before analysis. A small but measurable amount of labile organic phosphorus was extracted with charcoal from water collected at 6 m and a trace was found in samples collected at 3 m, but this fraction was not detected in surface samples.

TABLE IIIA. Percentage of soluble phosphorus fractions isolated from North Gate Lake water, August 6, 1969.

Depth (Meters)	Orthophosphate (P ₁)	Labile* Inorganic	Labile** Organic	Unidentified Organic
6	48.2	12.3	4.2	35.2
3	50.7	1.7	0.3	47.6
Sfc.	44.9	3.6	0.0	51.5

*Hydrolyzable in 1N acid at 100°C for 7 min (pyrophosphate and/or polyphosphate).

**Adsorbed by charcoal and eluted by alcohol-ammonia mixture (nucleic acid phosphorus).

Samples collected at 7 m soon after disappearance of the ice (May 19) had larger percentages of labile organic and inorganic phosphorus than was encountered in the August 6 series. A large fraction (23%) of ³²P activity was eluted from the charcoal. These findings were not consistent with the rou-

tine analyses reported above which indicated that little if any of the soluble phosphorus was in organic form at this time (May 19). A major difference in the two procedures was the addition of charcoal before filtration. There may have been labile compounds present which could be adsorbed on charcoal but which were filterable through a 0.45 μ membrane and hydrolyzed by the relatively mild hydrolysis used in analysis of SRP. Further studies of the chemistry of phosphorus compounds within the noncirculating strata are required since it is clear that there is an accumulation of labile compounds in this zone which must be related to biochemical processes that are in some way peculiar to ecosystems of this type.

DISCUSSION

Our observations of physical limnology of the lake during winter stagnation in early spring have not only contributed new data to our understanding of physical phenomena but also have important implications in regard to the cycle of nutrients within the lake basin. Demonstration of density currents moving along the basin slope and injecting mat water into the hypolimnion is a finding of considerable importance in interpretation of the nutrient cycles. Such water movements represent a way in which the leachates of the bog mat are injected directly into the noncirculating strata and await the spring overturn before they are brought into the photosynthetic zone. Thus although the input of nutrient-rich water from the mat is sizable and leads to high levels of inorganic phosphorus during summer and winter stagnation, it is clear that utilization of this phosphorus is dependent upon the amount of mixing that takes place during the overturn periods. In the case of the spring of 1969, a warm spring permitted a very limited circulation and large stores of phosphorus remained within the hypolimnion.

The observed chemocline at 7 m very likely results from density currents carrying leachate to the bottom of the lake during spring and fall when the surface water of the lake is still warm, the mat is flooded, and cool nights bring about the formation of ice on the mat surface. Such injections may continue as long as water remains on the mat and there has not been appreciable storage of heat within the mat. Injections of bog mat water into the bottom must be accompanied by a counter flow of surface lake water over the bog mat. It has been previously shown that there is a flow of surface water from the lake into the mat in late summer when transpiration of the surrounding vegetation is high. Actual phosphorus losses from the lake by such a flow must be small since at this time little soluble phosphorus is present. Flow from the mat to the lake surface might occur during the summer months with exceptionally heavy rainfall. Injections of this sort of bog leachate into the epilimnion might bring about temporary pulses of phytoplankton such as were observed in late July.

The overall picture which emerges from the data regarding the phosphorus cycle of bog lakes such as North Gate is as follows: In the spring and summer the photosynthetic zone is approximately 1 m thick. Its thickness is determined by the low transparency of the lake water, the relatively large amount of heating of the surface, and the great protection from wind action. Phosphorus input into this zone from the hypolimnion may be minimal during a warm spring when the circulation period is short and often incomplete. Flooding of the mat and warming of the mat bog water and flow of warm mat water over the surface may contribute to the relatively rapid heating and development of stratification. As the surface water warms, exchange with the mat is reduced and may be limited to the density current phenomena suggested above.

Influx of nutrient-rich water from the mat may continue in the early spring as long as there are heavy rains and there is a net transport through the lake into the surface drainage system. However, by early summer, the flow is away from the lake as the water table falls and transpiration of the bog forest increases. Thus during the major part of the growing season there would appear to be little influx of phosphorus except the allochthonous supply from wind drift and/or rain.

During this period the box experiments indicate that other factors are favorable for high production and phosphorus utilization within the epilimnion. They indicate that the photosynthetic organisms can utilize the large stores of SRP that have accumulated in the hypolimnion. These data also suggest that other organic fractions are metabolized and utilized in the food chains. Thus it would appear that only the shallow photosynthetic layer and not the bog mat lake system as a whole is nutrient limited. This zone depends upon infrequent injections from the mat system and from the bottom water but has no regenerating system of its own as in eutrophic lakes. Such instability of nutrient resources may lead to limited number of niches and low species diversity. In such a low diversity system the ecological role of Chaoborus appears to be predominant both from the point of view of energetics and from the standpoint of nutrient transport and cycling.

SUMMARY

Labeling of bottom water in a small dystrophic lake with ^{32}P and rhodamine WT provided new data regarding physical transport of water within lake basins. Evidence for horizontal transport under the ice of substances in the bottom layer is presented which suggests that these and other horizontal movements noted after the ice breakup may be the result of density currents flowing down the slope of the basin and moving horizontally in a zone of appropriate density. Evidence for the injection of mat-water in this manner into the bottom layers came from analysis of chemical conditions during the period when the surface of the lake was warming up but after temporary "cold snaps"

caused ice on the water that flooded the mat. Such injections carried phosphorus leached from the mat into the hypolimnion.

Under the ice the principal phosphorus fraction was soluble reactive phosphorus (SRP). However, at the overturn there was loss of SRP from the surface water, and rapid increase in soluble unreactive phosphorus (SUP). The SUP component increased at 3 and 6 m as the summer progressed. Both these and observations of changes in the box system show increases in SUP as SRP is used up by organisms.

The ^{32}P label equilibrated with the SRP of the 7-m water rapidly and little SUP appeared in this stratum. All of the labeled phosphorus at this depth remained dialyzable. There was little evidence indicating incorporation of the label into sediments and into organic compounds. There was a slow and steady decrease in both SRP and ^{32}P activity at 7 m which could not be accounted for by any processes taking place within this layer, but may have resulted from removal of SRP and ^{32}P by the phantom midge Chaoborus.

Experiments with the flow-through system indicated that a large fraction (up to 90%) of the SRP of the bottom water was removed during passage through a system containing phytoplankton, bacteria, and algae. Differential removal of SRP and ^{32}P suggests that there was some "metabolism" of organic fractions by the system which released SRP back into the effluent.

Preliminary analysis of the phosphorus components indicates that significant fractions of labile organic and inorganic phosphorus compounds exist in bottom strata.

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APPENDIX B

THE IMPORTANCE OF CHAOBORUS (CULICIDAE: DIPTERA) IN THE BIOLOGICAL
TRANSPORT OF PHOSPHORUS IN A MEROMICTIC BOG

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INTRODUCTION

On March 17, 1969, radioactive phosphorus (^{32}P) was put into the deeper layers of North Gate Lake, a small acid bog on the University of Notre Dame property in Gogebic County, Michigan (Hooper, 1969). After the ice went out on April 17, only the upper waters showed any vertical mixing, and a temperature gradient soon formed, stratifying the bog and closing off the bottom waters from the effect of vertical mixing by convection currents. A high concentration of ^{32}P remained near the bottom of the bog for the entire study.

After a body of water has become thermally stratified, vertical dispersal of material may be by diffusion of ions (Hayes and Beckett, 1955), by chemical reaction which leads to precipitation (Foster, 1959), or by biological action. However, if the materials to be dispersed vertically are in the bottom layers of the hypolimnion, chemical precipitation cannot be important. Also, certain biological action, such as sedimentation of plankton, will not be important. If there is evidence of upward dispersal of materials, and if one can show that dispersal is not caused by physical means, dispersal must be biological.

In North Gate Lake during the day the radioactive water in the hypolimnion was inhabited by the larvae of Chaoborus punctipennis (Say). At night these larvae migrated to the hypolimnion, and in the process carried ^{32}P into the epilimnion. This biological transport system becomes complex when mortality of Chaoborus in the epilimnion and horizontal movement of ^{32}P are considered. Since the radioactive phosphorus was in equilibrium with the stable phosphorus in the hypolimnion, the presence of ^{32}P in the epilimnion indicated that stable phosphorus might also be carried from the hypolimnion into the epilimnion.

Earlier studies on biological transport of phosphorus were chiefly concerned with downward movement (Hutchinson and Bowen, 1950; Coffin, et al., 1949). McCarter, et al. (1952), and Hayes and Beckett (1955) recognized the possibility that vertical migrations of zooplankton might carry phosphorus into the epilimnion from the hypolimnion. Hasler and Likens (1963) and Malueg (1966) demonstrated biological transport of radiiodine from the hypolimnion to the epilimnion. Main (1953) assumed that biological transport of phosphorus occurred and by measuring changes in stable phosphorus in flasks containing Chaoborus, calculated the excretion rate that should occur in the epilimnion.

Many possible mechanisms exist to explain how these aquatic animals become radioactive, but no one has demonstrated how Chaoborus gains activity if it does not ingest radioactive food. Malueg (1966) found that Chaoborus became radioactive with I^{131} in the hypolimnion, but he did not indicate the process involved.

METHODS AND PROCEDURES

STABLE PHOSPHORUS AND RADIOACTIVITY

Measurements of rhodamine WT, dissolved oxygen, pH, temperature, and radioactivity were used to monitor vertical mixing after the bog was ice-free (Hooper, 1969). Radioactivity measurements were made on water samples that had been used for stable phosphorus determinations and on water samples plated out directly from the bog. Water samples up to 15 ml in volume were placed in planchets and evaporated to dryness on a hotplate. For water samples greater than 15 ml, the water was evaporated down to 5-10 ml in an evaporating dish and transferred to a planchet. Distilled water was used to rinse out the evaporating dish, with the rinse being added to the planchet.

The methods described by Hooper and Brady (1968) for stable phosphorus determinations were used for both water samples and Chaoborus. No stable phosphorus determinations were made on plankton collections.

Nitric acid was added to samples that had been to determine stable phosphorus in order to avoid a crust forming on planchets. On water samples of 50 ml or more, nitric acid was added to digest the dissolved solids and thus give a better plating on the planchets. To measure activity, a fish stomach and Chaoborus samples were digested with nitric acid.

All radioactivity measurements were made with a Nuclear-Chicago low background beta counting system. A background count of 1.4 counts per minute was used in all results. This background count was used in order to insure that all results were significantly above background. Counting was for either 10, 20, or 30 min. Counts per minute were converted to picocuries by multiplying counts per minute by an efficiency factor of 1.2461. Picocuries were then corrected for decay so that results from various dates could be compared.

PLANKTON SAMPLES

The first plankton samples were collected on May 9 by pouring surface water through a 20 μ , Nyltex plankton net. Until May 25 control plankton samples were also collected from nearby lakes (Morris Lake and/or Hummingbird Lake). Plankton was filtered with a 0.45- μ millipore filter, the filter paper was placed on a planchet, dried, and counted. Narrow solder rings were used to hold the filter paper flat on the planchets. The plankton was weighed on a Mettler balance to the nearest mg, but since the filter papers in some instances had not been previously weighed, only estimates of weights could be made. Between 10 and 20 liters of North Gate water were strained through the nyltex plankton net for each sample.

Since the background for plankton could not be collected from North Gate

plankton, the values for Morris Lake and Hummingbird Lake were used.

On May 27-28 and on June 2-3, 20 liters of plankton samples were taken with a Kemmerer water sampler at four depths (1/2, 2, 4, and 6 m) at 3- to 4-hr time intervals and strained through the nytex plankton net. The number of Chaoborus in each sample was recorded.

The plankton from May 27-28 was divided into two aliquots; one for counting individuals, the other for measuring radioactivity. Only the larger zooplankton were counted. For copepods, nauplii, and cladocerans 60-100% of the plankton was counted while for rotifers only a few random fields were counted.

On June 24 and 25, 10 liters of North Gate surface water were poured through filtering devices of three mesh sized in order to see if the activity was concentrated in any size group of plankton. The smallest mesh size was 20 μ ; the largest was 1 mm².

CHAOBORUS SAMPLING

On six nights from June 2 through July 8, Chaoborus larvae and pupae were collected with an assortment of plankton nets. The size of the mouth of the plankton net affected the capture of Chaoborus. In July only large plankton nets could capture Chaoborus.

After making a plankton tow, Chaoborus were poured from the net onto a small screen, picked from the screen with fine forceps, put into a bottle of water, and then refiltered onto a clean screen. In this way the phytoplankton and other zooplankters could be removed. To measure activity losses, living Chaoborus were then put into filtered Morris Lake water and/or filtered surface water from North Gate Lake and/or tap water.

Measurements of losses were made on six dates between June 2 and July 8. On June 2 the length of time that Chaoborus were left in the water was not recorded. In other experiments the length of time in water varied, but for each date the time was the same for all bottles of water. The water was poured through a screen into an evaporating dish, and the Chaoborus were removed from the screen to a planchet for drying and counting. Nitric acid was added to the water in the evaporating dish, and when only 5-10 ml of water were left, the water was transferred to a planchet for further drying and counting. Approximately 400 of the Chaoborus collected at two different times on June 27-28 were weighed on a Mettler balance to the nearest mg, their activity measured, and they were then digested to measure stable phosphorus.

On July 9 and July 24 Chaoborus were collected in vertical tows with a plankton net having an opening of 0.2 m². Three individual lines were tied onto the rim of the net, and one was held taut as the net was lowered; this kept the mouth of the net in a vertical plane. At a depth of 4 m, the other two

lines were pulled taut, thus bringing the mouth into a horizontal plane. The net was then brought quickly to the surface by pulling on the three lines together as if they were one rope. Three Ekman dredge samples were taken in early June to sample Chaoborus in the mud.

RESULTS

THE RADIOACTIVITY OF PLANKTON IN NORTH GATE LAKE

All parameters measured indicated that vernal circulation in North Gate Bog was not complete. The deeper water where the radioactive phosphorus was placed was not affected by vertical circulation; thus radioactive phosphorus was not dispersed throughout the lake. However, on April 14 the particulate matter at the surface had a significant amount of radioactivity (Figure 1B). On April 16 when part of the lake was ice-free, a large number of dead Chaoborus punctipennis larvae were at the surface of the lake. At the time no connection was made between the observed activity and the dead Chaoborus. However, later, after the ability of Chaoborus to lose radioactivity to water was measured, I realized that the activity in the particulate matter came from dead and decaying Chaoborus larvae. Since the water was near freezing, the larvae could have remained well-preserved under the ice for several days. The radioactivity of Chaoborus was not measured at this time. Since plankton removed SRP almost immediately, no activity was present in filtered surface water.

Plankton samples showed that plankton in North Gate Lake had more radioactivity than plankton in nearby lakes (Table 1B). The plankton appeared to gain radioactive phosphorus as the summer progressed, although there was great variation in samples. Some of the difference between samples and sampling dates can be explained by not having precise background data. The data for May 27-28 and June 2-3 were for samples from the 0.5-m depth, and the surface might have had a higher concentration of plankton and radioactive phosphorus.

Activity measurements at this time showed that radiophosphorus was not moving vertically to the surface. At a depth of 18 ft there were traces of radioactivity, but at 15 ft the counts were at background level. On some dates the surface radioactivity measured in samples was not significant by above background but at all times there was a thick stratum of water with no radioactivity.

The radioactivity of plankton at various depths changed diurnally (Figures 2B and 3B). Since the weights of plankton and/or debris could not be accurately calculated for various depths, corrections for background could not be made. The presence of Chaoborus in some samples could affect results, but not enough to change the general trends. On June 2 the plankton at 6 m had an unusually high activity at 11:00 p.m.; this was probably a contamination from surface

Figure 1B. Activity of soluble and particulate phosphorus at various depths in North Gate Lake.

TABLE IB. Plankton activity. All samples are corrected to picocuries per m³. From May 9 - May 23, the background was calculated from plankton activity from other lakes; from May 25 - June 25, the background was estimated from the earlier background data.

Date	Depth	pc/m ³	$\bar{x}$ pc/m ³
May 9	Surface	38,550	33,225
	Surface	27,900	
May 19	Surface	162,700	216,350
	Surface	270,000	
May 23	Surface	194,900	308,600
	Surface	422,300	
May 25	Surface	220,000	220,000
May 27-28	1/2 meter	157,550	61,820
	1/2 meter	197,870	
	1/2 meter	158,250	
	1/2 meter	159,600	
	1/2 meter	99,050	
	1/2 meter	19,450	
	1/2 meter	28,950	
	1/2 meter	84,450	
	2 meters	120,850	
	2 meters	10,750	
	2 meters	80,600	
	2 meters	116,350	
	2 meters	69,550	
	2 meters	78,850	
	2 meters	12,650	
	2 meters	5,590	
June 2-3	1/2 meter	329,150	184,350
	1/2 meter	226,050	
	1/2 meter	215,550	
	1/2 meter	61,950	
	1/2 meter	71,350	
	1/2 meter	230,850	
	1/2 meter	155,550	
	2 meters	229,550	
	2 meters	112,120	
	2 meters	132,950	
	2 meters	81,150	
	2 meters	111,750	
June 24	Surface	2,404,900	2,404,900
	Surface	1,232,900	
June 25	Surface	1,232,900	1,232,900

Figure 2B. Activity of plankton, greater than 20 μ in size, from 20 liters of water collected at 4 depths every 3 hrs from noon May 27 through 9 a.m. May 28, 1969.

Figure 3B. Activity of plankton greater than $20\ \mu$ from North Gate Lake water collected at 4 depths intermittently from 8 p.m. June 2 through midnight June 3, 1969. (Water samples were 10 liters.)

water remaining in a malfunctioning Kemmerer water sampler. Most of the activity was in the epilimnion because the live plankton concentrations were greater there. If the background activities were known for the particulate matter at 4- and 6-m depths in the hypolimnion, the radioactivity at these depths would probably prove to be very low.

The radioactivity of plankton samples of June 24 and June 25 (Table IB) shows a tremendous variance and is much higher than in previous samples. Once again a better background estimate would have improved the data. Nevertheless, counts were well above background. The plankton was not gaining activity but neither was it losing activity. This is significant since the lateral movement of phosphorus in the surface waters would tend to be towards the Sphagnum mat (Hutchinson and Bowen, 1959; Hayes and Beckett, 1955; Hayes, et al., 1952; Hayes and Phillips, 1958; Pomeroy, 1960; etc.). Eventually isotopic equilibrium would be reached and the Sphagnum would no longer gain radiophosphorus from the open bog.

BIOLOGICAL VEHICLE FOR PHOSPHORUS TRANSPORT

Diurnal plankton samples on May 27-28 indicated that Chaoborus larvae and/or pupae were the only species capable of transporting radiophosphorus from the hypolimnion to the epilimnion. Rotifers (Figure 4B) showed a large change in numbers with depth within the epilimnion, but there was never a significant diurnal change in the small numbers recorded in the hypolimnion. Cladocerans (Figure 5B) were much like rotifers and did not show a migration from the hypolimnion to the epilimnion. Very few copepods (Table IIB) and nauplii (Table IIIB) were collected and therefore were probably not important in phosphorus transport. Chaoborus punctipennis larvae migrated to the upper waters at night and returned to bottom water during the day (Table IVB and Figure 6B).

Three Ekman dredge samples taken at 1:00 p.m. on June 5 had no macroscopic organisms. Thus larvae at the lake bottom do not live in the mud. During June many plankton tows were made at approximately 6 m, but Chaoborus were never caught until sundown. Hence it seems that during the day Chaoborus live in the water between the bottom and 6 m, a zone containing the highest concentrations of stable and radiophosphorus.

LABORATORY STUDIES OF CHAOBORUS

The absolute amount of radioactivity of Chaoborus decreased as the summer progressed; however, their activity corrected for decay remained fairly stable, showing perhaps a slight increase. When Chaoborus larvae were put in water, the water gained activity, while the larvae lost activity (Table VIB).

The amount of activity lost by Chaoborus larvae in water depended upon temperature, time in water, and whether the larvae were dead or alive (Table VB)

Figure 4B. Vertical distribution of rotifers in North Gate Lake, from noon, May 27 through 9 a.m. May 28, 1969. (After this graph was made, an error was found in the calculations, such that the number of rotifers per liter should be multiplied by 100.)

Figure 5B. Vertical distribution of Cladocera in North Gate Lake, from noon, May 27 through 9 a.m. May 28, 1969.

Figure 6B. Vertical distribution of *Chaoborus* in North Gate bog on May 27 through May 28, 1969 and on June 2 through June 3, 1969. Shaded area indicates that *Chaoborus* were present at that depth.

TABLE IIB. Depths at which Copepods were found in North Gate Lake, from noon, May 27 through 9 a.m. May 28, 1969. (Number of individuals per 20 liters.)

Depth in Meters	12 noon	3 p.m.	6 p.m.	9 p.m.	12 m.	3 a.m.	6 a.m.	9 a.m.
1/2	0	0	0	4	10	2		
2	0	0	1	0	2	0		
4	2	0	0	2	0	0		
6	1	2	0	0	0	0		

TABLE IIIB. Depths at which Nauplii were found in North Gate Lake, from noon, May 27 through 9 a.m., May 28, 1969. (Number of individuals per 20 liters.)

Depth in Meters	12 noon	3 p.m.	6 p.m.	9 p.m.	12 m.	3 a.m.	6 a.m.	9 a.m.
1/2	0	0	5	1	3	0	0	0
2	0	0	0	0	8	0	2	1
4	0	0	0	0	2	0	0	0
6	0	0	0	2	0	0	1	0

TABLE IVB. Number of Chaoborus in 40 liters of lake water at four depths at 3-hr intervals on May 27-28, 1969.

Depth	12 noon	3 p.m.	6 p.m.	9 p.m.	12 m.	3 a.m.	6 a.m.	9 a.m.
1/2 meter	0	0	0	0	0	2	0	0
2 meters	0	0	0	14	14	7	0	0
4 meters	0	0	0	10	2	1	0	0
6 meters	0	0	2	2	1	1	8	1

TABLE VB. Activity of Chaoborus larvae (picocuries/individual) removed from lake water and (a) plated immediately, and (b) transferred to activity-free water for varying periods before measurement.

Date	(a) Plated and Counted Immediately		(b) Transferred to Activity-free Water			
	Number	Mean Activity	Number	Mean Increase in Water Activity (pc/individual)	Activity of Larvae (End of Experiment)	Total Activity
June 2	24	159	28	59	106	165
June 12-13	40	216	133	11	205	216
June 21	61	278	48	88	178	266
June 27-28	388	231	200	49	-	-
July 7	25	304	50	30	265	295
July 8-9	67	269	47	38	285	323
Totals	605		582			
Weighted Means		239		35	216	251

TABLE VIB. Laboratory experiment to determine the loss of activity of Chaoborus larvae to water of a known activity.

Date	Number of <u>Chaoborus</u>	Number of Experiments	Average H ₂ O Temperature (°C)	Time in Water (hours)	Condition of <u>Chaoborus</u>	Average Increase in H ₂ O (pc/ <u>Chaoborus</u> /hour)
June 12-13	133	8	4.0	8	Live	1.31
June 21	34	2	17.5	17	Live	3.70
June 21	14	1	17.5	17	Dead	8.65
June 27-28	125	5	16.0	12	Dead	5.17
June 27-28	75	3	16.0	12	Live	2.31
July 7	25	1	20.0	12	Live	3.92
July 7*	25	1	20.0	12	Live	1.08

*Chaoborus put in water from bottom of North Gate Lake with high activity.

and Figure 7B). Dead Chaoborus lost activity twice as fast as live Chaoborus. In 24 hr, a dead Chaoborus could lose about 75% of its activity while a live Chaoborus could lose about 30% of its activity. The rate of loss of activity in live Chaoborus increased with an increase in water temperature. For a given date, the variation in water temperatures used was small.

On June 27-28 Chaoborus captured in the evening had a lower activity than those caught in the morning (Table VIIB). However, the Chaoborus caught in the morning had a higher phosphorus content so that the specific activity remained essentially the same. The mean specific activity for morning Chaoborus was slightly lower than for evening Chaoborus. In the epilimnion Chaoborus larvae were probably eating radioactive plankton. The difference in stable phosphorus between morning and evening Chaoborus (Table VIIB) indicated that about 0.6 mg of phosphorus per Chaoborus was taken from the epilimnion to the hypolimnion. However, live Chaoborus were losing about 2.31 pc per hour to the water in the epilimnion (Table VIB).

TABLE VIIB. Dry weight, activity, and phosphorus content of Chaoborus in North Gate bog on June 27-28, 1969.

Time	Number	<u>Chaoborus</u>			
		Average Dry Weight (mg)	Average Activity (pc)	Average Phosphorus (μg)	Specific Activity pc/μg P
10:15 p.m.	97	.247	221	6.29	35.14
10:15 p.m.	97	.247	220	6.04	36.42
4:15 a.m.	96	.250	244	6.81	35.82
4:15 a.m.	98	.253	238	6.84	34.80

THE EFFECT OF UMBRA LIMI ON THE PHOSPHORUS CYCLE

During the night of June 21, mudminnows, Umbra limi (Kirtland), were first observed. They came out of the Sphagnum mat, but did not venture far from the mat. Mudminnows were observed on all night experiments after the initial observation.

On June 21 fish were captured, and one stomach was digested for counting radioactivity. The entire stomach had a radioactivity of 12,400 pc. The other two fish were used for stomach analysis; they had 16 and 20 Chaoborus punctipennis larvae and pupae in them. Since 45 Chaoborus would be needed to give 12,400 pc, the stomach tissue probably had accumulated radioactive phosphorus. Coffin, et al. (1949), found that fish concentrated phosphorus in the gut tract area.

Figure 7B. The loss of activity from *Chaoborus* in activity-free water of various temperatures.

SIZE OF THE CHAOBORUS POPULATION AND ITS CAPABILITY FOR TRANSPORTING PHOSPHORUS

On July 9 and July 24, Chaoborus larvae were collected in vertical plankton tows; the tows averaged about 100 individuals/m² surface area (Table VIIIB).

TABLE VIIIB. Chaoborus collected in a 0.2 m² water column, from surface to 4-m depth; collections made between 1:30-2:30 a.m.

Date	Location	Number of Samples	Mean Number of <u>Chaoborus</u> per m ²
July 9	Near mat	5	108
July 9	Station B	3	72
July 24	Near mat	5	100
July 24	Station B	5	100
July 24	Random	5	129

On July 9 no small Chaoborus were present in the tows while on July 24 approximately half the Chaoborus were small Chaoborus of a new generation. On May 27-28, the density of the Chaoborus population was twice as large as on July 9. As Chaoborus pupae emerged and as larvae and pupae were eaten by fish, the population decreased. Some Chaoborus larvae died in the epilimnion, but dead larvae were only found near the center of the lake. Dead larvae were never found near the mat; mudminnows probably ate all the dead larvae in the area. In August small Chaoborus larvae could be caught in the epilimnion during the daytime.

The soluble radioactive and soluble inorganic phosphorus in the hypolimnion showed a rather steady decrease from May 12 to June 23 (Figure 8B). However, a large amount of radiophosphorus is required to account for the radioactivity of the plankton on June 24-25 (Tables IB and IXB). This activity must

TABLE IXB. Activity of three size fractions of particulate matter in surface water of North Gate Lake.

Date	Size Net or Filter	pc per Liter
June 24	1 mm ²	529
	#20 bolting silk	439
	20μ	1550
June 25	1 mm ²	90
	#20 bolting silk	348
	20μ	914

Figure 8B. Distribution of phosphorus in North Gate Lake at 4 depths from March 17 to August 7, 1969.

Figure 9B. Distribution of phosphorus in North Gate Lake, June 26, 1969.

come from the label, and only the hypolimnion had a large radiophosphorus pool. The activity present in the plankton on June 25 could account for between 16 and 48% of the activity lost by this date from the hypolimnion. Since some activity would have gone to equilibrate with the phosphorus of the bog mat, the loss of activity in the hypolimnion could be very near the gain in the epilimnion. It is of interest to attempt to determine if the Chaoborus population was large enough to account for the phosphorus change.

Radioactivity appeared to be present in plankton and in debris of all sizes (Table IXB). By using the stable phosphorus data from Hooper (1969) with the plankton radioactivity from large samples (Table IB), specific activities can be calculated. Specific activity varied from 2-13 pc/ μ g P between May 9 and June 13, but on June 26 the specific activity of the plankton was 73.82 pc/ μ g P, which was higher than the specific activity of Chaoborus. The specific activity of water at 7 m was 105 pc/ μ g P on June 26, but the soluble inorganic phosphorus had a specific activity of 236 pc/ μ g P.

Just how Chaoborus gained activity was not determined, although several mechanisms for gaining activity were apparent. When Chaoborus were put in water with high activity, they did not lose as much activity as Chaoborus put in activity-free water (Table VIIB).

DISCUSSION

Juday (1921) stated that the great abundance of Chaoborus punctipennis makes it a very important factor in the biological complex of a lake. Chaoborus are tolerant of extreme conditions (Eggleton, 1931) and often are the only important macroscopic benthic organisms. In North Gate Lake, the population size was never accurately estimated. The number of larvae and/or pupae migrating to the epilimnion was sampled in July, after the number of migrating larvae had dwindled because of emergence and mortality. Wood (1956) found that between 11 and 54% of the Chaoborus population migrated to the epilimnion at night from May through August.

Although the Chaoborus population in North Gate Lake was not as large as populations in nearby lakes it was perhaps more important because the lake did not have a complete overturn. The nutrients that accumulate in the bottom of the lake cannot be returned to enrich the surface waters if the bottom waters are not mixed. However, the system is not static and animals are capable of moving nutrients from one part of the lake system to another; this movement is called biological transport. Chaoborus punctipennis was capable of carrying phosphorus from the hypolimnion to the epilimnion and vice versa in North Gate Lake.

A large Chaoborus contained only 7 μ g of phosphorus. However, the large

number of individuals in the lake and rapid turnover could account for a significant displacement in North Gate Lake. The total phosphorus in the water, including plankton, on June 26 was about 1.25 kg. The total amount of phosphorus in Chaoborus on this date was estimated to be less than 10 g. Thus at any given time 1% of the phosphorus in the lake could be in Chaoborus.

Zooplankton is eaten by Chaoborus so that P is also carried downwards. However, the types of phosphorus may be different when going in different directions. Inorganic phosphorus might be going up, while a large portion of the phosphorus released in the hypolimnion could be organic P. Since there was no SRP in the epilimnion but a high concentration in the 7-m layer, SRP might well be lost by diffusion in the upper layers and gained by diffusion at the bottom. Thus there is a possibility of a net movement of SRP into the epilimnion as well as the observed transport of radioactivity. This does not mean that there was a net transfer of all forms of phosphorus, but only SRP. Further study of phosphorus excretion is needed to resolve this question.

In lakes where Chaoborus are limnetic, meromictic conditions are present (Hunt, 1958; Main, 1953); Teraguchi and Northcote, 1966; and Malueg, 1966). Some workers (Wood, 1956) have found Chaoborus both limnetic and benthic in the same lake, but most workers have found Chaoborus to be benthic. Juday (1921) suggested that since Chaoborus are excellent fish food, they avoid fish by burrowing in the bottom mud. However, Chaoborus live in the bottom mud of holomictic lakes when the oxygen and carbon dioxide tensions are highly unfavorable for fish.

The feeding habits of Umbra limi could be a very important factor in the phosphorus cycle of North Gate Lake because Umbra does eat Chaoborus pupae and larvae. In a laboratory experiment Umbra limi consumed between 60 and 100 mosquito larvae or pupae every 24 hr from mid-May until mid-June (May, 1968). In North Gate Lake possibly more Chaoborus larvae and pupae were eaten by mudminnows than emerged as adults during the summer. This would mean fewer emerging Chaoborus adults taking phosphorus to the terrestrial environment.

Peckham and Dineen (1957), Fish (1932), Gunning and Lewis (1955), and Keast and Webb (1966) all found that Umbra limi live in dense vegetation. Keast and Webb found that the body form of Umbra limi (stubby and cylindrical) favored life in dense vegetation. Umbra limi goes into the open water to eat Chaoborus and could lose some phosphorus there. However, since Umbra spends more time among the plants of the mat most of the eliminated phosphorus would be taken up by these plants, and the amount of phosphorus going into the open water and plankton would be less than if Chaoborus died in the center of the bog.

Since Umbra lacustris Grossinger does not live in water with oxygen tensions below 0.8 ppm (Geyer and Mann, 1939), Umbra limi probably does not go into the hypolimnion of North Gate Lake where oxygen tensions are as low as 0.0 ppm. Pearse and Achtenberg (1920) did find that Perca flavens invaded the deep water

to feed on Chaoborus larvae although the oxygen content was unfit for permanent occupation. Since Umbra limi are somewhat sluggish in the day and active at night (Spencer, 1939), there would be no advantage in going to the bottom of the lake to feed when Chaoborus are in the epilimnion at night. Also, all the other sources of food for Umbra in North Gate Lake were in the epilimnion. Almost certainly Umbra limi were not responsible for transporting phosphorus from the hypolimnion to the epilimnion; however, they were responsible for keeping SRP from the hypolimnion within the epilimnion.

Although Chaoborus might have been emerging in May, no pupae were noticed until June. Emerging Chaoborus certainly decreased the number of Chaoborus larvae. Emerging Chaoborus remove phosphorus from the entire lake system unless the adults carry the phosphorus to an area within the bog drainage so that the phosphorus is returned to the lake. Emerging Chaoborus and outflow of water are probably the most important mechanisms removing phosphorus from the lake.

The pupal skins from emerging Chaoborus have some activity, but this was only a minor input of phosphorus into the epilimnion. However, Chaoborus dying at the surface probably lose all of their activity to the epilimnion; if there are large numbers of dead Chaoborus, such as on April 14, the amount of activity gained by the epilimnion should be significant. No dead Chaoborus were tested at low water temperatures for phosphorus loss, but since decomposition would be slower at lower temperatures, phosphorus loss should not be as fast as at higher temperatures (Table VIIIB). At temperatures found in the surface water of North Gate Lake in late June, July, and August, dead Chaoborus would probably lose all of their radioactivity to the water in two days.

VERTICAL PHOSPHORUS CYCLE

When the biological transport of a biologically active nuclide was studied by Hasler and Likens (1963) and Malueg (1966), their main concerns were with how nutrients were taken out of the lakes by emerging adult insects. Malueg (1966) found that Chaoborus gained radioiodine in the radioactive hypolimnion and that when migrating to the epilimnion, probably lost some activity since the plankton had a small increase in radioactivity. He thought that this increase could have been caused by the pupal skins of the emerging Chaoborus or by excretion; he did not attempt to find out how much radioiodine was being lost by migrating Chaoborus in the epilimnion. Main (1953) without using a radioisotope determined how much phosphorus a migrating Chaoborus could lose in the epilimnion. However, since no phosphorus was labeled, there was no way of knowing that phosphorus from one area of the lake was actually lost (or transported) to another area.

This study in North Gate Lake gives strong evidence of biological transport of phosphorus from the hypolimnion to the epilimnion and vice versa by Chaoborus punctipennis. The major vertical flow of phosphorus via Chaoborus

can be summarized diagrammatically (Figure 10B). The upward movement of phosphorus into the epilimnion is followed by emergence of Chaoborus, deposition of pupal skins, mortality in the epilimnion (due in part to fish predation), and by excretion and exchange of phosphorus with water of the epilimnion. Phosphorus of the zooplankton that Chaoborus ate would be carried downward. Phosphorus could be eliminated from Chaoborus in the hypolimnion by excretion and exchange. However, due to the high concentration of SRP and radiophosphorus in the hypolimnion, ^{32}P might be gained if phosphorus diffuses in and out of Chaoborus. Some labeled and nonlabeled phosphorus atoms would diffuse out at the surface; even if there were not a net movement downward, the forms of phosphorus would continually be changing between the epilimnion and the hypolimnion.

The data from June 27-28 indicated that Chaoborus were excreting phosphorus in the hypolimnion; Main (1953), however, believed that phosphorus was excreted in the epilimnion. Since Main measured his animals, he was able to state that possibly phosphate excretion was related to the area of the body, much as is oxygen uptake. Possibly phosphorus can diffuse through the larval skin so that the concentration of phosphorus might be important in determining whether phosphorus is lost in the epilimnion. (Thus Main's excretion could be diffusion.)

Since the specific activity of Chaoborus is not as high as the specific activity of the water in the hypolimnion, the net movement of ^{32}P would be into the animal. The phosphorus in the hypolimnion is mostly soluble inorganic phosphorus, so that absorption would be feasible. In the epilimnion the soluble inorganic phosphorus is removed by the plankton so that in water with no soluble inorganic phosphorus the net diffusion from intact Chaoborus is outward. The plankton would take up this radioactive phosphorus, and as the summer progressed, activity of the plankton would increase. As the specific activity of the plankton increases, the specific activity of Chaoborus should increase since the zooplankters that Chaoborus eat have a higher concentration of radioactivity. The data indicated this general trend in that the radioactivity seemed to increase, but more data would be necessary to substantiate an increase in specific activity of Chaoborus since stable phosphorus was measured on Chaoborus on only one date.

The mode of uptake of activity by Chaoborus in the hypolimnion remains unknown and laboratory tests will be necessary to obtain conclusive results. Bacteria living in the gut or on the surface of Chaoborus might take up inorganic phosphorus from solution. Chaoborus might take up inorganic phosphorus directly from solution (absorption). Absorption can be by active uptake, diffusion, or merely exchange of ^{32}P at a site or sites in the body. Since the radioactive phosphorus in the hypolimnion is practically all soluble inorganic phosphorus, Chaoborus could not assimilate radioactive phosphorus from ingested materials.

North Gate Lake provided an excellent system for studying the phosphorus cycle because there were few organisms to complicate transfer. The only organism capable of carrying phosphorus from the hypolimnion to the epilimnion

Figure 10B. Schematic diagram of the vertical flow of phosphorus in North Gate Lake. Dimensions are not drawn to scale.

appears to be Chaoborus punctipennis. The only major predator of Chaoborus was Umbra limi. More data would make possible estimates of the amounts of phosphorus added to or taken from the various parts of the lake; the net transfer of phosphorus up or down by Chaoborus (Figure 10B) could be calculated ($\text{net } p = A+B+C+D-F$). Emerging Chaoborus would have to be considered as a loss of phosphorus from the hypolimnion, but they do not add phosphorus to the epilimnion. The horizontal movement of phosphorus would greatly complicate the phosphorus cycle, and undoubtedly much of the radioactive phosphorus brought to the epilimnion ended up in the Sphagnum and other plants of the mat. The phosphorus added to the hypolimnion by dead plankton appears to be quite low.

SUMMARY

Radioactive phosphorus put in the bottom waters of a small acid bog in late winter remained in the bottom waters after the ice went out because vertical circulation was not complete. Most of the labeled phosphorus in the deoxygenated bottom water was in the soluble inorganic form.

The radioactive phosphorus did not diffuse upward. There was always a wide layer of activity-free water, but particulate matter in the epilimnion gained radioactivity.

Plankton samples indicated that only Chaoborus punctipennis migrated between the radioactive hypolimnion and the epilimnion. Chaoborus were found to be radioactive. Further experiments showed that they could lose radioactivity in the epilimnion by diffusion and/or excretion of phosphorus.

The data indicated a somewhat complex vertical phosphorus cycle controlled by Chaoborus. The mudminnow, Umbra limi, eats Chaoborus, retains some phosphorus, and excretes some phosphorus. The excreted phosphorus most likely goes into the bog mat where the fish live. Chaoborus emerge and leave radioactive pupal skins in the epilimnion. Some Chaoborus die in the epilimnion, and with decomposition almost all of this phosphorus remains in the epilimnion.

Chaoborus eat zooplankton in the epilimnion and take phosphorus to the hypolimnion. If there were not fish, or if Chaoborus did not emerge, the net transport of phosphorus by Chaoborus would probably be to the hypolimnion. Regardless of the direction of net transport the large pool of soluble inorganic phosphorus in the hypolimnion seems to be enriching the epilimnion by biological transport.

The exact mechanism by which Chaoborus becomes radioactive in the hypolimnion was not determined.

Chaoborus were not found in the mud; this seems to be normal for meromictic lakes. The concentration of certain elements(s) in the water may determine whether Chaoborus enters the mud.

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