INORGANIC NITROGEN METABOLISM AND PHYTOPLANKTON PRIMARY PRODUCTIVITY IN A SUBARCTIC LAKE

A

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INORGANIC NITROGEN METABOLISM AND PHYTOPLANKTON

PRIMARY PRODUCTIVITY IN A SUBARCTIC LAKE

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ABSTRACT

The relationship between nitrogen utilization and primary productivity was studied in a subarctic lake, using the stable isotope ^{15}N to measure the uptake rates of ammonia, nitrate and molecular nitrogen. Primary productivity determinations employed the ^{14}C method. A combination of these methods with the quantitative determination of nitrogen nutrients during two seasons provided information on the role of these nitrogen sources in the productivity regime of this particular lake.

The study lake was Smith Lake, located on the University of Alaska campus. Its shallow depth, and lack of inlet and outlet during much of the productive season made it interesting from the point of view of an internal cycle study. The lake is characterized by two major growth periods for photosynthetic organisms. The first takes place under the ice, under low light conditions, and is predominantly composed of green algae and flagellates. The major nitrogen source at this time is ammonia. The major bloom takes place in June, and the dominant alga is Anabaena flos-aquae. Ammonia, nitrate and molecular nitrogen all play a part in the nitrogen supply for this bloom. Following this bloom, which declines abruptly, a low and more steady production continues through the summer, with ammonia providing most of the nitrogen. Periods of very high photosynthetic rates are always accompanied by high rates of ammonia assimilation. From the amount of ammonia

in the water, and the rate of removal, a very high rate of turnover for ammonia is established. Direct measurement of the rate of ammonia supply using an isotope dilution technique supported this conclusion.

ACKNOWLEDGMENTS

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Hutner and Provasoli, 1964

INTRODUCTION

Statement of the problem

The biogeochemical cycles of certain elements are intimately involved in controlling the potential biological productivity of an environment. These elements either are required directly as major nutrients or as quantitatively minor components of vitamins or enzymes. Nitrogen primarily belongs to the former category, and its complex cycle plays a role of great ecological importance.

A lake is a discrete ecological system in that it exhibits its own internal cycles, which are, however, affected by the influents and to some extent by the effluents. This concept of a lake as an ecological entity was first introduced by Forbes (1925). In the past, a large number of measurements have been made of the abundance of various compounds of nitrogen in lake water, since it was assumed that such information gave a good idea of the amount of nutrient available, and therefore of the fertility of the water. The current interest in nutrient cycles developed partly in response to the realization that this assumption is not valid, and that the standing concentration of a nutrient is in many cases less important than its rate of replenishment by advection and regeneration. Furthermore, the concentration of a nutrient in the water may have an inverse relationship with production, since a high concentration is unlikely to persist when the substance is being removed rapidly, but may accumulate when it is not. There is evidence, which largely comes

from work on the oceans, that the rate of regeneration in situ in the water column is much more important than had previously been considered (Ketchum, Ryther, Yentsch, and Corwin, 1958). Since there is no reason to believe that marine and lacustrine processes are fundamentally different, this may be equally true of fresh water.

Although standing concentrations alone cannot give an understanding of the availability of a nutrient which undergoes a cycle in transport, measurements of the actual rates of utilization can do so, because a rate of utilization implies a minimum rate of supply. This dissertation is the result of an attempt to investigate the relationships between the utilization of inorganic nitrogen sources and primary productivity, and at the same time to obtain insight into the role of the internal nitrogen cycle of a lake.

The populations in a water body are not static throughout a year, but tend to demonstrate seasonal changes which may be repeated from year to year. This annual repetition is only found where the lake as a whole is not changing significantly from one year to the next. These changes are believed to be in response to physical and chemical factors in the water and to alterations in these factors by the organisms present. There are available in the literature excellent and detailed descriptions of studies of such changes in terms of the composition of the populations and the abundance of the constituent organisms (Utermohl, 1925; Silvey and Roach, 1964; Prowse and Talling, 1957; Hartman and Graffius, 1960). These changes in population composition are accompanied by fluctuations in the primary productivity rates in the lake (Verduin, 1960); in turn, probably, they are accompanied

by variations in the patterns of utilization and regeneration of nitrogen compounds in the water.

The interactions resulting in net production are highly complex, but ultimately algae and bacteria interact in producing fluctuations in productivity, which may result in efficient utilization of the inorganic resources available. In order to obtain a reasonable picture of the nutrient regime of a lake, the study must be carried out throughout a considerable span of time. This has been attempted.

Ability of phytoplankton to assimilate various compounds of inorganic nitrogen

The work discussed here will deal with the relationship between the utilization of nitrogen and primary productivity. The experimental techniques employed in this study derive from the considerable information available from laboratory studies on the ability of algae to use various sources of nitrogen. Algae are, of course, the most important agents of primary productivity, both in terms of biomass and in their contribution of photosynthetically reduced carbon in pelagic water.

Nitrogen fixation. Molecular nitrogen represents a virtually inexhaustible reservoir for those organisms capable of carrying out its reduction to ammonia and thus of using it as a nitrogen source. Nitrogen is one of the major gases in the atmosphere, occupying 78$\pm$ 0.004 percent of its volume (Byer, 1959). The biological process of using molecular nitrogen is termed nitrogen fixation, and consists of an energy-consuming series of reactions. In a few organisms the processes of nitrogen fixation and photosynthesis are closely coupled

so that the energy trapped by the photosynthetic apparatus is also used directly or indirectly for the reduction of molecular nitrogen. The two groups demonstrating this relationship are the photosynthetic bacteria and the blue-green algae. Both groups are found in lake waters, but the blue-green algae are more likely to make a significant contribution to the nitrogen economy of the water. Photosynthetic bacteria appear to occur most abundantly in environments where fixed nitrogen compounds are present, and under these conditions bacterial nitrogen fixation is believed to be suppressed. Fogg (1956) therefore believes that although these bacteria may have had great importance early in the history of the earth, their nitrogen fixing ability remains as a facultative but not often important relict. A considerable amount of information is now available on the physiology and mineral nutrition of nitrogen fixing blue-green algae or Myxophyceae. Molybdenum is specifically required in higher amounts for nitrogen fixation than for the reduction of nitrate, although it is required also for the latter reaction (Wolfe, 1954); present information indicates that ammonia assimilation is independent of molybdenum concentration. Iron and boron are also required specifically for nitrogen fixation. Various other minerals, including calcium, sodium and cobalt, are required by nitrogen-fixing Myxophyceae for general growth. Among the requirements for nitrogen fixing activity, Webster (1959) includes the need for a vigorous metabolic rate. In photosynthetic nitrogen fixation this implies a high rate of photosynthesis, since the energy for the fixation process is derived from the photosynthetic process (Fogg and Than-Thun, 1960). A high rate

of photosynthesis also implies a high rate of removal of essential nutrients from the water. A steady supply of phosphorus and trace metals should be required for sustained high nitrogen fixation rates.

The physiology and ecology of photosynthesic nitrogen fixation by blue-green algae has been discussed by Allen (1956), and also in detail by Fogg (1956). A more recent review by Yocum (1960) emphasizes biochemical aspects of nitrogen fixation.

Ammonia. Ammonia is readily used by algae, and provides the optimal source from an energetic point of view, since all nitrogen compounds are brought to the reduction level of ammonia before assimilation into the metabolic pathways of the organism. In culture media, however, the unfavorable pH changes which accompany growth and the concomitant removal of ammonia from the medium frequently preclude its use. There is no evidence that any algae are unable to use ammonia in a natural situation. In the nitrogen cycle, ammonia is considered an intermediate, since, although it is released upon the breakdown of nitrogenous organic material and excreted in addition by animals, it is in turn oxidized through nitrite to nitrate. Nitrate is therefore the more stable end-product of the regeneration process. This oxidation process is called nitrification, and is carried out in soil and fresh water by bacteria belonging to the genus Nitrosomonas and the second step to nitrate by Nitrobacter spp. Nitrite, an intermediate step in this process, very seldom accumulates to any significant levels in water, and although algae are able to use it as a nitrogen source, it is seldom sufficiently abundant to be important. Under polluted conditions, where large

amounts of nitrite may occur, two factors are of importance. One is that nitrite is toxic under conditions of high concentration, so that it will not be used. Secondly, the oxidation of nitrite to nitrate is prevented by a high initial concentration of nitrite, so that the high concentration will tend to remain static or increase (Aleem and Alexender, 1960). In contrast, nitrate is often present in sufficiently large amounts to be significant.

Nitrate. Most algae are believed to be capable of using nitrate as a nitrogen source. The reduction of nitrate to nitrite is catalyzed by an enzyme termed nitrate reductase, which is a metallo-flavoprotein with molybdenum as the metal constituent. Nitrite reductase then catalyzes the subsequent reduction of nitrite to ammonia; here the metal components are iron and copper. Recently Nicholas et al., (1962) have implicated cobalt in nitrate reduction, although this work was not done with algae, but rather with Rhizobium. Light is beneficial to nitrate reduction in green plants, although some algae are capable of carrying out the process in the dark. Nitrite reduction is frequently coupled to respiration. Kessler (1964) has discussed the assimilation of nitrate by plants, mainly from the biochemical point of view.

In addition to the three sources of nitrogen already mentioned above, several organic compounds may serve as nitrogen sources for algae. Urea, uric acid and xanthine are often useful sources according to Hutner and Provasoli (1964). However, this ability to use organic sources is very variable between different genera and even between different species of algae, and with only the presently

available information it is difficult to evaluate its possible significance. In this research, therefore, only the three minor sources discussed above were used.

Previous work on nitrogen in the ecology of lacustrine environments

Nitrogen fixation. Blue-green algae are widely distributed and include species capable of surviving under rather extreme environmental conditions. According to Hutner and Provasoli (1964), workers have found that many blue-green algae were able to grow after storage for eight to nine weeks at -15°C. They are also highly resistant to desiccation, and their occurrence in hot springs at temperatures approaching the upper limit for living systems completes a picture of extreme versatility for this group. The presence of blooms of Anabaena in lakes has suggested that these algae may contribute substantially to the nitrogen budget of lakes by fixing molecular nitrogen. Hutchinson (1957) made this suggestion in connection with the Anabaena bloom in Linsley Pond. Guseva (1941) finds that nitrogen-fixing Anabaena are important in the biology of the Ucha Reservoir. In Crater Lake, Oregon, a bloom of Anabaena has been suggested responsible for the nitrogen supply of the lake (Utterback, Phifer, and Robinson, 1947); the phosphate to nitrogen ratio appears to be very high in this lake. Prowse and Talling (1957) attribute to nitrogen fixation by Anabaena a major role in determining the seasonal succession of algae in a reservoir on the White Nile. Here, a bloom of Anabaena during a period of low nitrogen

content was followed by algae which were apparently using nitrate. If this were so, it would indicate that the nitrogen fixed by the algae became available rapidly after its release following the death of the cells. In none of these cases was there any direct evidence to support the nitrogen fixation theory. Indirectly, however, the evidence strongly supported the conclusion, since in each case a large number of organisms with a high nitrogen content grew without any apparent supply of nitrogen.

Direct experiments to try to measure nitrogen fixation were carried out by Sawyer et al. (1941); he incubated lake water for long periods of time and measured the increase in nitrogen by the Kjeldahl method. Dugdale, Dugdale, Neess and Goering (1959) made preliminary attempts to measure nitrogen fixation in lakes by measuring the incorporation of ^{15}N labelled nitrogen gas into the particulate fraction in samples of lake water. Subsequently studies were carried out using the same technique, which showed that nitrogen fixation can be a major source of nitrogen to the phytoplankton in an eutrophic lake when blue-green algae of the genus Anabaena are dominant (Dugdale and Dugdale, 1962). A study of the rates of nitrogen fixation in several lakes in the vicinity of Madison, Wisconsin showed considerably varying roles for the process among lakes in a restricted geographical area (Goering 1962, 1964). In all these studies the behavior of the process in relation to light strongly indicated that photosynthetic processes were responsible for the nitrogen fixation either directly or indirectly. In each case when high rates of nitrogen fixation were measured, blue-green algae were present in large numbers. Fogg (1956),

in his review dealing with nitrogen fixation by photosynthetic organisms, mentions the possibility of a major role in the nitrogen economy of eutrophic lakes for nitrogen fixation by blue-green algae, but qualifies this by adding that many of the bloom-forming species do not fix nitrogen. Some species of Anabaena have been tested in pure culture and found to fix nitrogen, although not many of the components of the blooms discussed above have as yet been isolated in bacteriologically pure cultures.

As early as 1938, Fritsch and De recognized the role of Nostoc in the fertility of rice fields as a nitrogen fixing agent (Fritsch and De, 1938). De also recognized a similar role for Anabaena (De, 1939). Nostoc is also responsible for fixing nitrogen in tundra ponds in Northern Alaska (Dugdale and Toetz, 1961), whereas in the Kodiak, Alaska region, a lake exhibiting high rates of nitrogen fixation had a large population of Anabaena flos-aquae (R. C. Dugdale, personal communication). It is apparent, therefore, that significant nitrogen fixation occurs in waters ranging from tropical to the arctic. The hardiness of the blue-green algae does result in a wide geographical distribution.

Ammonia and nitrate utilization. A large part of the information on nitrogen utilization stems from an interest in its role as a limiting factor. Nitrogen, in combined form, is often present in sufficiently low amounts so that the rate of photosynthesis is directly controlled by its availability. Gerloff and Skoog (1957) have studied the role of nitrogen from this point of view, using

a combination of laboratory and field methods. They established from laboratory work that the cell nitrogen content of algae could be used as an index of the adequancy of the combined nitrogen supply in the medium, and then used this information in identifying instances of nitrogen limitation in the field. Hutchinson (1941), found, however, that even where a single nutrient appeared to be limiting in Linsley Pond, the situation was not that simple. The addition of nitrogen and phosphorus together in an enrichment experiment increased the growth rate considerably more than the addition of either of these nutrients alone, even though each had a stimulating effect also by itself. Goldman (1960) attributes the cline in productivity across Brooks Lake, Alaska to higher nitrate levels at one end than at the other. Unfortunately he did not measure nitrate levels in the water in connection with this work; such measurements would have helped to confirm the hypothesis. Goldman's work on Castle Lake (1960) similarly indicates at least a partially limiting role for nitrogen. There would seem to be difficulties inherent in assuming that one factor is actually solely responsible for controlling the rate of photosynthesis. A large number of factors, perhaps variably weighted in importance, would seem to be responsible. In the case of nitrogen, there should never be any limitation when suitable conditions for nitrogen fixation exist. At present the conditions favouring nitrogen fixation in natural waters are not known. Trace metals are probably important in addition to chelators which would make these metals as well as phorphorus readily available.

Additional components of the nitrogen cycle to be considered in this study

The process of the regeneration of inorganic forms of nitrogen, ultimately nitrate, from organic material was discussed briefly above in connection with the supply of various forms of nitrogen available to algae. The fate of the nitrate produced varies, and it may under some circumstances be completely lost to the biological system by the process of denitrification. This bacterial process is a form of respiration, in which nitrate serves as the ultimate hydrogen acceptor, and is reduced to nitrogen gas or to N_2O. This takes place only under anoxic conditions, where the oxygen content is below 0.1 ml/liter (Koyama, personal communication, R. C. Dugdale). Under some conditions the nitrate is reduced only to nitrite, but it is not clear whether this type of process ever results in reduction to ammonia. This type of reduction is sometimes termed 'dissimilatory' in contrast to assimilatory processes in which the nitrate is reduced and then used for biosynthetic purposes. Denitrification results in loss of nitrogen to the biosphere, and thus acts in an opposing manner to nitrogen fixation.

A source of nitrogen which has been ignored in the above discussion is the nitrogen content of the influents. The magnitude of this supply can be easily estimated from the volume of influent water and its nitrogen concentration. Rainfall will also bring in a small amount of combined nitrogen. Both of these sources will be largely ignored

here, since no attempt is being made to draw up any complete nitrogen budget for the lake, but rather to study one aspect of this budget in the biological utilization of nitrogen compounds. The nature of the experiments allows the estimation of uptake rates while ignoring the effects of advection, since the samples are enclosed in bottles and thus isolated for the duration of the experiment. Overall nitrogen budget studies have been made, as in the study of the nitrogen budget of Lake Mendota (Rohlich and Lea, 1949). The internal cycle has not been studied. Ryther and Guillard (1959) have pointed out that we know very little about the rates of regeneration in situ in the surface waters of the sea. The same statement can be applied with equal validity to our knowledge of lakes.

Experimental approach

The stable isotope of nitrogen, ^{15}N was used to measure nitrogen uptake for this work. Previously ^{15}N was used for measuring rates of nitrogen fixation by Neess et al., (1962), and subsequently the technique was extended to include measurements of the uptake rates of nitrate and ammonia by Dugdale and Dugdale (1965). The use of isotope techniques in this study was necessary in order to attain sufficiently high sensitivity using short incubation times. In this way an approach to measuring an instantaneous rate can be made. A radioactive isotope of nitrogen having a sufficiently long half-life for field use is not available. ^{13}N has been used in physiological work on bacterial nitrogen fixation, but its use is only practical in a laboratory with the facilities for producing the isotope (Nicholas

et al., 1961).

Measurements of the rates of utilization of molecular nitrogen, ammonia and nitrate were made periodically in the lake during two years. Simultaneously, primary productivity was measured, using the ^{14}C method of Steemann Nielsen (1952). Chlorophyll *a* was used as an index of plant biomass. Particulate nitrogen was used in a similar way and also was a necessary parameter for calculations of the absolute rates of nitrogen uptake. Determinations were made of the nitrate, nitrite and ammonia content of the water for each experiment. Analyses for phosphorus content were also included.

The information obtained by the procedures described should suffice for the purposes of this study. However, routine limnological observations were made in addition, so that a more complete interpretation of the environmental influences on the nitrogen regime could be made. These will be described below.

The lake

The lake selected for this work is small with a surface area of about 30 acres, and is located on land belonging to the University of Alaska. The surrounding land is largely a wildlife study area, and therefore is not used extensively for recreational purposes by the public. Some areas of the watershed are under cultivation, and it is possible that fertilization of these lands may affect the nutrient content of the lake during the time of year when there is considerable runoff. Smith Lake has a maximum depth of three meters and an estimated mean depth of two meters. However, the depth undergoes fluctuations

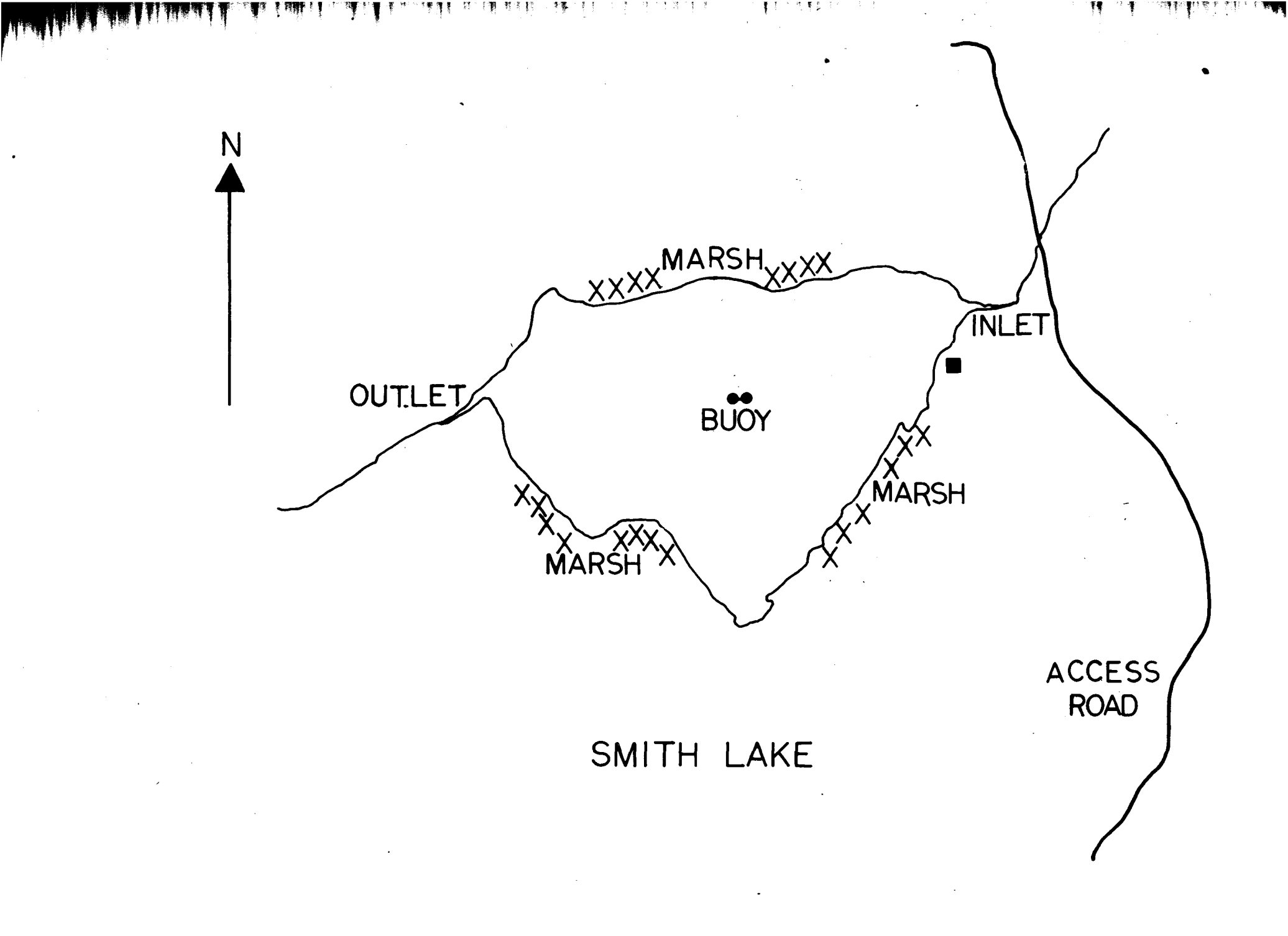
seasonally, with a considerable loss of water due to evaporation during the summer. The lake occupies a depression in a region of spruce, white birch, aspen and alder forest which is dry during the summer, but is essentially a marsh during the spring thaw period. There is no permanent inlet, but during the spring thaw much of the incoming drainage water enters through two distinct streams. There is a single outlet, also functional only during the Spring. During the summer, rainfall provides the only significant source of incoming water.

During the early part of the season there is very little rooted vegetation. In late summer, the rooted and submerged shore vegetation assumes an increasing relative importance.

The lake water shows a strong brown color which is often characteristic of arctic and subarctic lakes. This material appears to be similar to that described by Shapiro (1964). It is very stable, not readily precipitated and it absorbs strongly in the shorter wave lengths, showing no peak, but only end-absorption. The intensity of color is directly related to pH. Thin-film chromatography of this material resulted in a separation whereby a yellow component separated and moved ahead of the other components on the chromatogam.

Figure 1 is a simple outline map of Smith Lake which shows the position of the buoy which was used for incubating samples and also as the major sampling site.

Figure 1. - Outline map of Smith Lake, Alaska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SMITH LAKE

METHODS

Nitrogen-15 tracer methods

The method for measuring the rates of nitrogen fixation in water samples is a simple application of the tracer method used for protein metabolism studies by Rittenberg et al. (1939) and applied to studies of biological nitrogen fixation by Burris and Miller (1941). The modification for lake water work has been described in detail by Neess et al., (1962). A reprint of this paper, which includes details of the techniques and of the special glassware required, is attached for reference (Appendix A). Details are not repeated here. In brief, the process involves removing the nitrogen gas present from an enclosed water sample by flushing at reduced pressure with a helium-oxygen mixture (80:20), and then replacing the removed gas with nitrogen containing a known enrichment of ^{15}N. After incubation for a prescribed period of time, the particulate fraction is recovered and analyzed by mass spectrometry for enrichment in ^{15}N.

The procedure for measuring the uptake rates of ammonia and nitrate is based on a similar principle. It is necessary to add labeled KNO_3 or NH_4Cl to the water sample, incubate, and then isolate the particulate fraction and analyze it for ^{15}N content. Ordinary one liter Pyrex reagent bottles are used for these experiments. The isotope solutions are prepared in bulk and dispensed into ampoules, with each ampoule normally containing two μg-atoms ^{15}N in one ml

solution. These ampoules are autoclaved immediately after preparation. The amount of labeled substance added to a lake water sample in an experiment is usually close to ten percent of the amount of the same form of nitrogen present in the volume of lake water used. At very low concentration in the water, it is impractical to follow this rule.

The post-incubation procedure consists of filtering the water through a Hurlburt glass filter (984-H ultra filter; Reeve, Angel and Co.), and then drying the filter. In this way samples can be stored for a reasonable period of time in a dessicator without deterioration. The mass spectrometry was carried out using a Bendix Time-of-Flight mass spectrometer (Model 17-210). The high sensitivity of this machine is of great value in handling samples of variable nitrogen content. The inlet system of the machine was connected via a liquid nitrogen trap to a modified Coleman nitrogen analyzer, so that by using the Dumas combustion in the analyzer, the evolved nitrogen was introduced directly into the mass spectrometer. Details of this modification can be found in Dugdale and Barsdate (in press). The samples are ground up with a small amount of cupric oxide, with no effort made to separate out the glass filter, since these filters have been found to contain only a small and consistent amount of nitrogen. The sample is then introduced into the combustion tube of the nitrogen analyzer.

Incubation procedures

During the first year of the study, incubation was generally carried out *in situ*, with the bottles suspended in the lake from a buoy. During the second year, a variety of methods were employed. Incubation in a constant temperature room at 20°C under standard light conditions became the usual method, with additional bottles incubated in a tank on the roof of the building, under natural light conditions, but with rather variable ambient temperatures. Nitrogen fixation flasks were in addition incubated in the lake whenever possible, since nitrogen fixation was found to be more sensitive to differences in light and temperature than were nitrate or ammonia uptake.

Calculations

The isotope ratios calculated from the mass 29 and mass 28 peaks are converted into atom percent values according to the formula:

$$A_f = \frac{100R}{2 + R}$$

where A_f is the atom percent of the particulate material at the end of the experiment. From this the atom percent of normal unenriched nitrogen is subtracted. This value is determined from

isotope ratio blanks run on unenriched filtered lake water samples.

The atom percent of the nitrogen supplied at the beginning of the experiment must be known in order to calculate the amount of nitrogen taken up. In the case of nitrogen fixation experiments, where all the unlabeled nitrogen is removed, it is simply the enrichment in the supplied gas. For nitrate and ammonia experiments, the amount of unlabeled nitrate or ammonia in the water must be included in the calculation. The atom percent of the available nitrogen at the beginning of the experiment will be designated A_i. The amount of nitrogen taken up during the experiment is then:

$$N_f = \frac{N_t A_f}{A_i}$$

where N_t is the total particulate nitrogen in the sample. This method of calculation is valid as long as the change in N_t during the experiment is very small. In practice the change in N_t during an experiment very seldom exceeds 1%. In order to calculate the rate, the total uptake is divided by the incubation time.

Proportional uptake is sometimes used:

$$\text{Fractional uptake} = \frac{A_f}{A_i}$$

The formula for calculating percent uptake is:

$$\text{Percent uptake} = 100\ \frac{A_f}{A_i}$$

All these methods of calculation will be used below.

Primary productivity measurements

Primary productivity was measured by the ^{14}C method of Steemann Nielsen (1952), using similar incubation procedures to those described for the nitrogen uptake experiments above. However, in situ depth series were included periodically. Dark and light 125-ml bottles were used, and the ^{14}C was added from ampoules, with reasonable precautions to shield the bottles from direct light when this was done in the field. Each ampoule contained 5 c in one ml of solution, and the absolute counts per minute per ampoule was obtained on the Tracerlab scaler (Model Sc-33A with SC-10A sample holder and TGC-2 end-window Geiger tube) used for sample counting by the $BaCO_3$ precipitation methods described by Strickland and Parsons (1960). Following incubation, the samples were filtered through an HA Millipore filter dried and counted. A dilute HCl rinse was used after filtration to remove any inorganic carbon precipated as carbonate. The following formula was used to calculate the amount of carbon fixed:

$$\text{Mg C assimilated} = \frac{\text{Final counts per minute (light-dark)}}{\text{Counts per minute supplied}} \times 1.06 \times C_a$$

Where 1.06 is the correction for the isotope effect which favors the uptake of ^{12}C, and C_a is the total available carbon in mg per unit volume in the water, calculated from the alkalinity and pH information. This result is then divided by the incubation time in hours to obtain an hourly rate.

Particulate nitrogen determinations

Particulate nitrogen was measured using a Coleman nitrogen analyzer. A known volume of the sample was filtered through a glass filter (similar to that used in the isotope experiments), the filter was dried and then introduced into the nitrogen analyzer in its entirety after grinding with cuprox (Coleman grade cupric oxide). Blanks were determined on several filters for each batch of analyses and their mean value was subtracted from the individual sample values to compensate for the nitrogen content of the filters.

Chlorophyll *a*

The method used for determining chlorophyll *a* levels in the water was a conventional chlorophyll extraction using acetone as the solvent. The sample was filtered through a Whatman glass micropore filter, the filter placed in a centrifuge tube and 5 ml of a 90% acetone:water mixture was added. The volume of water filtered was generally one liter; however when there was a large amount of particulate material present a smaller volume was filtered to insure rapid filtration. A few drops of a $MgCO_3$ suspension was added during filtration. The centrifuge tube was then tightly sealed, and the extraction was carried out in a refrigerator in the dark for at least twenty-four hours.

The percentage transmission of the extracts obtained in this way were read in a Beckman DB recording spectrophotometer. One-

centimeter cells were used, with a 90% acetone:water mixture in the reference cell. Wavelengths between 750 and 450 mμ were scanned and recorded. In this way percentage transmission curves were obtained, usually for three depths, with each experiment.

There has been discussion recently about the relative merits of various ways of calculating the results of chlorophyll analyses, and a lot of criticism of the conventional procedures in the literature. In particular, the validity of the values obtained by the Richards with Thompson (1952) equations have been questioned (see, for instance, Riley, 1963). Strickland and Parsons (1963) have revised these equations to correct for low specific absorption coefficients used in the original work and to correct for an overestimation of chlorophyll c. The results calculated by these revised equations do not agree with those obtained by using the formula of Arnon (1949), whose equation for total chlorophyll has in turn been revised by Bruinsma (1961).

In this work, the method of calculation uses the specific absorption coefficient for chlorophyll a given by Bruinsma (ibid.) The formula obtained is:

$$\frac{O.D._{658} \times 14.9 \times \text{volume of solvent}}{\text{volume filtered}} = \text{mg/liter Chl. a}$$

The optical density at 750 mμ is first subtracted from the optical desity at 658 mμ, and then the calculation is carried out as above. The results obtained in this way are a little lower than those obtained by Bruinsma's calculation method for total

chlorophyll using the same water sample. Although this almost certainly does not give an exact result in terms of chlorophyll *a* concentration, the results obtained in this way appear to be consistant and to reflect the changes in chlorophyll *a* in the water, and indeed are probably fairly close to the true concentration. Further refinements in calculation do not seem justified for the purposes of this work, especially in view of the confusion and controversy in the current literature.

Methods for the quantitative analysis of nutrients in the water

Nitrogen compounds. Nitrate was measured by a modification of the Mullin and Riley procedure (1955) similar to that described by Strickland and Parsons (1960). In this method, nitrate is reduced to nitrite by hydrazine sulfate in the presence of copper as a catalyst, and the resulting nitrite is measured spectrophotmetrically after coupling with sulfanilamide to form an azo dye. An additional precaution proved essential for this particular study, since the lake water had a high but variable complement of an organic material which made copper less available as a catalyst for the reduction. It was necessary to compensate for this by adding an experimentally determined additional amount of the metal.

Nitrite was determined using the same method, in this case omitting the reduction step. For both nitrate and nitrite determinations, compensation was made for the variable water color by using internal standards rather than comparing the values with a

distilled water standard curve. Such standards were run for each individual sample, so that variability from sample to sample would not affect the results. A Beckman DU spectrophotometer was used for these determinations; cells with a light path of 1, 5 or 10 cm were used as required for accuracy. The samples for these analyses were micropore-filtered immediately after collection and stored in polyethylene bottles in a freezer prior to analysis.

The samples for ammonia determinations were frozen unfiltered in polyethylene bottles. No significant changes in ammonia concentration were found when samples were stored frozen for periods of time up to six months. Vacuum distillation or diffusion methods were used to concentrate the ammonia in the samples, with apparently good recovery. The analysis for ammonia, which was collected in .01 N HCl, was done by the sodium phenate method of Riley (1953). A new method developed by Richards and Kletsch (1964) was used for samples collected later in 1964. This method is based on the oxidation of ammonia to nitrite using sodium hypochlorite in strongly basic solution, and the subsequent determination of the nitrite.

Phosphorus determinations. Phosphorus as dissolved phosphate was measured by the method described by Stephens (1963), in which the molybdenum blue complex is extracted into isobutanol. It was necessary to use this method because the brown organic material in the water masked the blue color at the wavelengths used for reading, but fortunately was not extracted to any great extent into the isobutanol. This method also has the advantage, at low phosphate concentrations, of concentrating the color prior to reading, resulting

in a high sensitivity.

Total phosphorus was measured by concentrating the sample and digesting with perchloric acid, followed by the use of the standard phosphate procedure. Dissolved phosphorus (total) was done in the same way, but using micropore filtered samples.

Techniques used for other analyses

Temperature was measured at half-meter depth intervals using a Whitney thermistor thermometer. Light penetration determinations also employed a Whitney underwater instrument. pH was measured in the laboratory using a Beckman expanded scale pH meter. Specific conductivity was read at approximately 20°C using a Serfass conductivity bridge (Model RCM 15).

Alkalinity titrations were carried out using .01 N HCl, and a Beckman expanded scale pH meter to determine the end-point. From this information and the pH, the total available carbon for the ^{14}C uptake calculations could be calculated. Oxygen determinations used the Alsterberg modification of the Winkler method, with samples fixed in the field and titrated in the laboratory.

Phytoplankton samples were preserved by adding Lugol's solution described by Verduin (1960) to lake water in a polyethylene bottle.

PHYTOPLANKTON

Qualitative information about the dominant algae during 1964 in Smith Lake indicates that there is indeed a variation in population composition accompanying the variations in the nitrogen uptake regime. Counts were not made, and thus quantitative data are not available. In addition, any relatively rare components of the algae flora would have been missed by the method used, which involved filtering a small aliquot of lake water, preserved or freshly collected, through either a millipore filter (HA) or a Gelman GM-6 filter, pore size .45 μ. The filter was examined microscopically after drying and clearing with cedar oil.

During the winter months, only zooplankton were present in detectable numbers. These disappeared with the absence of oxygen in the water in spring. In April the algae which were subsequently present throughout the first productive time appeared, and were most numerous in the warmer water near the bottom. These were dominated numerically by Euglena sp., Chlorella sp., and small flagellates. A few Gymnodinium and Gloeocapsa sp. were found in addition. This population persisted during the entire ice-covered period, and also subsequently during the first few days of June, with Selenastrum sp. and a colonial form tentatively identified as Pyrobotrys sp. appearing at this time. The first appearance of large numbers of algae in April corresponded in time with the presence of traces of oxygen in the water, and the reappearance of large numbers of immature zooplankton.

On June 6, Anabaena spp were found for the first time, and within a few days Anabaena filaments were abundant. During this building-up phase, the filaments were long, with no differentiated cells, and all the cells had the appearance of dividing actively. These algae were restricted to the surface meter. At the bottom the early spring population persisted in small numbers, again dominated by Chlorella and colonial forms. The thermal and chemical stratification of the water was thus also reflected in a biological stratification at this time. On June 10, the Anabaena in the surface were very numerous and the population was unialgal. There were very little zooplankton present. Within a few days, the Anabaena had formed heterocysts and akinetes, and the bloom began to form scum on the water surface. In the deeper water the spring populations persisted, with some algae now attached to decomposing Anabaena filaments. Following this, the Anabaena appeared to deteriorate in condition, and free akinetes were abundant in the water as well as Anabaena filaments which were surrounded by large numbers of bacteria, clearly visible when fresh material was examined with phase-contrast microscopy. Very few algae were found in the deeper water now. The akinetes were large, often equivalent in length to six normal cells in the filament. As the bloom declined, Euglena sp., Chlorella sp., and Selenastrum sp. reappeared, although a few Anabaena filaments were still present.

During July, Selenastrum sp., Gloeocapsa sp., and Euglena sp. were the main constituents of a very small algal population. Aphanizomenon sp. had now appeared at one meter depth, and Gymnodinium sp. was also present at two meters. Large zooplankton, mostly copepods,

appeared as soon as the Anabaena bloom declined. These persisted during the entire remainder of the summer.

August represented a period in which the phytoplankton was dominated by a dense population of Aphanizomenon sp. although a few Selenastrum sp. and Chlorophyceae were found, in addition to occasional Anabaena sp. In September Selenastrum was almost unialgal in the water, whereas in October only Gymnodinium sp. was found in abundance.

A continuous variation in dominant genera was evident; however, all components of the population frequently seemed to be present in small numbers even when they were not dominant.

RESULTS - PHYSICAL AND CHEMICAL BACKGROUND

Since the biological events in a lake cannot be divorced from their physical and chemical environment, the description of environmental variations logically preceeds the discussion of experimental results.

Thermal regime

The tabulated temperature information is given in Tables 1 and 2 in Appendix B.

In spite of the cold winter climate in interior Alaska, Smith Lake does not freeze to the bottom. The water becomes gradually concentrated as the ice increases in thickness, eventually reaching a thickness of about one and a half meters. At this time only about two meters of water remain at the region of maximum depth. The minimum temperature reached at the mud surface is 3.0°C. A rather steep temperature gradient is found at this time, and the water column is probably characterized by high stability resulting in very little water movement. During the early spring, conditions at the time of melting are important in determining the temperature profile early in summer. In 1963 the ice melted from the edges producing an island of ice in the center of the lake with wide leads surrounding it. This open water was warmed before all the ice was gone, so that a slight wind was all that was needed to completely melt the ice and mix the entire lake. In 1964 the melting took place in a different

way, and a thick layer of very thin vertical spicules of ice covered the entire lake with very little melting around the shore regions. These vertical columns of ice had channels between them, and apparently particles trapped in these channels were responsible for heat absorption and the increase in diameter of the channels. Eventually the whole system collapsed and melted, but all the melted ice initially remained in the surface region. There was apparently not complete mixing even as the water warmed through an isothermal state, probably because of the density difference between the two layers resulting from the high concentration of dissolved substances in the lower layer. A stable, warm layer of water was thus formed, with a sharp thermocline between 0.5 and 1 meters. This boundary gradually mixed down, but complete mixing of the surface 2 meters was not achieved until the last day of June. Even then there was very little mixing to a depth of 2.5 meters.

The warming of the surface layers is very rapid in Spring, with maximum summer temperature levels reached within two weeks of ice-free conditions.

Dissolved oxygen

The oxygen concentrations during the 1963-1964 period are shown in Tables 1 and 2. In a shallow lake of this type large departures from equilibrium with the atmosphere are not to be expected during the ice-free season. However, there is some reduction in oxygen content in the deep water during the summer, although this condition does not persist for long. The temperature profiles showed that

Table No. 1.- mg/liter dissolved oxygen, Smith Lake, 1963.

Depth meters	5/21/63	5/30/63	6/05/63	6/11/63
0.0	6.82	7.57	7.95	10.50
0.5				
1.0	7.62	7.40	7.90	8.30
1.5		7.44	7.88	6.94
2.0	1.94	5.80	7.90	7.54

	6/19/63	6/26/63	7/01/63	7/15/63
0.0	8.70	6.00	8.51	6.2
0.5	8.30			
1.0	7.53	5.92	8.00	5.6
1.5	5.38	6.20	8.80	4.53
2.0		6.24	6.30	2.23
2.5				

	7/23/63	7/30/63	8/07/63	8/12/63
0.0	7.00	7.00	6.90	7.10
0.5	7.0	6.75	6.90	7.45
1.0	4.8	6.80	7.05	7.45
1.5	4.0	6.80	7.10	6.80
2.0		6.75	6.85	6.60
2.5				

	8/20/63	8/27/63	9/25/63	12/09/63
0.0	7.58	7.75	7.20	Ice
0.5	7.40	6.33		
1.0	6.80	7.68	7.80	6.0
1.5	6.90	7.70		
2.0	6.52	7.00	8.35	2.2
2.5				

Table No. 2.- mg/liter dissolved oxygen, Smith Lake, 1964.

Depth meters	1/03/64	2/03/64	2/19/64	2/26/64	3/18/64
0.0	Ice	Ice	Ice	Ice	Ice
0.5	Ice	Ice	Ice	Ice	Ice
1.0	4.0	.93	.12		0.008
1.5		.55			
2.0					
2.5	1.65	.15	.12	0.58	0.0
	3/24/64	4/08/64	4/14/64	4/20/64	5/01/64
0.0	Ice	Ice	Ice	Ice	Ice
0.5	Ice	Ice	Ice	Ice	Ice
1.0	0.0	0.5	0.0	0.0	1.06
1.5					
2.0					
2.5	0.0	0.0	0.0	0.0	Trace
	5/05/64	5/12/64	5/18/64	5/23/64	5/27/64
0.0	-	-	-	-	-
0.5	-	-	-	-	-
1.0	2.32	6.27	8.57	5.25	9.6
1.5	-	-	-	-	9.8
2.0	-	-	-	0.6	9.3
2.5	0.00	0.50	1.74	-	5.1
3.0			-	-	0.7
	6/02/64	6/08/64	6/10/64	6/12/64	6/16/64
0.0	8.95	9.65	9.75	11.6	11.48
0.5	-	-	-	12.6	10.64
1.0	8.29	8.68	9.59	9.4	10.16
1.5	-	-	-	7.7	4.28
2.0	4.2	4.02	3.30	1.9	1.69
2.5	1.9			.3	.19

Table No. 2.- (continued)

Depth meters	6/18/64	6/23/64	6/25/64	6/30/64	7/07/64
0.0	8.66	6.68	6.90	7.90	8.07
0.5	8.84	6.50	6.90	7.88	-
1.0	6.44	6.41	6.70	7.86	7.89
1.5	5.84	6.48	6.60	7.31	-
2.0	1.2	6.24	6.60	4.17	1.37
2.5	0.0	.17	4.00	0.82	-

	7/16/64	7/21/64	7/28/64	8/04/64	8/11/64
0.0	7.64	7.08	8.05	7.7	7.95
0.5	-	-	-	-	-
1.0	7.18	6.34	7.84	7.7	7.78
1.5	-	-	-	-	-
2.0	1.94	1.28	2.89	3.9	4.13

	8/27/64	9/10/64	9/22/64
0.0	8.25	9.57	6.87
0.5	-	-	-
1.0	8.01	9.18	9.04
1.5	-	-	-
2.0	8.01	9.28	8.80

complete mixing to the bottom is not the rule during the summer in this lake. During the winter the oxygen concentration gradually becomes lower in the water under the ice, and finally completely anoxic conditions obtain. During this oxygen-free period, which lasted through March and April in 1964, hydrogen sulfide is produced in the water, resulting in a characteristic odor. Oxygen reappears under the ice early in May, coincidentally with the first appearance of large numbers of algae. The effect of this anoxic period on the nitrogen regime will be discussed below.

Specific conductivity

Representative examples of the depth distribution of conductivity from the summer of 1964 are shown in Table 3. The higher conductivity at the two meter level in June is the result of the concentrated water under the ice, whereas the dilute layer above is largely melted ice and influent water. On June 30th mixing resulted in a more even distribution with depth.

pH

Smith Lake has a pH which fluctuates around 7.0 with very small deviations, .6 pH units being an extreme. The water at the bottom tends to have a slightly lower pH than that at the surface, probably as a result of CO_2 released by decomposition or organic material. During the summer the pH is a little higher than during the winter - the reduction in winter again being possibly due to CO_2 released under the ice. In addition, during the summer photosynthetic removal

Table No. 3.- Specific conductivity - Micromhos.

Depth	6/16/64	6/18/64	6/22/64	6/30/64	7/07/64
0.0	75.8	83.9	76.4	88.6	92.4
1.0	77.2	85.1	80.3	89.2	93.2
2.0	103.0	113.4	110.5	97.2	105.2

	7/18/64	7/21/64	7/28/64
0.0	95.0	89.2	92.1
1.0	91.9	96.6	87.7
2.0	108.5	95.0	91.0

of CO_2 in the surface waters tends to raise the pH.

The only exception to the relatively constant pH takes place during June at the peak of the phytoplankton bloom. At this time the pH rose rapidly to 9.5 in 1963 and 9.8 in 1964. This increase is very temporary, and within a few days normal values are found again.

The tabulated data for depth distribution of pH for the 1963-1964 period are found in Table 3 and 4 in Appendix B.

Light

The penetration of light into Smith Lake water is reduced by the strong brown color. However during periods of low productivity, the depth of penetration of 1% of surface light reaches almost to the bottom. During the June flowering, the depth of 1% penetration lies somewhere between 0 and .5 meters. The effect of such a shallow euphotic zone could be considerable in controlling the maximum productivity during the bloom.

Particulate nitrogen

Particulate nitrogen is used here as an indicator of biomass, since the major interest lies in the uptake of nitrogen, and thus its incorporation into the particulate organic nitrogen fraction in the water. The increase in particulate nitrogen should reflect the net assimilation of nitrogen by the phytoplankton.

Information on the depth distribution of particulate nitrogen is given in Tables 5 and 6 in Appendix B. The seasonal variations

Table No. 4.- Light penetration - percent reaching each depth.

Depth meters	6/02/6	6/12/6	6/16/6	7/28/6	8/04/6	8/11/6
0.0	100	100	100	100	100	100
0.5	20.6	.5	.6	16.8	72.0	62.5
1.0	.5	.1	.2	4.5	45.6	8.3
1.5	.4				20.0	2.9
2.0					1.12	

in particulate nitrogen in Smith Lake surface water are shown for 1963 in Figure 2 and for 1964 in Figure 3. The 1963 distribution shows a large maximum in June, and relatively low levels during the remainder of the year. There is evidence of a slight rise in fall. The particulate nitrogen data for 1964 are more complete, and show a peak in May in addition to the June peak. The depth at which this early peak occurs is one meter rather than surface since the lake surface is ice covered at this time. The June peak appears to be smaller in 1964 than in 1963, but this is not really the case. The maximum at this time was found at one meter rather than at the surface, and therefore does not show up on this plot of surface concentrations. The particulate nitrogen level shows a decline after the June maximum, and then a temporary rise after mixing, so that the total base width of the peaks for the two years is similar even though the decline in 1964 was more rapid.

Chlorophyll *a*

The data for chlorophyll *a* concentrations in Smith Lake are given in Tables 7 and 8 in Appendix B. Surface concentration plots have been made on a seasonal basis and are shown in Figures 2 and 3. The 1963 plot shows the peak of 100 μg per liter at the start of the summer's work, the abrupt drop in concentration and then the low levels maintained in the lake during the remainder of the summer. The 1964 data, again follow closely the behavior of the particulate nitrogen, and show the rise in May under the ice, the decline followed by the June maximum, and then the sharp decline following the bloom termina-

Figure 2. - The seasonal distribution of chlorophyll *a* and particulate nitrogen concentrations in Smith Lake - 1963.

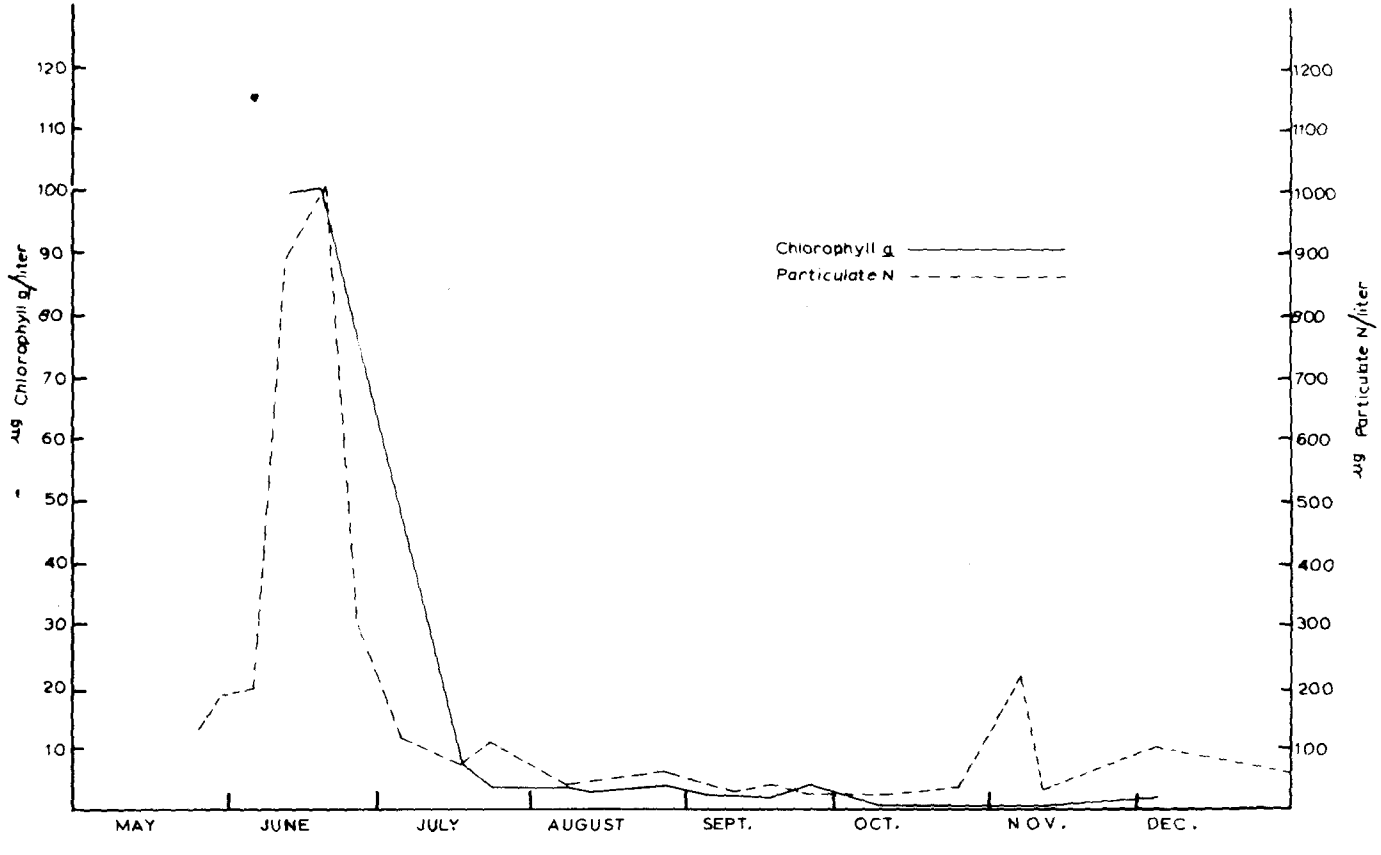
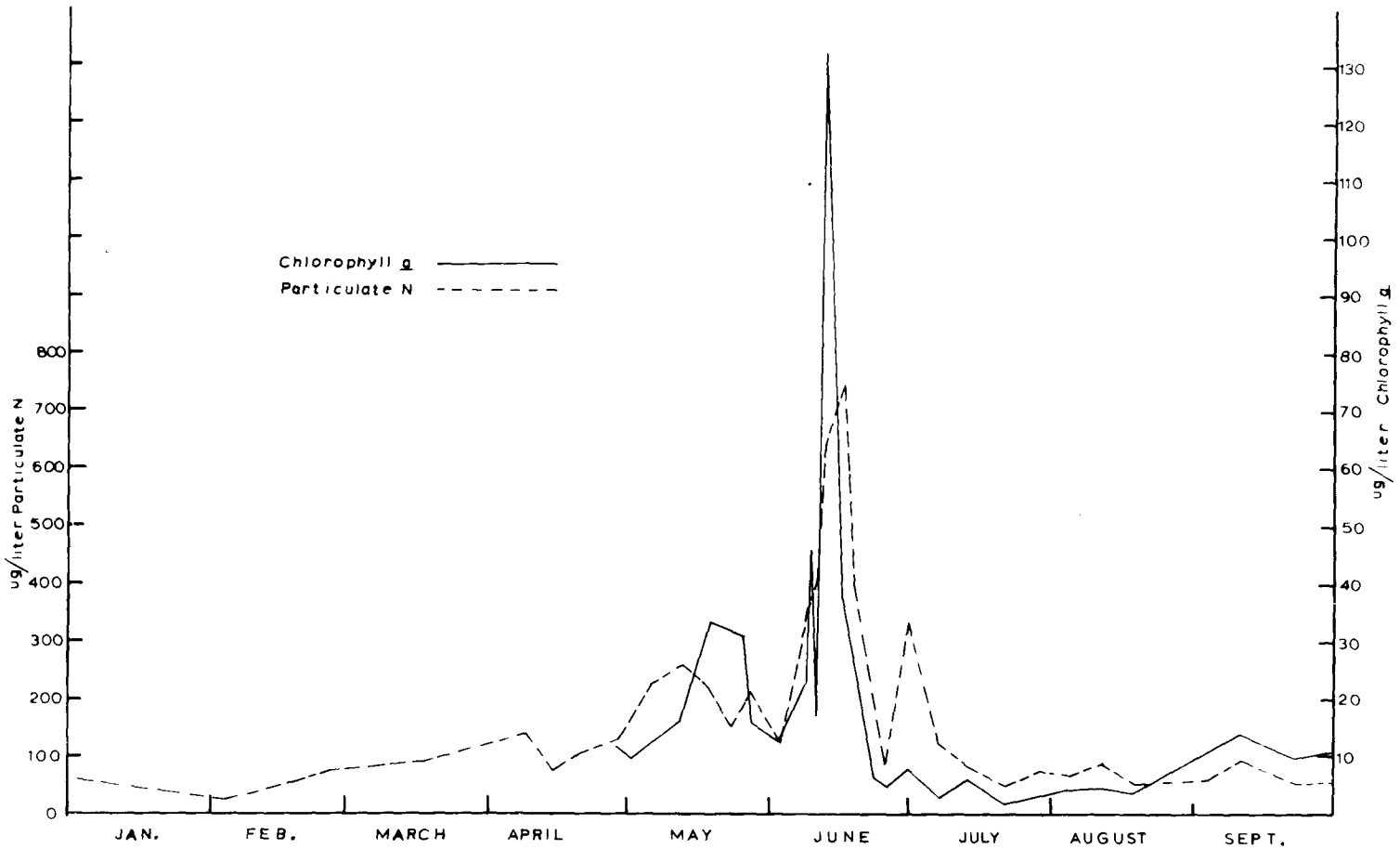

120
110
100
90
80
70
60
50
40
30
20
10
µg Chlorophyll a/liter
1200
1100
1000
900
800
700
600
500
400
300
200
100
µg Particulate N/liter
Chlorophyll a
Particulate N
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.
DEC.

Figure 3. - The seasonal distribution of chlorophyll *a* and particulate nitrogen concentrations in Smith Lake - 1964.


Chlorophyll a
Particulate N
ug/liter Particulate N
ug/liter Chlorophyll a
0
100
200
300
400
500
600
700
800
10
20
30
40
50
60
70
80
90
100
110
120
130
JAN.
FEB.
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPT.

tion. During this year there is a trend towards an increase at the end of the summer which was not found in 1963.

Nutrient chemistry

Ammonia. Ammonia is the more important inorganic nitrogen source from the point of view of availability in Smith Lake water. It is present in detectable amounts at almost all times. During the June bloom in 1963 it was absent in the surface water, but reappeared very quickly after the decline. In September the concentration was again temporarily reduced.

During the fall and winter, a slow increase in ammonia in the water is evident, representing a balance between ammonia released from nitrogenous organic material and nitrification. After the oxygen in the water is exhausted, the rate of increase increases, probably both because nitrification is inhibited in the absence of oxygen, allowing the ammonia released by decomposition to accumulate, and also possibly due to ammonia relased from the mud as a result of the anoxic conditions (Mortimer, 1941-1942). A maximum of 46.4 μg-atoms per liter of ammonia is present in the water near the bottom in mid-April. The level then drops to complete exhaustion by May 27th. The gradual increase follows leading to a maximum on June 25th, followed again by a decline to exhaustion on August 11, followed again by a slight increase. A constantly significant rate of ammonia production with a variable removal rate would explain the seasonal distribution of ammonia in Smith Lake. Table 5 shows the analytical results for ammonia in Smith Lake.

Table No. 5.- NH_3-N µg-atoms/liter, Smith Lake, 1963-1964.

Depth meters	6/12/63	6/19/63	6/26/63	7/07/63	8/20/63
0.0	0.0	2.78	.94	5.71	3.57
1.0		1.49	2.80	9.14	1.43
2.0		7.74	3.69	5.71	5.71
	8/27/63	9/03/63	9/10/63	9/17/63	9/25/63
0.0	3.58	.46	6.48	5.54	2.84
1.0	4.03		2.53		1.91
2.0	4.69	.51	2.77		6.00
	10/09/63	10/25/63	10/23/63	11/07/63	12/04/63
0.0	4.59	6.61	5.34	3.17	8.28
1.0		4.76			-
2.0		6.30			11.23
	1/03/64	2/03/64	2/19/64	2/26/64	3/18/64
0.0			-		
1.0	6.80	5.29	7.64	19.9	13.37
2.0	23.5	6.34	10.24		11.37
	3/24/64	4/08/64	4/14/64	5/05/64	5/13/64
0.0					
1.0	29.74	12.13	32.14	8.39	7.18
2.0		32.67	46.40		
	5/18/64	5/23/64	5/27/64	5/30/64	6/08/64
0.0				.36	.65
1.0		3.13	0.0		
2.0		2.74			

Table No. 5.- (continued)

Depth meters	6/10/64	6/25/64	6/30/64	7/07/64	7/13/64
0.0	2.15	16.47	6.10	6.02	5.77
	7/16/64	7/21/64	7/28/64	8/04/64	8/11/64
0.0		5.10	.58	.15	0.00
1.0	2.58				
2.0					
	8/18/64	8/27/64	9/03/64	9/04/64	9/10/64
0.0	1.49	2.71	2.46	.60	.60
1.0					
2.0					
	9/22/64	10/06/64			
0.0	.31	-			
1.0					
2.0					

Nitrate. The nitrate concentrations in Smith Lake are shown in Table 6. The first depth series in June 26, 1963 shows a high concentration of nitrate, probably resulting from the nitrification of ammonia released from the Anabaena bloom. During the winter the nitrate level gradually rises to a maximum early in February. At this time the oxygen becomes exhausted, and the nitrate level drops rapidly. The most likely explanation for this drop is denitrification, since nitrate, in the absence of oxygen, can serve as a hydrogen acceptor for certain bacteria. On the basis of the loss of nitrate, therefore, an idea of the amount of denitrification occuring can be obtained.

Figure 4 was obtained in the following manner. The nitrate concentrations at the one and two-meter levels were plotted for the beginning of oxygen depletion, one week later, one month later and one additional month later. The two depth points for each date were then connected by a straight line, since there was no way to get a more realistic estimate of the shape of the depth distribution curve. Area ODEP represents the nitrate concentration at the start of the low oxygen period, OCFP representing the same one week later, and area CDEF is the difference between the two and can be taken as an approximation of the amount of nitrate lost by denitrification during that time. This assumption is reasonable, since the only other anoxic dissimilatory process which is likely to remove nitrate from the water is reduction to nitrite. No significant increase in nitrite was found in the water during this time, and therefore it can be assumed that the reduction went to N_2 or N_2O. The area CDEF represents 53 µg-atoms lost under a surface area of 100 square centimeters. This is equivalent

Table No. 6.- NO_3-N µg-atoms/liter, Smith Lake, 1963-1964.

Depth meters	6/12/63	6/26/63	9/25/63	10/09/63	11/07/63
0.0	-	7.83	1.2	3.25	3.00
1.0		12.71	1.1	3.75	
2.0		10.83	1.2	4.00	
	11/11/63	12/04/63	12/09/63	1/03/64	2/03/64
0.0	3.0	6.42	-	-	-
1.0	-	-	7.7	8.97	13.01
2.0	-	4.42	5.7	5.30	8.99
	2/19/64	2/26/64	3/18/64	3/24/64	4/08/64
0.0	-	-	-	-	-
1.0	10.0	4.68	1.28	0.32	-
2.0	6.8	1.65	0.51	-	0.19
	4/14/64	4/20/64	4/28/64	5/05/64	5/12/64
0.0	-	-	-	-	-
1.0	0.52	0.26	0.66	3.02	0.26
2.0	-	-	-	-	-
	5/18/64	5/23/64	5/27/64	6/02/64	6/10/64
0.0	-	-	0.00		0.13
1.0	0.24	0.11	0.00	0.26	0.04
2.0	0.32	0.33	0.51	0.33	0.48
	6/12/64	6/16/64	6/18/64	6/23/64	6/25/64
0.0	0.23	0.16	0.08	0.27	0.34
1.0	0.05	0.22	0.09	0.33	0.40
2.0	0.30	0.59	0.40	0.45	0.51

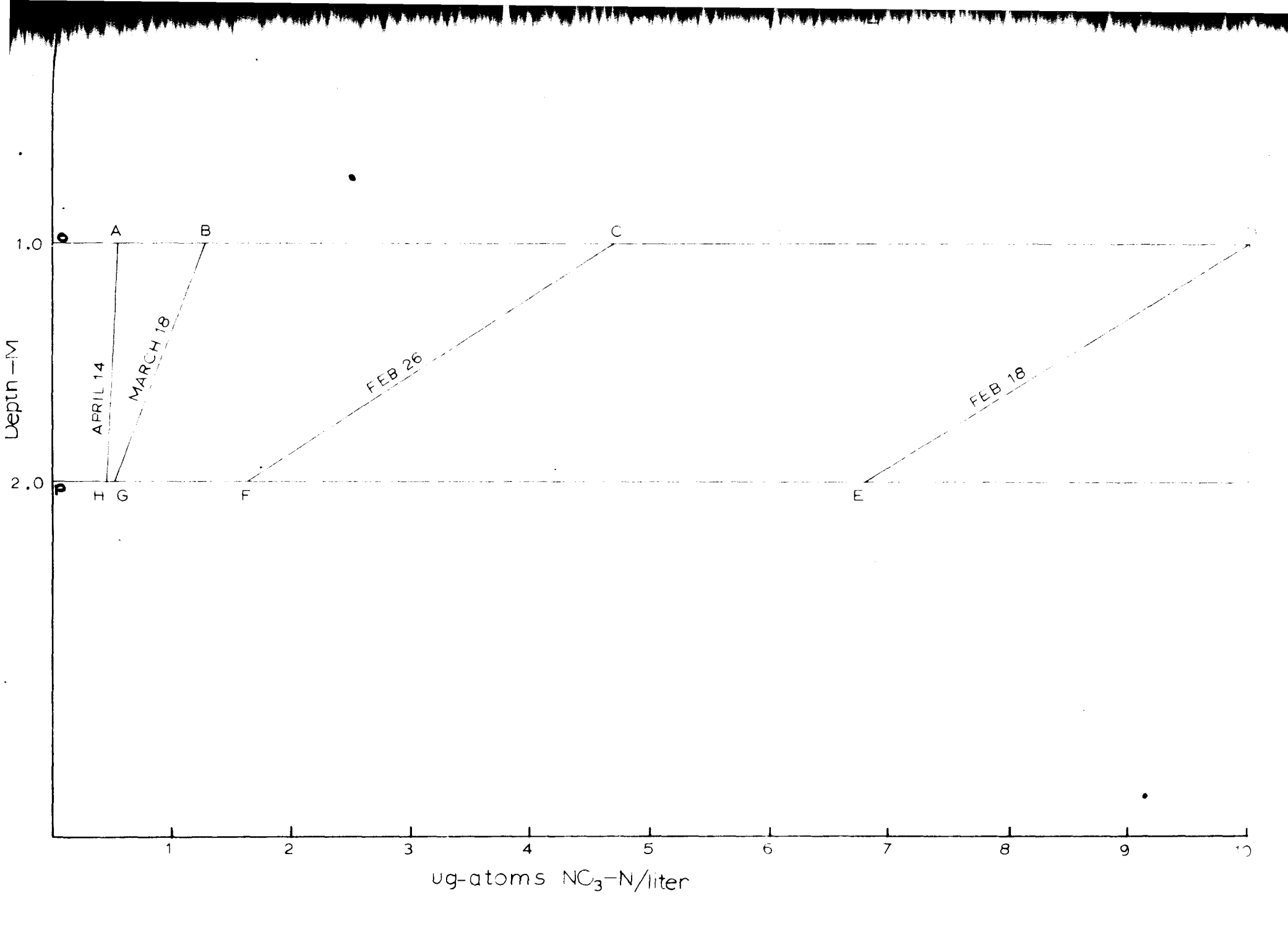
Table No. 6.- (continued)

Depth meters	6/30/64	7/07/64	7/13/64	7/21/64	7/28/64
0.0	1.00	0.49	0.33	0.65	0.07
1.0	0.86	0.67	0.53	0.83	0.04
2.0	1.70	1.78	1.65	0.96	0.80
1.7					

	8/04/64	8/11/64	8/18/64	8/27/64	9/03/64
0.0	0.01	0.08	0.09	0.10	0.16
1.0	0.67	0.01	0.08		0.21
2.0	0.82	0.58	0.22		1.20

	9/10/64	9/22/64	10/06/64
0.0	0.09	0.31	0.06
1.0	0.20	-	0.11
2.0	0.23	0.00	0.08

Figure 4. - Disappearance of nitrate in Smith Lake in the spring of 1964.


Depth—M
1.0
2.0
A
B
C
D
E
F
G
H
APRIL 14
MARCH 18
FEB 26
FEB 18
1
2
3
4
5
6
7
8
9
10
ug-atoms NC_3—N/liter

to 5.3 µg-atoms per liter.

The difference between areas OCFP and OBGP represents the amount of nitrate lost during the next month. This amounts to 1.9 µg-atoms per liter. The total loss of nitrate is given by area ADEH, which can be obtained by obtaining the area ABGH and adding this to the sum of the previous results. Area ABGH represents .39 µg-atoms per liter.

The total amount of nitrate lost during this period is 7.6 µg-atoms per liter, the bulk of this being lost during the first anoxic week. This estimate is probably conservative since there was some loss of nitrate prior to the date taken as starting point. On an annual basis, there are other occasions during the summer when the oxygen tension is sufficiently low to allow denitrification.

As a result of this nitrate loss, there is very little nitrate present at the time of the first significant phytoplankton growth. Both the under-ice and the June bloom take place under low nitrate conditions, and the first significant appearance of nitrate takes place after the decline of the Anabaena bloom.

Nitrite. Nitrite levels in the water varied from undetectable to about .4 µg-atoms per liter. Since the water color made accurate determination at these low concentrations difficult, nitrite will not be discussed here. There was no significant concentration of nitrite at any time.

Phosphorus

The phosphorus data for Smith Lake can be found in Appendix B,

Tables 9 and 10. The dissolved phosphorus content of Smith Lake water shows considerable variation with the seasons. The dissolved inorganic fraction shows the drop which is expected during periods of high productivity, dropping from 2.4 µg-atoms per liter down to .25 µg-atoms per liter between mid-May and Mid-June. The surface concentration also shows a drop simultaneously, but owing to the initially lower concentration at this depth the reduction is less spectacular. The minimum surface concentration found was .2 µg-atoms per liter. At 1.5 meters no reduction in concentration is apparent, whereas at 2 meters the correlation with productivity appears to be negative. Very high phosphate concentrations of 6 µg-atoms per liter are found at 2.5 meters under the ice in early Spring, with a slow reduction evident during June.

A variable but considerable amount of dissolved organic phosphorus is present in Smith Lake water at all depths. A reduction in the amount found at one-meter depth is evident at the end of May, followed by a slight peak corresponding to the time of the peak of the June bloom.

EXPERIMENTAL RESULTS

Primary productivity

In Smith Lake the highest rates of carbon fixation are found in the early spring and summer. The first major productivity peak is found under the ice in May and is probably connected in some way with the termination of the anoxic state of the water. During the first year's work this peak was not observed, because the sampling was not started early enough. At that time only one major peak in production was found, due to a bloom of Anabaena sp. which took place during both years soon after the ice had melted.

Figure 5 shows the ^{14}C results for 1963, and represents the integrated results of in situ depth series incubations, so that each value plotted represents the total amount of carbon fixed under a square meter of water surface in an hour. The peak value of 10 mg of carbon per square meter per hour was attained at the end of the first week in June. This was followed by a steady decline, with low rates of photosynthesis occurring during the rest of the summer, with the exception of a minor peak in August. Figure 6 also represents ^{14}C uptake data from 1963, which are in this case expressed on a unit volume basis, from the results of experiments using surface water. The pattern is similar to that shown by the depth integrated curve, with the possible exception of an additional small peak at the end of May.

The results of in situ experiments during 1964 are shown in

Figure 5. - Primary productivity results for Smith Lake, 1963.

Figure 6. - Surface photosynthesis measurements in Smith Lake, 1963.

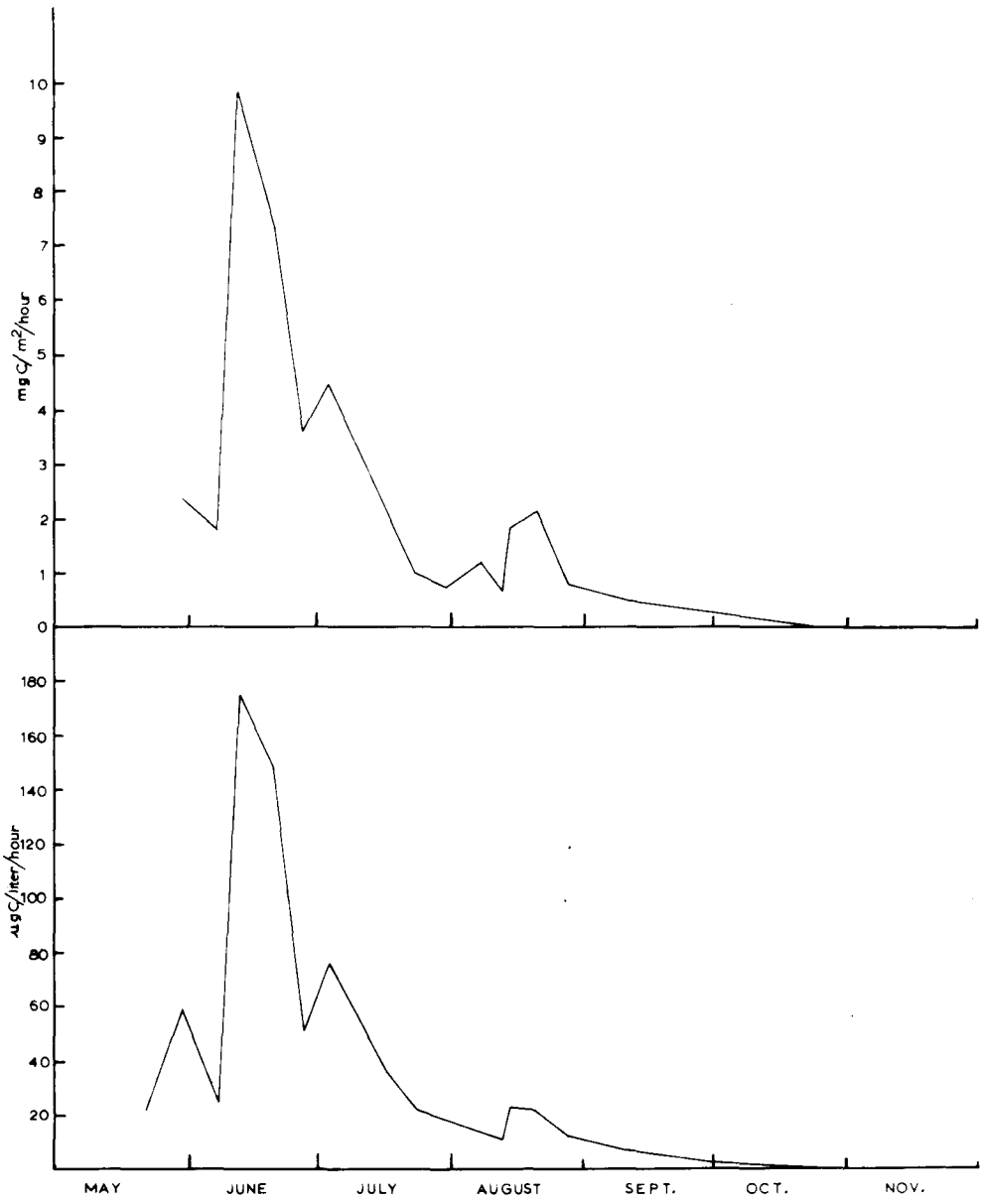
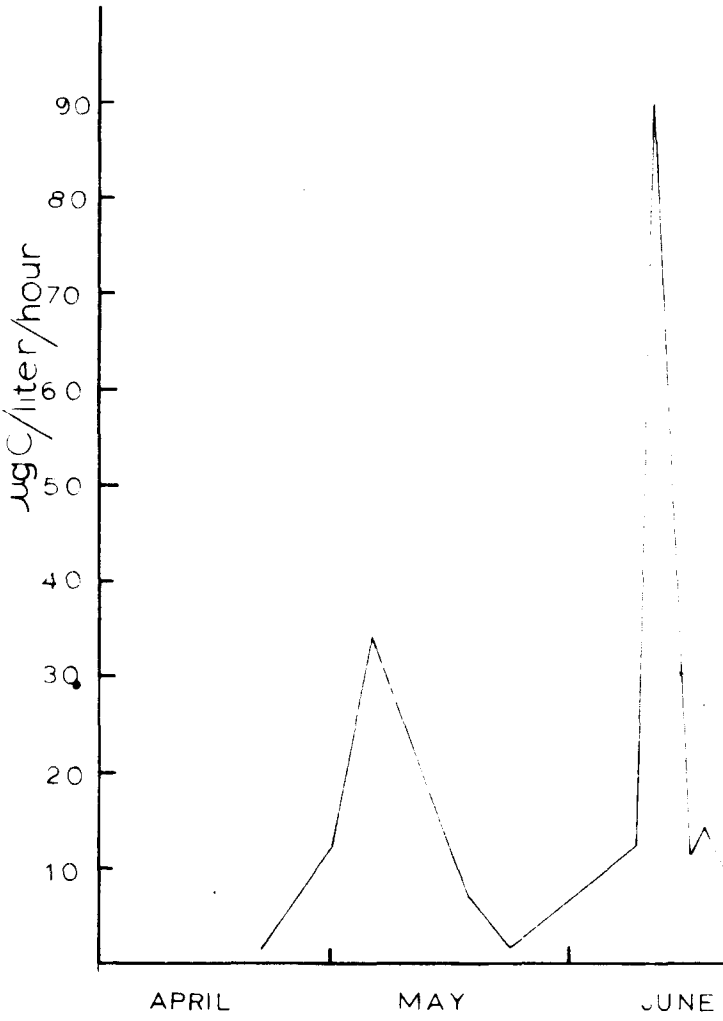

mgC/m²/hour
0
1
2
3
4
5
6
7
8
9
10
µgC/liter/hour
20
40
60
80
100
120
140
160
180
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.

Figure 7. These values were obtained from surface water incubated just below the surface. The pattern is quite similar to that for 1963, but in this case a sufficiently early start in spring produced a clear picture of the early spring growth under the ice cover. The Anabaena sp. bloom occurred at the same time as it had on the previous year, the peak being reached on June 12 in both years. The maximum rate of photosynthesis during 1963 had been 175 µg of carbon per liter per hour, whereas in 1964 it was only 90 µg of carbon per liter per hour. The rate of increase in the photosynthetic rate for the two years was approximately similar, but in the latter year the decline was much more rapid. The actual height of the peak is of limited significance, since light conditions on the particular day can be of greater importance in determining this than actual differences between the two years. However, the sharper rate of decline does indicate that conditions in 1964 were not as favourable, and caused the bloom to terminate more precipitously.

Figure 8 also shows data for 1964, in this case the results of experiments which were incubated at constant temperature (20°C), and light. The patterns, as would be expected, are similar to the in situ patterns, with the exception of slightly lower peak values, slightly higher midsummer values, and the appearance of an increase in fall which did not show up in the in situ incubated samples. This late summer increase seems to indicate that the fall bloom which is commonly found in more southern latitudes would also occur here, were the light and temperature conditions suitable. It is not possible to guess which of the two parameters is critical in this case.

Figure 7. - Results of *in situ* ^{14}C experiments in Smith Lake, 1964.


90
80
70
60
50
40
30
20
10
µgC/liter/hour
APRIL
MAY
JUNE

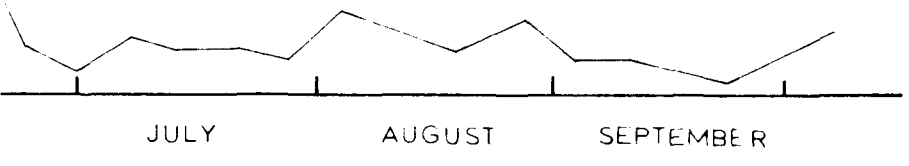
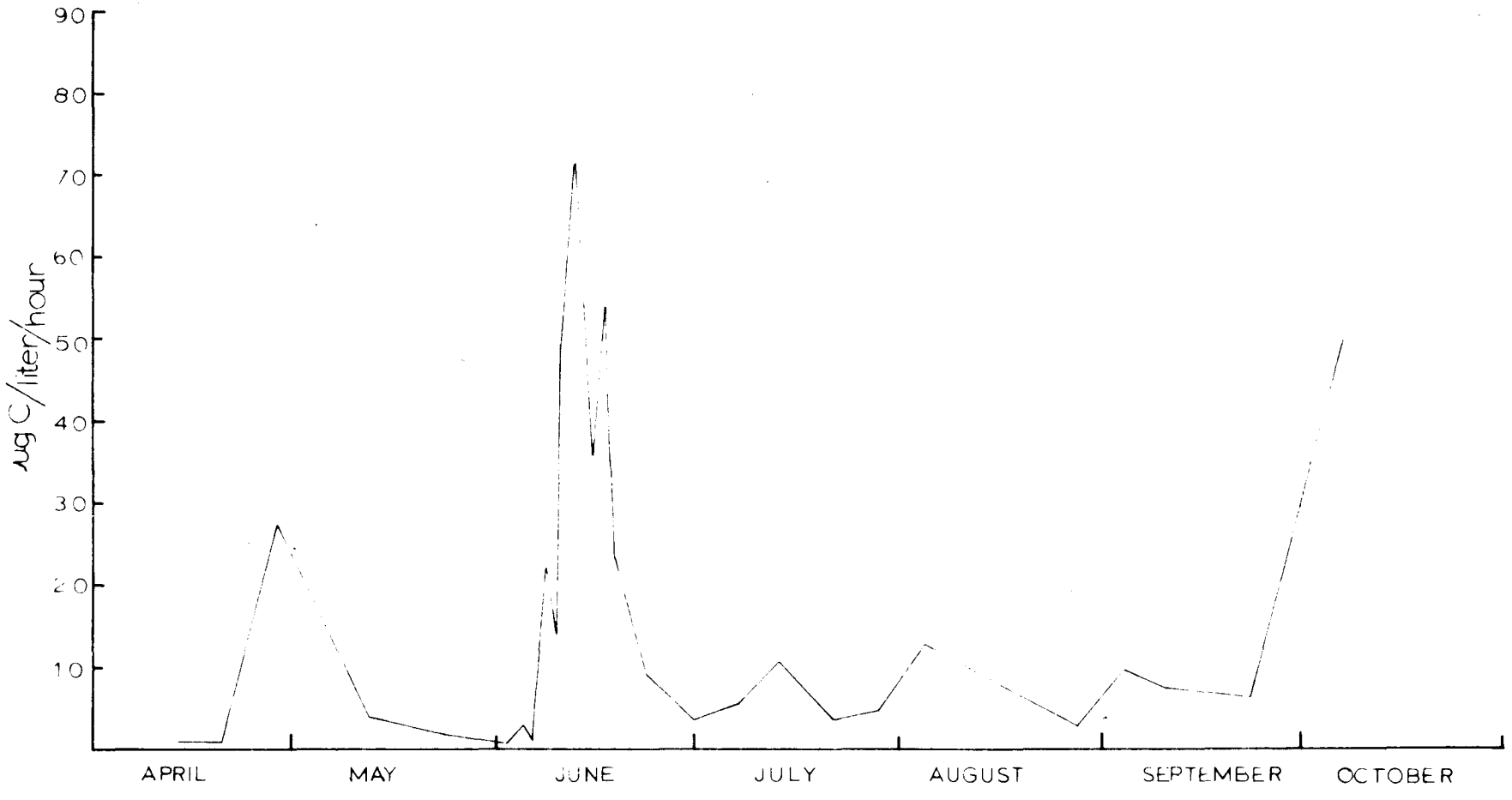

JULY
AUGUST
SEPTEMBER

Figure 8. - Results of ^{14}C experiments incubated under constant light at 20°C - Smith Lake surface water, 1964.


90
80
70
60
50
40
30
20
10
ug C/liter/hour
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

The third method of incubation, in which bottles were placed in a tank on the roof, did not produce results which were greatly different than those obtained by the other methods used. There was a tendency for the rates to be somewhat lower than with other incubation methods, and light inhibition may have been a problem. These results, therefore, are not included. There is actually remarkably little difference between the results obtained by all three incubator methods. On cloudy days the 20°C incubation produced higher rates, but under optimal conditions on sunny days the in situ results were highest.

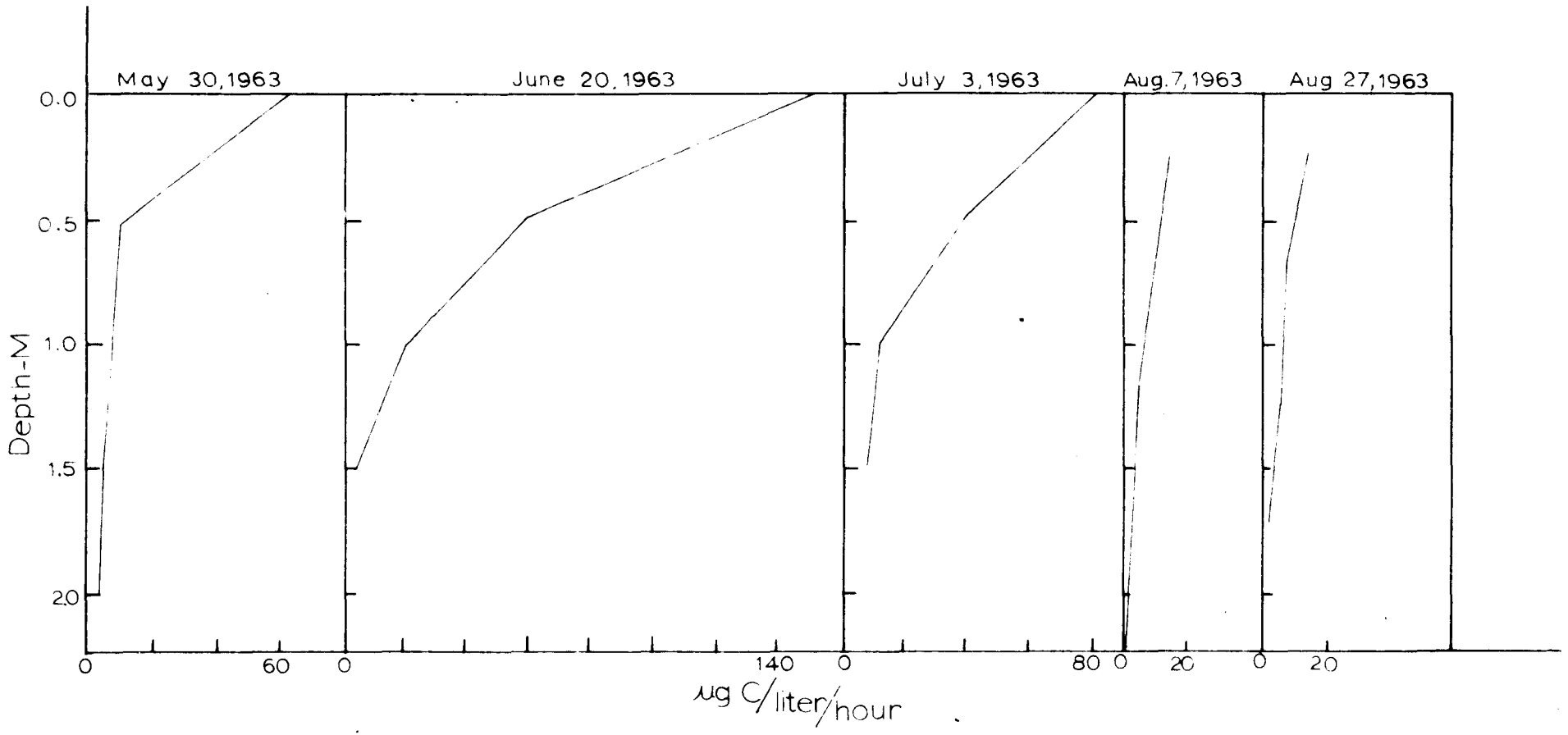
Depth distribution of ^{14}C uptake in Smith Lake

Figure 9 shows representative plots of the depth distribution of photosynthesis in the lake. During the period of high productivity, the reduction of the photosynthetic rate with depth is great, but even at other times the high extinction properties of the lake water prevent much photosynthesis below a depth of one meter.

Winter productivity

It was not practical to attempt in situ measurements in the winter in Smith Lake. However, periodically, water samples were brought into the laboratory and incubated with ^{14}C to see whether the photosynthetic ability to fix carbon was there. At no time were zero results obtained, the lowest value measured in this way being .1 μg carbon per liter per hour. In February, water collected

Figure 9. - The depth distribution of photosynthesis in Smith Lake - representative curves for 1963.


May 30, 1963
June 20, 1963
July 3, 1963
Aug. 7, 1963
Aug 27, 1963
0.0
0.5
1.0
1.5
2.0
Depth-M
0
60
0
140
0
80
0
20
0
20
ug C/liter/hour

in the lake and brought into the laboratory and incubated at 4°C with a gro-lux lamp as the light source showed a rate of carbon assimilation of 1.4 µg carbon per liter per hour.

The efficiency of photosynthesis

Assuming that a relatively constant relation exists between the amount of chlorophyll in the water and the rate of photosynthesis, and that photosynthesis-light curves can be determined for phytoplankton communities, chlorophyll content can be used as a measure of the photosynthesis in the water. Ryther and Yentsch (1957) introduced this method in oceanography, and from experimental data and from data found in the literature, they adopted a value of 3.7 g carbom assimilated per hour per g of chlorophyll. Since that time, considerably more information is available concerning similar values for both marine and freshwater communities, and recently much of this has been collected and tabulated for both environments by Ichimura and Aruga (1964). For lakes, they concluded that values from 2-6, 1-2 and 0.1-1 g of carbon per g of chlorophyll per hour were the light-saturated photosynthetic rates for surface plankton from eutrophic, mesotrophic and oligotrophic lakes respectively. These values are for gross photosynthesis, however. Table 7 has been compiled from similar information for Smith Lake, but represents in situ results for net photosynthesis. Considerable variation is found here, but a surprisingly high rate of carbon assimilation per unit chlorophyll is found under the ice in May. Light conditions cannot be very favourable at this time, but nutrient concentrations are very high.

Table No. 7.- The relationship between chlorophyll *a* and photosynthesis.

Date	A Chlorophyll *a* µg/liter	B ^{14}C uptake, µg/liter/hour	B/A
5/01/64	10	13.75*	1.38
5/01/64	12	33.5 *	2.79
5/18/64	16.4	6.92*	.42
5/23/64	7.1	2.24*	.31
5/27/64	4.5	2.26*	.51
6/01/64	6.8	.84	.12
6/08/64	22.6	34.10	1.50
6/10/64	17.1	29.65	1.73
6/12/64	132.3	89.74	.68
6/15/64	62.9	35.68	.57
6/18/64	29.3	14.91	.51
6/23/64	5.9	5.29	.90
6/30/64	7.7	3.34	.43
7/07/64	3.2	5.76	1.80
7/13/64	5.7	5.49	.96
7/21/64	2.3	4.54	1.96
8/04/64	4.5	9.06	2.00
8/18/64	4.0	5.38	1.34
9/10/64	14.0	4.34	.31
9/22/64	10.0	2.39	.24
10/06/64	11.7	7.32	.63

* Incubated at 1 meter under ice.

Experimental results - nitrogen uptake

The nitrogen uptake experiments carried out during 1963 serve mainly to establish the seasonal patterns of nitrogen utilization and especially to show the occurrence of a Spring nitrogen-fixing bloom of Anabaena sp., principally of the species Anabaena flos-aquae. The incubation facilities during that year were inadequate, and in addition the nitrate and ammonia uptake data had to be rejected for the early productive period since the amount of nitrogen-15 added for the experiments at that time was too low and was apparently instantly used up, resulting in low apparent uptake rates.

The results of the experiments for the 1963 season are shown in Table 8. Very little nitrate uptake is evident; however, ammonia is used during most of the summer with several minor peaks in uptake rate. Nitrogen fixation is only important in June.

The seasonal distribution of nitrogen fixation is shown in Figure 10, in which the particulate nitrogen levels in the surface water are also shown. The two peaks coincide in time, and the drop in both fixation and particulate nitrogen levels following the peak is spectacular.

During 1964, improved facilities for controlled incubation allowed the collection of more consistent, reliable data. These are shown in Figure 11. The first major peak in uptake coincides with the photosynthesis peak under the ice in May, and comprises predominantly ammonia uptake at a maximum rate of 13 µg per liter per hour.

Figure 10. - Nitrogen fixation rates and the particulate nitrogen concentration in Smith Lake surface water, 1963.

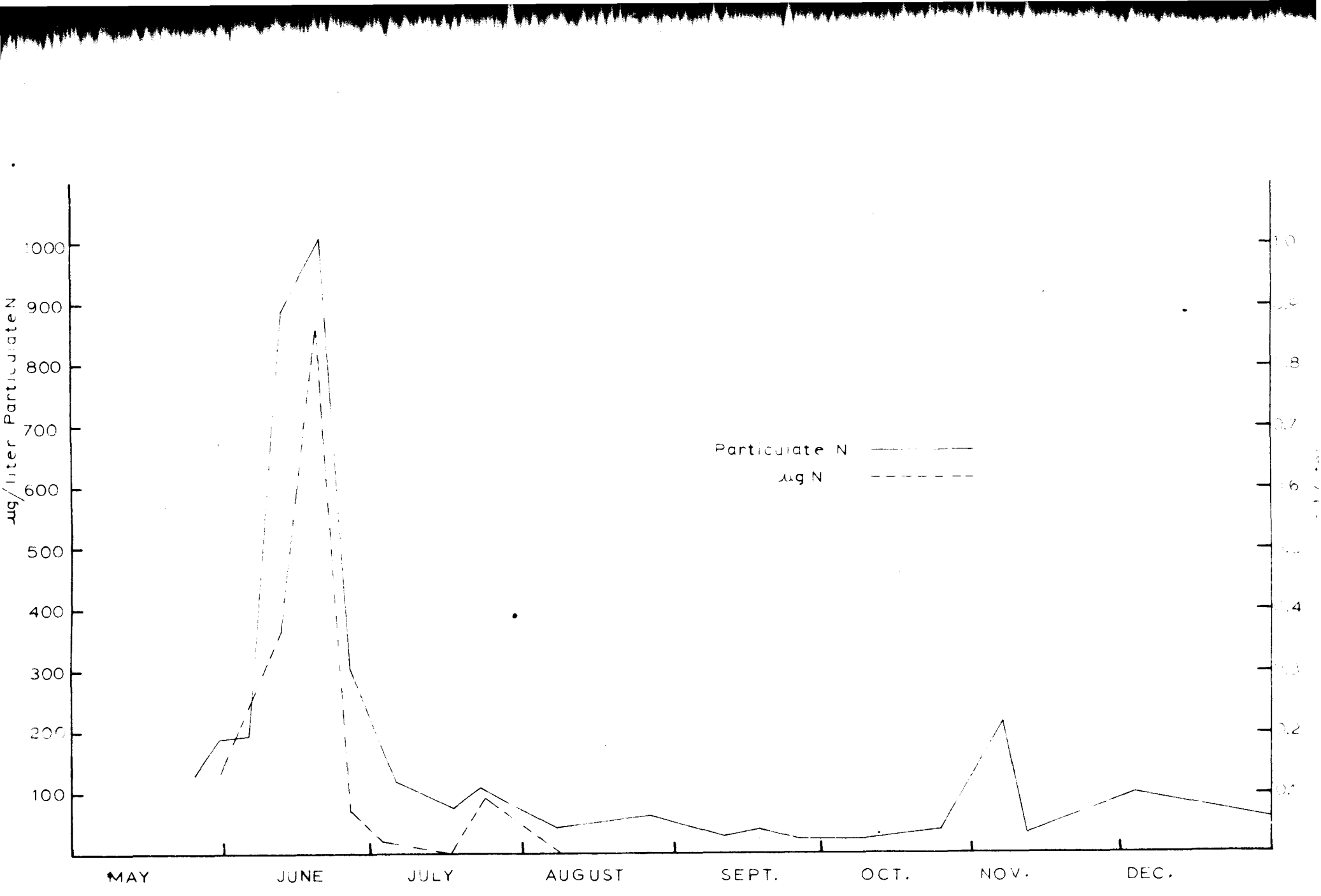

µg/liter Particulate N
1000
900
800
700
600
500
400
300
200
100
Particulate N
µg N
MAY
JUNE
JULY
AUGUST
SEPT.
OCT.
NOV.
DEC.

Table No. 8.- The uptake of nitrogen compounds. Results of tracer experiments, Smith Lake, 1963, surface water.

µg N/liter/hour

Date	NH_3-N uptake	NO_3-N uptake	N_2 uptake
5/30/63	-	-	.13
6/12/63	-	-	.37
6/19/63	-	-	.86
6/26/63	.61	.26	.12
7/02/63	.59	.32	.02
7/16/63	.19	.01	.00
7/23/63	.79	-	.09
7/30/63	.14	.01	.00
8/07/63	.18	.01	.00
8/12/63	.31	.11	-
8/20/63	.37	.02	-
8/27/63	.72	.02	-
9/03/63	.36	.05	.00
9/10/63	.30	.00	-
9/17/63	.29	.00	.00
9/30/63	.10	.00	.00
10/25/63	.00	.00	-
11/13/63	.02	.00	.00

Table No. 9.- The uptake of nitrogen compounds. Results of tracer experiments, Smith Lake, 1964.

Date	N uptake - µg/liter/hour			
	NH_3-N	NO_3-N	N_2	Total N uptake
2/26/64	.20	-	-	.20
3/18/64	.07	.01(dk)	-	.08
3/31/64	.05	.00	-	.05
4/13/64	.56	.01	-	.57
4/28/64	1.00	.03(dk)	-	1.03
5/01/64	1.40	.06	-	1.46
5/05/64	2.24	.05	-	2.29
5/12/64	13.03	1.42	-	14.45
5/18/64	6.01	1.56	-	7.57
5/23/64	.45	.01	-	.46
5/27/64	.95		.06	1.01
6/01/64	1.22	.01	-	1.23
6/04/64	.82	.36	.02	1.20
6/06/64	17.23	4.88	1.76	23.87
6/08/64	5.99	4.12	2.45	12.56
6/10/64	6.41	.75	2.88	10.04
6/12/64	8.21	.37	2.28	10.86
6/15/64	1.70	.15	.67	2.52
6/18/64	4.76	.15	.19	5.10
6/23/64	.94	.00	.02	.96
6/30/64	5.36	.13	.30	5.79
7/07/64	.94	.00	.02	.96
7/13/64	1.66	.00	.01	1.67
7/21/64	1.78	.02	.00	1.80
7/28/64	1.30		.01	1.31
8/08/64	1.92	1.16	.03	3.11
8/11/64	1.48	0.45	0.13	2.06
8/18/64	0.97	0.16	0.00	1.13
8/27/64	0.93	0.17	0.01	1.11
9/03/64	0.78	0.03	0.00	0.81
9/10/64	1.52	1.22	-	2.74
9/22/64	0.88	0.69	-	1.57
10/06/64	0.64	0.35	-	0.99

A lower but still significant nitrate uptake was found at the same time. The nitrogen regime in Smith Lake in Spring appears to be determined by the availability of ammonia as a result of the anoxic Spring period. The population appears to build up at a time very little light could be reaching the water, especially since most of the chlorophyll increase is initially at the bottom in water immediately in contact with the mud. It is possible that the nutrition during this initial build-up in population is predominantly heterotrophic, since dissolved organic material is certainly abundant in the water at this time. The decline in phytoplankton, as seen in the chlorophyll and particulate nitrogen information, as well as the reduction in photosynthesis and nitrogen assimilation rates, corresponds in time to the exhaustion of the ammonia supply in the water.

The second major period of nitrogen uptake, then, takes place shortly after the ice is melted, representing a period of rapid increase in temperature and available light.

The first point that becomes obvious in connection with the June bloom in 1964 is that even though a high rate of nitrogen fixation was found, amounting to 3 μg per liter per hour, this source appears insignificant when compared with the rate of ammonia assimilation found during this flowering. The peak ammonia and nitrate uptake rates, respectively 17.2 and 5 μg per liter per hour, took place several days before the peak in nitrogen fixation. The nitrogen fixation peak corresponded in time with the photosynthesis peak. The maximum chlorophyll a concentration was found a few days later, followed by

Figure 11. - The uptake of inorganic nitrogen sources in Smith Lake in 1964 - the results of stable isotope tracer experiments on surface water, incubated at 20°C and constant light.

the peak in particulate nitrogen. Since the particulate nitrogen concentration is in a sense the integral of the uptake rates, this delay is to be expected. The ammonia assimilation rate was reduced to 6.4 μg per liter per hour by the time that nitrogen fixation reached its maximum rate, and the nitrate uptake rate had been reduced to .8 μg per liter per hour.

The rates of nitrogen fixation over a period of ten days during June account for the contribution of about 500 μg per liter of newly fixed nitrogen to the surface water of Smith Lake. These rates are among the highest hitherto measured in any lake, and are of a similar order of magnitude as those measured during a fall bloom of Anabaena in Sanctuary Lake (Dugdale and Dugdale, 1962). It is therefore surprising to find at the same time a high rate of ammonia utilization. It is possible that during nitrogen fixing activity, ammonia uptake will always appear to be high. Where the ammonia concentration in the water is low, any labeled ammonia added will significantly increase this concentration, and since the metabolic rate of the nitrogen fixing organisms is probably high they may rapidly absorb this added ammonia, even though they had not been using ammonia previously. The uptake rate measured then would not represent an actual in situ rate, but could rather be an experimental artifact, due to the addition of the tracer. However, the increase in dissolved ammonia in the water as soon as the ammonia uptake rate dropped (see Table 5) indicates that ammonia had indeed been removed rapidly previously. Since the ammonia concentration in the water was not high during this high uptake period, this uptake was probably the result

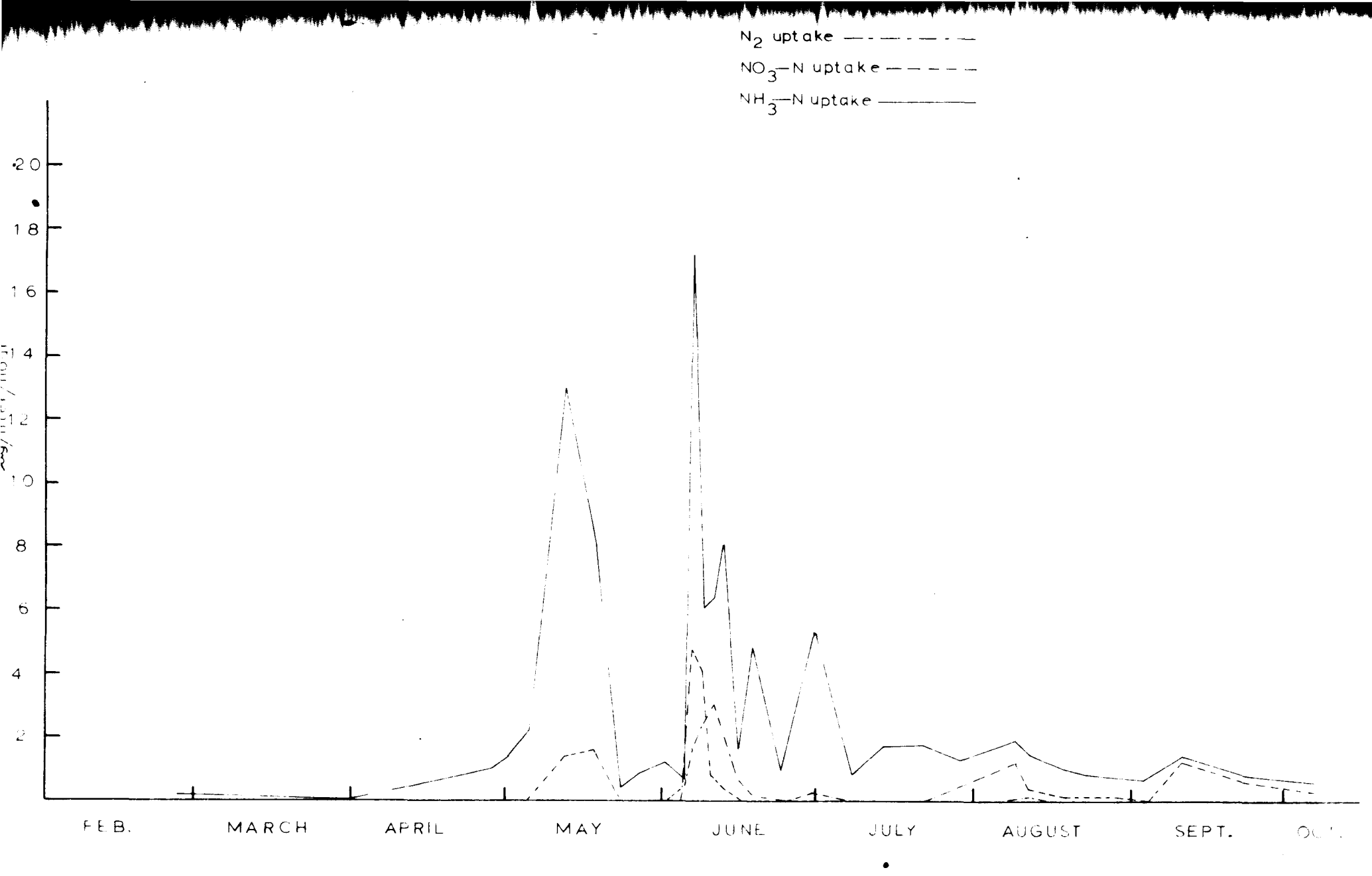
of a high rate of recycling. The amount of particulate nitrogen present in the water during May and the amount newly fixed in June are together sufficient to account for the peak value in particulate nitrogen present in the water in June.

The total uptake of all three forms of nitrogen for 1964 is shown in Figure 12. It is apparent, by comparing this with Figure 10, that the total uptake very closely corresponds to the uptake of ammonia, and that clearly ammonia is the most important source of nitrogen to the phytoplankton in Smith Lake. The uptake of nitrate is not very important in Smith Lake; however on two occasions in August and September, nitrate was being used at about the same rate as ammonia.

The excretion of newly-fixed nitrogen by phytoplankton

Reports on nitrogen fixation have included estimates of the amount of nitrogen excreted into the medium by blue-green algae (Fogg, 1952). In Smith Lake, experiments were carried out using the same samples as those used for measuring nitrogen fixation, so that a relationship between the amount of nitrogen fixed and the amount appearing in the medium could be established. Following incubation and removal of the particulate fraction, the ammonia fraction in the sample was collected by distillation, and its isotope ratio determined by liquid conversion to nitrogen gas using alkaline hypobromite as described in Neess, et al. (1962). The remaining water was then concentrated by boiling, and then digested by the micro-Kjeldahl method in order to collect the dissolved

Figure 12. - The total uptake of inorganic nitrogen in Smith Lake, 1964.


N_2 uptake
NO_3—N uptake
NH_3—N uptake
20
18
16
14
12
10
8
6
4
2
FEB.
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPT.

organic fraction. This was collected as ammonia following the digestion, converted to nitrogen gas as above and its isotope ratio measured.

Table 10 shows the results of these determinations. It must be kept in mind that the lake water has an unknown amount of dissolved organic material of unknown nitrogen content, so that the dissolved organic isotope ratios could represent a considerable dilution.

The ammonia concentration in the water was increasing during the course of these experiments, so that to some extent the decrease in ^{15}N enrichment in the ammonia fraction is compensated, and the actual excretion of ammonia is probably increasing.

Significantly high isotope ratios in the dissolved organic fraction were found on June 12th, corresponding to the time of akinete differentiation in the Anabaena filaments. On this day, all three incubation methods were used, and the highest excretion of both ammonia and dissolved organic nitrogen was found in the flask which had been incubated on the roof. The reduction in nitrogen fixation rate in this flask when compared with the other two indicates suboptimal conditions with this incubation method, and possibly this is connected with the higher excretion rates. However, one experiment alone is insufficient to establish any such connection. There is also a possibility that the 24-hr incubation was sufficiently long for some breakdown in cells, and that some material could have been released to the medium in this way.

Table No. 10.- The excretion of dissolved nitrogen during nitrogen fixation.

Date	Incubation method (24 hours)	Atom percent excess ^{15}N in particulate fraction	Atom percent excess ^{15}N in ammonia fraction	Atom percent excess in dissolved organic fraction
V1-08-64	In situ	3.2799	0.1180	0.0069
V1-10-64	In situ	3.1240	0.1266	0.0000
V1-12-64	Roof tank	2.1070	0.6048	0.1276
V1-12-64	In situ	4.2455	0.1601	0.0717
V1-12-64	20° room	4.5601	0.0930	0.1051
V1-18-64	20° room	0.5761	0.0338	0.0000

Determination of the rate of ammonia supply in Smith Lake water

In many cases the ammonia uptake rate was higher than the ammonia concentration in the water would imply. Rates of ammonia uptake, of course, provide an idea of the rate of supply; however, a more direct method of evaluating this was also employed.

The technique used was designed to measure the rate of dilution of labeled ammonia added to the liquid fraction. 11.9 µg-atoms per liter of nitrogen-15 labeled ammonia was added to lake water, and immediately an aliquot was filtered to remove the particulate material. The ammonia in the filtrate was collected by distillation and its isotope ratio determined. This represented the initial isotope ratio for the experiment. The techniques for converting the ammonia to nitrogen and obtaining the isotope ratio were similar to those used in handling the liquid samples in the excretion experiments described above.

The sample was now incubated, and then the ammonia was collected as above and its isotope ratio determined. From these two isotope ratios the rate of dilution was calculated:

$$\text{Fractional rate} = \frac{A_i - A_f}{t \cdot A_a}$$

where A_a = the average isotope ratio during the experiment, and t = incubation time.

Any reduction in the isotope ratio of the ammonia fraction over this initial value has to result from the addition of unlabeled ammonia from another nitrogen fraction in the system, and therefore represents ammonia supply.

Results: A_i = 84.54%

A_f = 14.18% August 11, 1964

t = 9.25 hr

Fractional rate of supply = .1541.

Percentage rate of supply = 15.4 per hour.

Absolute rate of ammonia supply 1.83 μg-atoms per liter per hour.

The measured ammonia uptake rate at this time was 1.48 μg per liter per hour (or .11 μg-atoms per liter per hour) and there was no detectable ammonia in the water at this time.

DISCUSSION

The production under the ice

It is clear from the inorganic nitrogen results that a large amount of ammonia appears in the water under the ice in spring, and that the first population of algae depend largely on this source for their nitrogen supply. The sources of this ammonia have been discussed above. The ammonia concentration is reduced by the algae from 46.4 μg-atoms/liter to undetectable levels, which in this case represent concentrations somewhat lower than .2 μg-atoms/liter. At the peak of this high assimilation, 13 μg/liter/hr (.93 μg-atoms/hr) of ammonia nitrogen was being removed, and the amount available in the water was 7.18 μg-atoms/liter. This amount would be exhausted in one day at the measured rate of removal. The processes which release ammonia to the water apparently allowed the population to use a larger amount than was present in the water, although both the rate of removal and the standing concentration in the water gradually declined until the ammonia was completely exhausted.

During the most active period of ammonia uptake which covered a period of 13 days, an average of 140 μg/liter/day (10 μg-atoms/liter/day) was assimilated, whereas the decline in the water amounted to 6.41 μg-atoms/liter for the whole period. This decline averages out to .49 μg-atoms/liter/day. Therefore 9.5 μg-atoms/liter/day more ammonia was used than was apparently available. Part of this discrepancy could represent the

difference between gross and net uptake of nitrogen, since the tracer method probably measures gross uptake where short incubation times are used. In addition, bacterial breakdown of nitrogenous organic material was undoubtedly continuing during this time. Possibly a major part of the ammonia came from this source. In any event, these figures indicate that ammonia supply is important during such periods of high production, and that the decline in ammonia in the water is not a good measure of the amount used, since 95 percent of the ammonia used would have been missed by such methods. Only 27.3 percent of the nitrogen taken up showed up in the particulate nitrogen at its maximum concentration during this period. The ratio of particulate nitrogen in μg-atoms/liter to chlorophyll a in μg/liter was 1.52, which is similar to the 1.65 ratio given by Harris (1959) for Long Island Sound during the build-up of a spring flowering.

The first increases in chlorophyll a appeared in the deep water when there was still more than one meter of ice on the lake surface covered by several inches of snow. These organisms therefore grew under low light conditions. Possibly part of this growth, at least in the initial phases, was heterotrophic. Rodhe (1963) describes a true arctic lake, Juoblatjokkojaure, in which there are large numbers of nannoplankton in spite of extremely low temperatures and otherwise seemingly inhospitable conditions. It is interesting to note that here, as in Smith Lake, Chlorella is among the most numerous components, although in Rodhe's case dinoflagellates dominated in bulk. Rodhe feels that heterotrophic processes must be allowing the growth, and

his preliminary tests with ^{14}C labeled glucose seemed to substantiate this. The system, then, depends on energy stored during the previous summer through autotrophic processes to initiate the spring grwoth, so that when light conditions become suitable the population is already present and can switch to autotrophy. In Smith Lake, by mid-May, sufficient light may penetrate the ice and snow to allow this. Earlier, the low sun angle results in high albedo, and further reduces the penetration of light into the water. Chlorella is capable of growth heterotrophically, mixotrophically and autotrophically, and its behaviour under these different growth conditions has been studied by Eyster, Brown and Tanner (1958) in terms of mineral requirements. Mixotrophy could allow the efficent use of any light penetrating the ice. In any event, heterotrophy is a possible mechanism allowing the early development of phytoplankton under the ice. A similar argument was used by Wright (1964).

The Anabaena sp. bloom

The bloom in June is of interest as the only period in which nitrogen fixation supplies a portion of the nitrogen for the phytoplankton. All the inorganic nitrogen in the water in combined form had been used up prior to this bloom. In each of the years, this bloom occurred at the time of maximum day-length, when the water was rapidly warming to its summer temperature.

It is not so easy in this case to calculate the contribution of nitrogen from various sources, since newly fixed nitrogen may be immediately

released to the water and then reassimilated. There was no nitrate or ammonia in the water at the start of the bloom, and therefore the uptake of nitrogen in these forms must represent the regeneration of nitrogen from the previous growth period or the recycling of newly fixed nitrogen. The excretion information discussed above indicates that the latter may not be important; however, the incubations for the experiments described in Table No. 10 were 24 hr, and if the turnover time is much shorter, recycling would not necessarily be detected in this way.

The cause of the termination of the bloom is of interest, since precipitous decline seems to be a property of spring blooms regardless of latitude. Lund (1950) was interested in this problem in several lakes of the English Lake District, and he attributed the spring bloom decline to silica shortage confirming the suggestion of Pearsall (1932). The organisms in this case were predominantly Asterionella formosa Hass. In Smith Lake the earlier bloom under the ice declined probably as a result of ammonia exhaustion, and the regeneration rate at the low temperatures under the ice may not have been adequate to support further growth. The bloom in June, however, was dominated by Anabaena sp., the dominant species being Anabaena flos-aquae. Since nitrogen fixation was being carried out, presumably by these algae, nitrogen exhaustion cannot be a cause of the bloom termination in itself. The most plausible explanation still lies in exhaustion of some other nutrient.

If nutrient limitation is a factor terminating the spring bloom

in Smith Lake, the most likely critical elements involved are phosphorus, iron or molybdenum. The first is, of course, an essential nutrient which has been implicated as a limiting factor in many cases. The amount required for normal growth is not great, however, and Benoit and Curry (1961) give the critical concentration of phosphorus for blue-green algae as about 10 ppb. This is equivalent to about 0.2 μg-atoms/liter, which is similar to the minimum concentration found in Smith Lake during the bloom (see Table 10, Appendix B). Coffin, et al. (1949) have shown that estimations of soluble phosphate are not reliable indices of fertility in some lakes, because there tends to be an equilibrium between supply and demand. Hutchinson and Bowen (1950) have found that the rate of regeneration of phosphorus after uptake by phytoplankton is very rapid. Rigler (1956) confirmed this experimentally. He used radioactive phosphorus, and showed that phosphorus was being taken up by organisms even though the amount detected analytically remained constant, and from this concluded that the phosphorus was being turned over very rapidly. The calculated turnover time for orthophosphate in the trophogenic zone of the lake was only 3.6 minutes. Later work by the same investigator (Rigler, 1964) confirmed this result, and in addition showed that in a highly productive lake, the turnover time could be as short as 1 minute. It seems unlikely that in Smith Lake lack of phosphorus causes the decline of the bloom, since the phosphorus pool in the lake is large, and even in 1963, when there was contact between the mud surface and the trophogenic region during the bloom, the termination occurred in a

similar manner, although less abruptly.

The other two elements mentioned were suggested for the following reasons. Iron is required in high amounts by nitrogen fixing blue-green algae, and by the time the bloom declined, nitrogen fixation was providing 50 percent of the nitrogen for growth. Maximum fixation of nitrogen takes place at a concentration of 10 mg/liter of ferric iron according to Carnahan and Castle (1958), and is inhibited at both higher and lower concentrations in the blue-green alga Nostoc muscorum. Shapiro (1964) has shown that yellow organic material of the type found in Smith Lake water binds iron increasingly firmly as the pH increases, and it is notable that the bloom decline coincides in time with an increase of the pH of the water. The consequent unavailability of a metal would provide a possible connection. The behavior of molybdenum in the presence of organic material is not known, but it is required in ecologically large amounts for nitrogen fixation, and preliminary enrichment experiments with this metal resulted in 100 percent increase in the ^{14}C uptake after one day incubation with additional molybdenum. The addition of phosphate at this time had no great effect. The effect of pH could also be direct in reducing the amount of free CO_2 in the water, and therefore inhibiting photosynthesis. During the peak of the bloom, ^{14}C uptake experiments were carried out in which ^{14}C was added to samples which were buffered at pH 8.5 and 9.2 as well as controls at lake water pH of about 7.4. This was during the peak nitrogen fixation time. A relationship resulted, in which ^{14}C uptake increased with the increase in pH in

a linear manner:

	µg C/liter/hr	
pH 7.4	12.38	
pH 8.5	19.12	June 10, 1964
pH 9.2	23.46	

It is felt that this indicates that very little organic carbon was indeed present, resulting in a high proportional uptake which increased with pH as the available carbon decreased, producing an apparent increase in photosynthesis with pH, which was in fact an artifact.

The original value for alkalinity was used in calculating the available carbon, and since some of the carbon was probably lost to the system by precipitation of carbonate, this overestimate of amount of carbon could result in a high proportional accumulation rate also appearing to be a high absolute uptake rate. The amount of free bicarbonate may be the most readily available source. However, it is possible that the photosynthetic ratio does indeed increase with pH in this range. Wright (1960) feels that CO_2 limitation is bound to be in effect where nutrient limitation is not controlling the rate of chlorophyll synthesis. This limitation would be density dependent.

Another possibility for the decline of the bloom is the algae themselves excreting a toxic substance which accumulates as the population increases in both density and age. Such a substance, termed an exotoxin, may initially serve to prevent the growth of other

algae, but eventually it is possible that the species which produces it can no longer tolerate its own growth-inhibiting substances, and this could result in a sudden crash in population. Anabaena flos-aquae, the dominant algae in the Smith Lake bloom has been studied by Gorham et al., (1964), who find that it excretes substances toxic to mammals and birds. It is not known to what extent these substances are toxic to zooplankton, other algae or the Anabaena itself. However, it is striking to note that at the peak of the bloom Anabaena is almost unialgal, and there are very few zooplankton. However, it is not unusual to find fewer grazers at the peak of a bloom. Ryther (1954) has discussed such relationships.

A virus which is able to cause lysis in blue-green algae has been reported by Safferman and Morris (1963), and this is a possible mechanism for bloom termination in lakes. Silvey and Roach (1964) suggest that this may be an explanation of sudden disappearance of blue-green algal cells that have attained high densities.

In the Smith Lake bloom, the precise timing for the two years was similar, and yet the peak was much sharper in 1964 than in 1963. This difference in peak shape could have been due to the stratification of the lake in the former year, in causing nutrient depletion to become critical more rapidly. In 1963, contact with the deeper water and the bottom mud allowed a longer period of high production. After the bloom, however, the production in 1964 was higher, confirming the assumption that mixing following the bloom replenished a nutrient, which had remained in the water because it had not been available during the bloom.

The post-bloom production

Violent fluctuations in algae populations are characteristic of control by nutrient depletion. In Smith Lake during July, productivity rates, nitrogen uptake rates and nitrogen nutrient levels remain almost constant, with only small and gradual fluctuations. A different kind of mechanism was then operating. Ammonia remained the principal source of nitrogen during this time, and its uptake varied directly with photosynthesis; thus, nitrogen was probably not controlling production. The amount of ammonia present at any time was sufficient to allow uptake at the rates measured for one or two days. Much of the ammonia used, again, must have come from recycling. Very little nitrate was present during this time.

On August 11th, the ammonia concentration fell below the lower limit of detectability, and yet an uptake rate of 1.48 μg/liter/hr was measured. However, the ammonia supply experiment described above for the same date suggests a rate of replenishment in the water which is even higher than would be assumed from the uptake measurements. At this time of year the rooted vegetation around the shore is well developed, and there is probably movement of nutrients from the open water to these plants. Horizontal movement could be important at this time.

During the more or less steady-state period in the lake, zooplankton could have provided both the control mechanism for algal population size and also the mechanism for recycling ammonia directly and rapidly.

This type of mechanism was described by Harris (1959) in Long Island Sound. This author points out that the time required for such return of nutrients by the animal population is negligible when compared with the time required for bacterial processes, and that this mechanism returns nutrients to the phytoplankton and thus bolsters up the population rapidly, providing food for the expanding animal population. A rapid positive feedback thus operates between phytoplankton and zooplankton. Zooplankton were abundant in Smith Lake during most of the summer following the spring flowering, building up to about 30 individuals per liter at the surface in early September.

In August, the increase in both ammonia and nitrate uptake resulted in depletion of the ammonia. Following this a lower steady ammonia uptake with constantly detectable but low nitrate uptake rates continued for the remainder of the month. Nitrate was used almost as rapidly as ammonia in September, at a time when the dominant alga was Selenastrum sp. In October, ammonia and nitrate were used, in the ratio 2:1, the population at this time consisting almost exclusively of Gymnodinium sp. Control by grazing was the explanation invoked by Anderson, Comita, and Engstrom-Heg (1955) for such inverse relationships between zooplankton and phytoplankton in two Washington lakes.

Nitrate as a nitrogen-source in Smith Lake

The lack of importance of nitrate as a nitrogen source in Smith Lake may be due in part to its unavailability. However, there is evidence that significant rates of nitrification do take place in the

surface water of the lake during the summer months. In 1963, nitrification was anticipated following the release of nitrogen from the June bloom, and with the cooperation of Dr. Stanley Watson enrichment cultures designed to isolate nitrifying organisms were set up. These produced cultures with very strong nitrifying ability within a few days, indicating that the organisms initially were probably present in large numbers in the water. The high nitrate levels in the water at this time suggest that there was indeed active nitrification going on. In 1964, the small nitrate increase following the bloom coincided with the first mixing of the lake, and so it is not easy to establish its origin. However, previously there had been very little nitrate at any depth, and a steady slow increase from June 18 to June 30 took place, so that probably some nitrification was being carried out.

Steemann Nielsen (1959) has pointed out that in shallow waters in contact with the bottom, temperature is an important factor governing productivity, because the bacterial enzymatic processes responsible for nutrient regeneration are temperature dependent. Since nitrification is an important process during much of the winter under the ice in Smith Lake, it should be even more so at the higher summer temperatures. Nevertheless, most of the time the ammonia is apparently used rapidly and the rate would suffice to prevent significant accumulation. In 1963, the nitrate accumulation following the June bloom could be explained by the complete depression of uptake, since the decline in photosynthesis and algae population was so extreme

in that year.

The behavior of nitrate provides confirming evidence for the idea that depletion of another nutrient is the terminating cause for the June bloom. The exhaustion of molybdenum, for instance, would prevent the utilization of nitrate. In 1964, mixing from the bottom obviously replenished essential nutrients, and allowed the nitrate to be used, whereas in 1963 the entire water column was apparently depleted.

Interrelationships with microorganisms

Unfortunately very little is known about the relationship between algal productivity cycles and other microorganisms in the water. It is reasonable to assume that nitrifying organisms, for instance, increase in numbers following a period of organic production. Less obvious relationships must surely also occur. Silvey and Roach (1964) describe microbiotic cycles in surface waters, and point out that there are cyclical changes in both bacteria and actimomycete populations accompanying those in the algae. With blue-green algae blooms, they find an accompanying increase in gram-negative bacteria, whereas following the blue-green algal decline, actinomycetes attack the remains of the algae, and reduce the bacterial population, presumably by antibiotic production. Following this, gram-positive bacteria take over, and then the cycle may be repeated with another algal bloom. This type of interrelationship could explain the productivity patterns in many lakes. The nature of the relationship between the gram-negative

bacteria and the blue-green algae, is not known but it is well known that many blue-green algae are not easy to grow in the absence of bacteria.

A comparison of Smith Lake and Sanctuary Lake, Pennsylvania

In Sanctuary Lake, Pennsylvania, preliminary work had been done in detecting nitrogen uptake using similar methods to those used here (Dugdale and Dugdale, 1965). Sanctuary Lake has some features in common with Smith Lake, especially depth and the marshy nature of the surrounding drainage basin. In Sanctuary Lake, however, there is continuous flow through the lake, and much of the cyclical behavior of the uptake patterns can be correlated with variations in nitrogen and phosphorus income from its major inlet. It was not necessary there to assume any high regeneration rate of nutrients to account for the uptake regime. In Smith Lake, we are considering an isolated internal cycle, which instead of income apparently undergoes loss of nutrients from the open water to the rooted vegetation. In both lakes it is apparent that high ammonia and nitrate uptake rates accompany nitrogen fixation, and for the spring blooms in both lakes, the proportion of the nitrogen contributed by the three sources is very similar. Sanctuary Lake has a fall bloom which is heavily dependent on nitrogen fixation, whereas no such nitrogen fixing fall bloom is found in Smith Lake.

In the Sanctuary Lake data, the expected inverse relationship

between utilization and dissolved fixed nitrogen levels shows up clearly.

Both lakes drain marshy areas, and it has been pointed out that the humic materials which are brought into a lake by drainage from such land could be very important ecologically, since they are known to stimulate production in culture. Phinney and Peek (1961) have attributed the unexplained high productivity of Klamath Lake to these materials, and they suggest that the major role of this material is chelation. In attempting to isolate the Anabaena flos-aquae from the Smith Lake bloom, it became apparent that it would grow much more readily in culture media in the presence of a few drops of concentrated Smith Lake water. A relationship may exist between significant nitrogen fixation and such materials in a lake.

CONCLUSIONS

1. Ammonia is the most important source of nitrogen to the phytoplankton in Smith Lake. It is produced in large amounts by bacterial breakdown of nitrogenous organic material under the ice during the winter, and then further accumulates as a result of the suppression of nitrification during anoxic conditions in early spring. This ammonia serves as the principal nitrogen source for the production under the ice in early spring, and all becomes incorporated into organic material prior to the melting of the ice. A maximum ammonia uptake rate of 13 μg/liter/hr was measured during this growth period. At the same time a photosynthetic rate of 33 μg C/liter/hr was measured *in situ* in the lake. This represents a C:N ratio of 2.5:1.

2. Nitrate is not an important nitrogen source in Smith Lake, although it accumulates slowly as a result of nitrification during the winter. By early spring a large amount of nitrate is present in the water, but is lost when the oxygen becomes depleted. The most probable fate of this nitrate is loss by denitrification, and if this is the case, about 8 μg-atoms/liter of nitrogen may be lost to the lake during this anoxic time. Although nitrification probably continues during the summer, there is never any accumulation of nitrate. The nitrification rates are apparently sufficiently slow so that even a very low uptake, or perhaps bacterial reduction, prevents any

discernible accumulation. Occasional pulses of nitrate utilization accompany ammonia uitilization, but nitrate is never the sole or even the more important source of nitrogen.

3. The nitrogen-fixing bloom of Anabaena sp., dominated by Anabaena flos-aquae, occurs in June with temporal reproducibility. As this bloom builds up, ammonia provides the primary nitrogen source, although nitrate is used at the same time. As the photosynthetic rate reaches its maximum, nitrogen fixation increases in relative importance. The source of the ammonia for this rapid uptake is not clear. There is abrupt termination of the bloom, which, although accompanied by a unique rise in pH, is more likely to be determined by nutrient depletion. This bloom accounts for the addition of about 500 μg/liter of newly fixed nitrogen to the lake surface water. The algae form akinetes, and largely become dormant for the remainder of the year, although an occasional individual filament is found subsequently in the water. The ratio between carbon and a total nitrogen uptake during the peak of this bloom was 3:1.

4. The remainder of the growing season is characterized by a more steady but modest rate of photosynthesis and nitrogen utilization rates, and an isolated experiment to determine ammonia production rates indicates that rapid recycling of ammonia takes place, and that this probably involves a direct phytoplankton-zooplankton relationship in addition to bacterial processes.

5. High productivity rates are invariably accompanied by high ammonia uptake rates, and for this lake similar seasonal patterns are obtained when primary productivity, ammonia uptake or total nitrogen uptake is considered.

6. The nitrogen utilization regimes in lakes would appear to be similar over a wide geographical distribution. The similarity of uptake patterns during the spring bloom in subarctic Smith Lake, Alaska and temperate Sanctuary Lake, Pennsylvania are striking. It is possible that nutrient cycle studies in one region can thus produce generalizations which may be applicable to similar lakes in other parts of the world.

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APPENDIX A

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NITROGEN METABOLISM IN LAKES I. MEASUREMENT OF NITROGEN FIXATION WITH N^{15} [1]

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ABSTRACT

A method for measuring the rates of nitrogen fixation in samples of lake water using N^{15} as a tracer is described, and a detailed description of glassware required is provided. The nitrogen present in the water is removed by flushing with He-O mixture and then replaced by the labeled nitrogen. After incubation and treatment, the isotope ratio in the organic material is determined on a mass spectrometer. Analyses of lake water and $(NH_4)SO_4$ as isotope ratio blanks are described and a résumé of statistical data from these analyses is presented.

INTRODUCTION

In several papers, one or the other of us has recently reported on the results of direct measurements of rates of nitrogen-fixation in samples of natural fresh or marine water (Dugdale, *et al.* 1959; Dugdale and Neess 1960; Dugdale, Menzel and Ryther 1961; Neess, *et al. in press*). Each of these lacked a complete description of the method used. The purpose of this communication is to supply such a description along with certain comments that will make it possible to interpret the results more critically.

Heavy nitrogen (N^{15}) has been used successfully in the study of the nitrogen metabolism of organisms and in the study of biological nitrogen fixation (Rittenberg, *et al.* 1939; Burris and Miller 1941; Burris, *et al.* 1942). Our method is identical in principle to those previously used, and mechanically similar in many respects. Such modifications as we have introduced are in keeping with our objective of treating essentially unmodified natural water, with its biota intact and functional, simply as a reagent for fixing nitrogen, and of studying its properties as such, largely ignoring the particular organisms responsible for the activity and their individual physiologies.

METHOD

Free nitrogen dissolved in an enclosed volume of natural water is completely removed by aeration with minimum disturbance of the concentration of dissolved oxygen. Nitrogen gas more or less heavily enriched with N^{15} is then added, and the sample is incubated for a time under a particular set of conditions. After incubation total Kjeldahl nitrogen is recovered from the sample as ammonia and its quantity determined. Thereafter the ammonia is oxidized to free nitrogen and the proportions of the isotopes in it determined with a mass spectrometer. If the proportion of N^{15} in the originally introduced nitrogen gas and the total amount of "fixed" nitrogen in the sample of water are known, then it is possible to compute the amount of free nitrogen converted to Kjeldahl nitrogen in the course of the incubation.

Collection of the sample and treatment before incubation

In Madison, our samples have been collected in the ordinary way, usually with a Kemmerer bottle. It would probably be desirable to use a plastic or non-toxic sampler, but we have not always done this, sometimes using one made of brass. At Pittsburgh glass or plastic samplers have been used exclusively. Samples are taken as quickly as possible to the laboratory. Whether or not the samples are exposed

[1] This work was supported by the National Institutes of Health; in the early stages some funds were provided by the Faculty Research Fund of the University of Kentucky. We are indebted to R. H. Burris of the University of Wisconsin and to R. Abrams of the Montefiore Hospital Institute of Research, Pittsburgh, for the use of the mass spectrometers in their laboratories.

[2] University of Wisconsin.
[3] University of Pittsburgh.

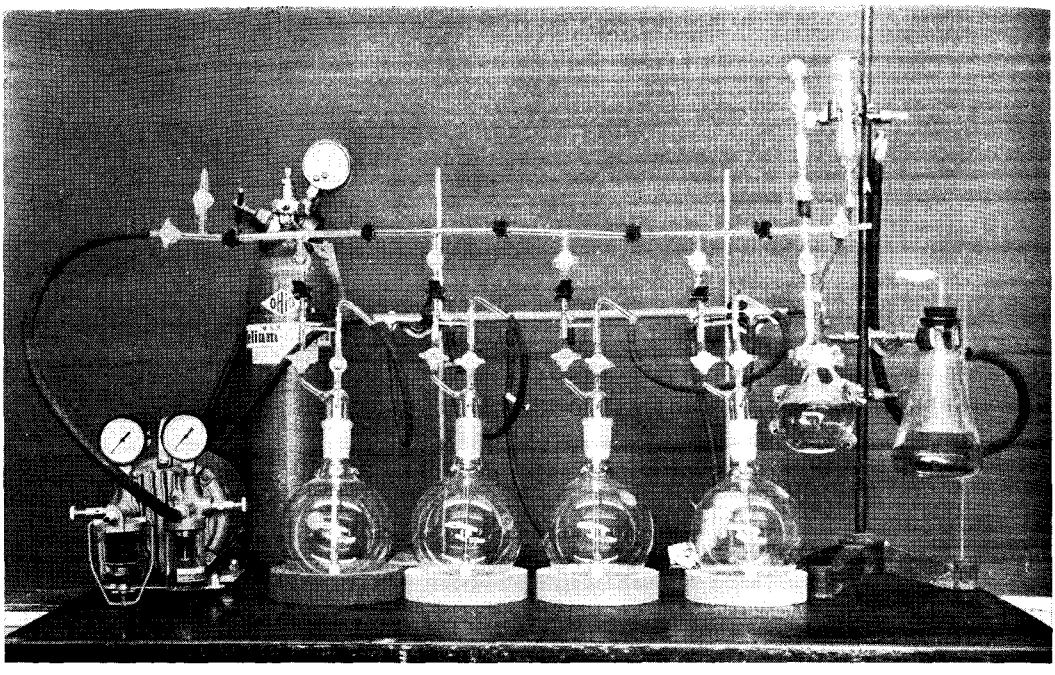
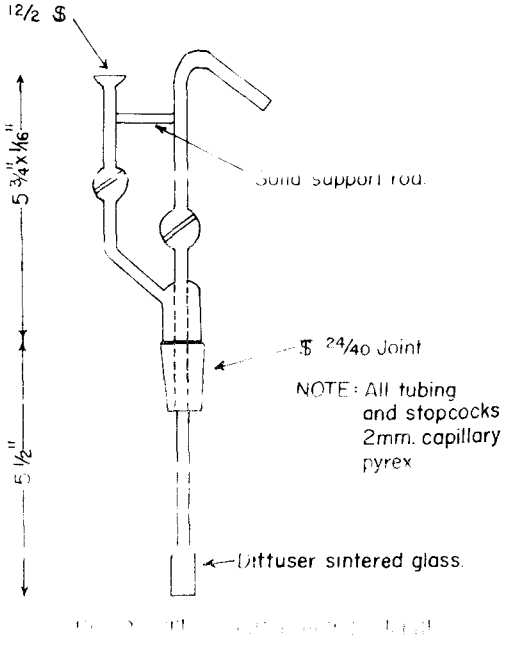


FIG. 1. Apparatus for replacing the dissolved nitrogen in water samples with N^{28}.

to weak or strong illumination before incubation may affect the results later obtained; depending upon the particular circumstances of an experiment, control of illumination of the sample may be desirable.

Preliminary treatment of the samples is carried out in the apparatus shown in Figure 1. Samples of water, untreated in any way, are enclosed in each of the flasks. These may be of any practical volume; we have generally used those holding 1 L, sometimes 250 or 500 ml. The flasks have necks fitted with standard-taper ground joints to receive the one-piece aeration units, shown in place in Figure 1 and in detail in Figure 2. Each of the latter has a gas-inlet extending to the bottom of the flask and ending in a sintered glass sparger, and a gas-outlet entering at the very top of the flask when it is closed. Inlet and outlet are fitted with stopcocks. When the flasks are filled with water, with aeration units in place but with stopcocks on inlet and outlet open, pressure is maintained at 0.8 atm at the outlet with an air-pump, and a mixture containing 0.8 vol of helium and 0.2 vol of oxygen is introduced at the inlet. This is accomplished by connecting the flasks to the manifolds shown in Figure 1. The inlet manifold is a simple large-diameter one-



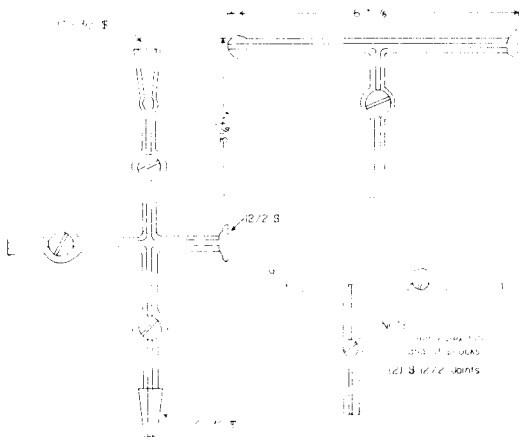


FIG. 3. Connecting and end units for the manifold.

piece affair to which the flask inlets and cylinder containing the He-O mixture are connected with rubber tubing. The outlet manifold is made up of individual units constructed of capillary tubing; the units connect to each other and to the gas-reservoir and sample-bulb at one end with ball-and-socket ground joints. The units are illustrated in Figure 3.

The He-O mixture leaves the spargers in small bubbles and carries nitrogen, along with CO_2 and atmospheric constituents other than oxygen, out of the flasks. With equipment designed as ours, with 1-L samples of water and a reasonably vigorous stream of gas, 15 min is sufficient to remove any nitrogen determinable by mass spectrometric analysis from the outlet gas. The behavior of macroscopic zooplankters such as copepods and *Daphnia* is apparently unaffected by this treatment.

After aeration, inlet and outlet stopcocks are closed in that order, leaving a residual total pressure in the flasks of 0.8 atm and an atmosphere containing only helium and oxygen. The outlet manifold is then exhausted to a high vacuum and disconnected from the vacuum pump. Nitrogen gas enriched in heavy nitrogen (up to 99.7 atom per cent N^{15}) is introduced into the evacuated manifold at a pressure slightly above 1 atm from the reservoir (Fig. 1). A formula for the confining fluid is given below. A sample is taken from the manifold for mass spectrometric analysis, and some gas is admitted into the flasks by opening each outlet stopcock briefly. The finally sealed flasks enclose total pressures of slightly more than 1 atm and contain atmospheres with about 0.20 atm partial pressure of nitrogen, about 0.64 app of helium and about 0.16 app of oxygen. We have obtained nitrogen gas enriched in N^{15} from the Isomet Corporation, Palisades Park, New Jersey.

When sealed, the flasks are disconnected from the manifold and shaken by hand or with a wrist-action mechanical shaker to equilibrate liquid and gas phases.

Flasks are incubated under various conditions and for various periods. Whenever possible, we have suspended them in the lake from which the samples originated and at distances from the surface corresponding to the depths at which the water was collected, or otherwise, in the laboratory under artificial illumination or in the dark.

After incubation, the flasks are opened, the contents acidified with a small quantity of H_2SO_4 and boiled down to a volume small enough to be accommodated in a 30-ml micro-Kjeldahl digestion flask. Subsequent digestion of the sample is carried out for 18 hr at the boiling point of a mixture of 2 ml of H_2SO_4, 1.2 to 1.4 g K_2SO_4 and about 50 mg $HgSO_4$. Digestion under such severe conditions is necessary to obtain correct results in subsequent stages of the procedure (see Rittenberg, *et al.* 1939). Nitrogen is recovered from the digest as NH_3 by a completely standard micro-Kjeldahl distillation into 2% H_3BO_3 acid, followed by titration with 0.01 N HCl in the presence of a methyl red-methylene blue indicator. The result of the titration is the total Kjeldahl nitrogen of the sample of lake water. After titration, the sample is re-distilled into HCl to rid it of the indicator. The second distillation need not recover the nitrogen of the sample quantitatively, and may be eliminated entirely if the original titration is done with a pH-meter instead of the indicator.

Free nitrogen is obtained from the result-

ing NH_4Cl by treating it with alkaline hypobromite under vacuum. This is accomplished in a special piece of apparatus so arranged that the hypobromite solution and the sample distillate are mixed by tilting part of the apparatus after it has been evacuated. Our apparatus is exactly like that described by Rittenberg, *et al.* (1939); formulas for hypobromite solutions are given below. The nitrogen gas released is then moved with a Toepler pump into a gas-sample bulb arranged to connect at the sample-inlet of the mass spectrometer, or it is sealed into a 3-cm piece of glass tubing according to the method of Weinhouse (1946). No further purification is necessary. For assay of isotope content we have used Consolidated Model 21-201 Isotope Ratio Mass Spectrometers.

Reagents

All reagents to be made up with fresh glass-distilled water.

1. Confining fluids. 15% Na_2SO_4 + 5% H_2SO_4. To 15 g Na_2SO_4 (reagent grade) add 80 ml H_2O and 3 ml reagent grade H_2SO_4.
2. Kjeldahl digestion mixture.
   1.2–1.4 g K_2SO_4 (reagent grade) and 50 mg $HgSO_4$ (reagent grade) and 2 ml H_2SO_4. The salts may be mixed and placed in the micro-Kjeldahl flasks before the sample is added.
3. Indicator for Kjeldahl distillation (used if pH meter is not in use) methyl-red methylene blue.
   0.248 g methylene blue and 0.375 g methyl red dissolved in 300 ml 95% ethyl alcohol.
4. 40% NaOH—add 100 g NaOH crystals to 225 ml of freshly distilled water, cool and store in a rubber-stoppered bottle.
5. Boric acid 2%.
6. 0.01 N HCl—this should be accurately standardized.
7. Alkaline hypobromite.
   a. 8 ml bromine and 35 ml of 11 M NaOH and 70 ml distilled water
   b. We have experienced difficulty in preparing low nitrogen samples for mass spectrometry using the above hypobromite preparation as a result of spontaneous O_2 production, which becomes a significant component of small gas samples. By using the hypobromite preparation of Rittenberg, *et al.* (1939) this difficulty has been largely overcome.

Calculations

The amount of nitrogen fixed is calculated by the expression

$$N_f = \frac{A_f \cdot N_t}{A_i} \ldots \qquad (1)$$

where N_f = nitrogen fixed (mg).
A_i = atom per cent excess N^{15} in enriched N_2 supplied at the beginning of the experiment.
N_t = total reduced nitrogen at the end of the experiment as determined by the micro-Kjeldahl procedure (mg).
A_f = atom per cent excess N^{15} in N_t.

The term atom per cent excess refers to the atom per cent N^{15} in a sample above some arbitrary standard, for instance, the normal atom per cent N^{15} found in biological materials or in the atmosphere. This value is usually taken to be 0.358.

The results are also sometimes expressed as "per cent nitrogen fixed." This refers to the amount calculated by:

$$\frac{N_f}{N_t} = \frac{100\,A_f}{A_i} \qquad (2)$$

The expressions given are simplified and hold as long as the change in N_t is held to a fairly small value during the course of the experiment.

Selection of Standards

The importance of getting an accurate estimate of A_f is obvious from the above discussion; the selection of the standard above which the excess is calculated becomes a critical matter since isotope-ratio mass spectrometers are not ordinarily calibrated to read absolute isotope ratios. Several standards are in common use: 1) laboratory air, 2) tank N_2, 3) N_2 obtained by conversion of some chemical compound

TABLE 1. *Statistical résumé of data obtained from standards and blanks*

	Wisconsin Mass Spectrometer			
	$(NH_4)_2SO_4$ standards	Lake water autoclaved blanks N^{15} added	Lake water blanks Lake Mendota	Lake water blanks Lake Wingra
N	17	13	13	15
$\bar{x}$	0.3668	0.3696	0.3694	0.3680
s^2_x	0.000012058	0.000044872	0.000011367	0.000040082
s_x	0.00347	0.00670	0.00379	0.00633
$s_{\bar{x}}$	0.00084	0.00186	0.00010	0.00016

	Pittsburgh Mass Spectrometer		
	Tank N_2	$(NH_4)_2SO_4$	Lake water
N	19	8	11
$\bar{x}$	0.3508	0.3528	0.3531
s^2_x	0.000001165	0.000000722	0.000000271
s_x	0.00108	0.00085	0.00052
$s_{\bar{x}}$	0.00024	0.00030	0.00016

of ammonia, and 4) a control from the experiment. Because the enrichments obtained in the early experiments were small, and were expected to remain at relatively low levels, measurements were made on the mass spectrometer comparing the results obtained from tank N_2, converted $(NH_4)_2SO_4$ and boiled down lake-water blanks from two lakes. A single bulb of N_2 taken from a tank of compressed, water-pumped nitrogen was used as primary standard using the following procedure:

(1) Successive determinations were made on tank N_2 until the isotope ratio no longer declined, but varied about some low value.
(2) The sample to be read (lake-water blank or converted $(NH_4)_2SO_4$ was admitted and the ratio determined.
(3) Another tank N_2 sample was read.
(4) Repeat of steps (2) and (3).

A similar series was run on water from Lake Mendota and Lake Wingra, which also included a number of autoclaved lake-water samples to which N^{15} was added in the usual way. The results of an analysis of these data are given in Table 1. Tank nitrogen is quite obviously a less suitable standard than is converted $(NH_4)_2SO_4$. Presumably impurities are added during conversion, bringing the resulting ratio of the latter up to that observed from the lake water samples subjected to the same treatment.

The least significant difference, at the 95% confidence level, is 0.0027 atom per cent, or practically 0.003 atom per cent for the Pittsburgh mass spectrometer, and similarly 0.0038 atom per cent for the Wisconsin mass spectrometer. Since the amplifiers in the Pittsburgh machine have recently been rebuilt by the manufacturer, resulting in greatly improved performance, it may be assumed that these figures are more or less optimal.

Controls

The data from the standards indicate that routine controls are not essential and that ammonium sulfate is an adequate standard for the isotope-ratio determination. Nevertheless, periodically autoclaved lake water is included in the experiments and more frequently samples of lake water are boiled down for isotope-ratio analysis.

DISCUSSION

It is entirely unlikely that the positive results we have obtained using these methods represent anything but active biological fixation of nitrogen which occurs during the incubation. We have obtained no evidence that the distribution of nitrogen isotopes in unautoclaved lake-water blanks differs

significantly from the distribution in a sample of Fisher A. R. $(NH_4)_2SO_4$ used as a primary standard for "ordinary" nitrogen. Although the matter of isotope effects in biological transformations of nitrogen is not yet completely settled, it seems safe enough to take most currently available data to suggest that there is no isotope effect in biological nitrogen fixation (Hoering and Ford 1960), and also that the nitrogen of biological materials does not in general differ strikingly or consistently in isotopic composition from that of non-biological ones. We conclude that we have made no overestimates of rate of nitrogen fixation due to isotope effect.

Autoclaved lake water in contact with nitrogen gas enriched in N^{15} has never in our experience incorporated N^{15} into its Kjeldahl nitrogen, suggesting that no non-active processes, such as atomic exchange, have led to our results. It remains possible, though unlikely, that the autoclaving itself has interfered with some non-active process leading to net uptake of N^{15}.

During incubation the partial pressure of nitrogen at equilibrium with the sample is about 0.2 atm, about one-fourth its normal value. In view of the findings of Burris and Wilson (1946) that the half maximum rate of nitrogen fixation by *Nostoc muscorum* occurs at a partial pressure of 0.02 atm, we have considered that reducing the pressure has led to no more than minor underestimation of the rates that would have occurred in the flasks had normal concentrations of free nitrogen been available; the cost of each determination has been appreciably reduced thereby.

Ordinarily the time of incubation has been no greater than 24 hr, often less. Some of our early results (Dugdale, *et al.* 1959) suggest that the rate of fixation inside one of the flasks tends to rise with increasing time of incubation and that therefore the latter should be kept as short as possible. We have never found evidence for a change in rate within 24 hr. Further investigations of the effects of prolonged incubation are in progress, but it is possible now to say little more than the above. It is to be expected that any prolonged confinement of a sample of lake water will bring about changes in its behavior; it would be useful to know how long its normal nitrogen-fixing ability persists under these conditions.

Along with nitrogen, CO_2 is exhausted from the flasks during aeration, with a resulting displacement of the carbonate-bicarbonate equilibrium. We have never made measurements of the changes in pH during aeration, nor estimated the losses of CO_2. It is entirely possible that our measured rates of nitrogen fixation are lower than those that would have been realized in the same samples of water had the amount of carbon available to photosynthetic organisms not thus been reduced. A useful modification of the method would be the inclusion of CO_2 at something like the normal atmospheric partial pressure in the aerating gas mixture, although this could not be expected to leave the concentrations of carbonate and bicarbonate steady in all samples of lake water.

It is also no doubt possible that the elevation of pH consequent upon aeration of some samples would lead to injury to some of the organisms present. Normally, as stated above, the activity of visible zooplankters is not changed during aeration, and indeed usually remains unchanged through the entire incubation period, indicating no violent damage to the biota. We have occasionally observed damage to the phytoplankton such that the healthy green color turned yellow, and suspect a correlation with unduly prolonged aeration. The question of pH change mentioned above may be important here.

Inasmuch as nitrogen combined as nitrite or nitrate is lost from an unmodified Kjeldahl determination, it is possible that we have made small errors in estimating the total quantities of nitrogen fixed in some samples owing to the movement of newly fixed nitrogen into nitrite or nitrate during incubation. In view of the short periods of incubation, this seems very unlikely to have occurred. Denitrification cannot be expected to occur during incubation in the presence of abundant oxygen.

but is furthermore unlikely to have any large effect upon measured rate of fixation because of the large excess of free nitrogen in the flasks (5–10 mg of free nitrogen compared to something of the order of 1 mg of total combined nitrogen, only a very small fraction of which is ordinarily in the form of nitrite or nitrate at the beginning of incubation). Under exactly the right circumstances, this conclusion might have to be revised.

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APPENDIX B

APPENDIX B TABLE 1

SMITH LAKE 1963

TEMPERATURE
(DEGREES CENTIGRADE)

DEPTH (M)	MAY 21	MAY 30	MAY 31	JUN 05
0.0	06.5	13.4	13.4	12.8
0.5	06.5	12.8	13.4	12.8
1.0	06.2	12.6	13.4	12.8
1.5	05.4	11.4	13.4	12.8
2.0	04.9	09.3	12.1	12.8
2.5	04.0	07.3	08.4	12.6
	JUN 07	JUN 11	JUN 12	JUN 19
0.0	16.0	19.0	18.7	19.4
0.5	15.9	18.4	18.5	17.4
1.0	13.2	15.6	16.9	16.0
1.5	12.7	14.1	14.4	14.7
2.0	11.9	12.8	13.0	13.8
	JUN 26	JUL 01	JUL 07	JUL 15
0.0	16.1	18.0	22.9	20.7
0.5	15.3	18.0	22.3	19.7
1.0	15.1	18.0	19.0	19.4
1.5	15.0	18.0	17.7	19.0
2.0	14.2	11.1	17.0	18.5
	JUL 23	JUL 30	AUG 07	AUG 20
0.0	19.3	16.8	15.8	15.1
0.5	19.3	16.7	15.6	14.5
1.0	18.5	16.7	15.3	14.2
1.5	16.7	16.7	15.1	13.8
2.0	16.4	16.3	15.0	12.4
	AUG 27	SEP 17	SEP 25	SEP 30
0.0	14.6	08.0	06.7	08.0
0.5	14.5	.	.	.
1.0	14.4	.	06.3	.
1.5	14.0	.	.	.
2.0	13.9	.	06.2	.

APPENDIX B TABLE 1 CONTINUED

DEPTH (M)	OCT 09	OCT 25	NOV 06	NOV 13
0.0	07.0	00.0	.	.
0.5	.	02.2	02.3	.
1.0	.	02.9	03.1	02.8
1.5	.	03.6	03.7	.
2.0	.	04.0	04.0	03.9

	DEC 04
0.0	.
0.5	.
1.0	02.5
1.5	03.6
2.0	04.1

APPENDIX B TABLE 2

SMITH LAKE 1964

TEMPERATURE
(DEGREES CENTIGRADE)

DEPTH (M)	JAN 03	FEB 03	FEB 19	FEB 26
0.0				
0.5				
1.0				
1.5	02.1	01.9	01.6	02.3
2.0	03.2	03.7	02.6	02.9
2.5	03.9	04.1	03.4	03.4
MUD	04.0	04.5	03.9	03.6

	MAR 18	MAR 24	MAR 31	APR 03
0.0				
0.5				
1.0				
1.5	01.6	01.6	01.4	01.5
2.0	02.6	02.6	02.4	02.2
2.5	03.1	03.1	03.0	03.3
MUD	03.4	03.7	03.8	03.7

	APR 08	APR 14	MAY 01	MAY 05
0.0				
0.5				
1.0				
1.5	01.5	01.6	01.7	01.8
2.0	02.4	02.4	02.1	02.2
2.5	03.4	03.4	03.2	02.9
MUD	03.9	03.9	04.0	04.0

	MAY 12	MAY 18	MAY 27	MAY 30
0.0	.	.	.	13.5
0.5	.	.	04.2	08.2
1.0	03.8	03.2	04.8	05.6
1.5	03.1	03.3	04.2	05.2
2.0	03.0	03.2	04.0	04.3
2.5	03.3	03.3	03.5	03.6
3.0	03.7	.	03.4	.
MUD	04.0	03.9	03.6	03.5

APPENDIX B TABLE 2 CONTINUED

DEPTH (M)	MAY 31	JUN 01	JUN 02	JUN 04
0.0	13.9	17.8	17.3	18.3
0.5	12.0	16.8	16.6	16.3
1.0	08.0	15.6	10.1	14.0
1.5	06.4	07.6	07.2	10.6
2.0	05.4	05.3	05.8	06.5
2.5	.	04.6	05.1	05.5
3.0	.	04.0	04.6	05.1
MUD	03.9	04.0	04.4	05.0

	JUN 06	JUN 08	JUN 10	JUN 12
0.0	19.0	20.6	18.2	20.5
0.5	18.8	18.8	18.2	17.9
1.0	18.8	17.2	18.2	16.5
1.5	10.2	09.7	17.9	12.3
2.0	08.3	07.8	08.6	08.8
2.5	05.9	05.9	06.6	06.1
3.0	05.7	05.5	05.5	05.7
MUD	.	05.4	.	05.6

	JUN 16	JUN 18	JUN 23	JUN 25
0.0	20.4	22.2	18.8	17.2
0.5	20.2	21.5	18.9	17.2
1.0	18.6	18.7	18.8	16.7
1.5	13.3	15.8	18.8	15.6
2.0	10.2	10.7	09.6	15.0
2.5	07.2	07.5	.	09.5
3.0	06.1			
MUD	05.9	06.3	.	06.8

	JUN 30	JUL 02	JUL 07	JUL 13
0.0	18.4	17.5	20.9	19.7
0.5	18.4	17.5	19.0	19.2
1.0	18.4	17.5	18.7	18.0
1.5	16.2	16.9	16.8	16.4
2.0	13.6	14.0	14.8	15.1
2.5	10.5	11.1	11.4	11.5
MUD	09.8	08.6	10.3	10.3

	JUL 16	JUL 21	JUL 28	AUG 04
0.0	24.7	18.8	19.8	19.0
0.5	21.7	18.6	19.7	18.9
1.0	20.3	17.3	19.1	18.2
1.5	17.0	16.6	17.9	17.8
2.0	14.9	15.8	15.9	16.5
2.5	11.8	12.3	12.7	12.3
MUD	.	10.2	.	11.8

APPENDIX B TABLE 2 CONTINUED

DEPTH (M)	AUG 11	AUG 18	AUG 27	SEP 03
0.0	19.3	18.1	14.2	12.2
0.5	19.3	18.0	14.3	12.2
1.0	18.8	17.8	14.3	12.2
1.5	17.6	17.2	14.3	12.2
2.0	16.8	16.6	14.3	12.2
2.5	14.8	13.5	14.3	12.2
MUD	11.8	.	14.1	12.2

	SEP 10	SEP 22	OCT 06
0.0	12.0	08.5	04.4
0.5	11.0	07.7	04.4
1.0	10.7	07.5	04.3
1.5	10.5	07.5	04.3
2.0	10.3		
2.5	10.3	07.5	04.3
MUD	10.3	07.7	04.6

APPENDIX B TABLE 3

SMITH LAKE 1963

PH

DEPTH (M)	JUN 05	JUN 19	JUN 26	JUL 01
0.0	7.0	9.5	7.2	7.1
0.5	.	9.3	.	.
1.0	7.3	7.7	7.0	7.0
1.5	.	7.1	7.1	7.1
2.0	7.2	.	7.1	8.0

	JUL 15	JUL 23	JUL 30	AUG 07
0.0	7.3	7.2	7.2	7.3
0.5	7.2	7.3	7.2	7.3
1.0	7.3	7.1	7.2	7.3
1.5	7.1	7.1	7.2	7.2
2.0	.	.	7.3	7.2

	AUG 20	AUG 27	SEP 17	OCT 25
0.0	7.2	7.1	7.4	7.2
0.5	7.2	7.1	.	.
1.0	7.2	7.1	.	7.2
1.5	7.1	7.1	.	.
2.0	7.1	7.2	.	7.1

	NOV 13	DEC 09
0.0	7.1	.
0.5	.	.
1.0	7.0	6.9
1.5	.	.
2.0	6.9	6.9

APPENDIX B TABLE 4

SMITH LAKE 1964

PH

DEPTH (M)	JAN 03	FEB 03	FEB 19	MAR 18
0.0	•	•	•	•
1.0	6.9	6.8	6.9	6.9
1.5	•	6.8	•	•
2.0	6.9	•	•	•
2.5	•	6.8	6.9	6.9
	MAR 24	APR 08	APR 13	APR 20
0.0	•	•	•	•
1.0	7.0	6.8	6.8	6.8
2.0	•	•	•	•
2.5	•	•	•	6.8
	APR 28	MAY 05	MAY 12	MAY 18
0.0	•	•	•	•
1.0	6.9	6.9	6.9	7.0
2.0	•	•	•	•
2.5	•	6.8	•	6.8
	MAY 23	MAY 27	JUN 02	JUM 08
0.0	•	•	7.1	7.2
1.0	6.4	7.2	7.1	7.1
1.5	•	•	7.0	•
2.0	6.7	•	6.9	6.7
2.5	•	•	7.0	6.7
3.0	•	6.9	•	•
	JUN 10	JUN 12	JUN 15	JUN 18
0.0	7.8	8.8	9.8	7.9
1.0	7.4	7.9	9.9	7.6
2.0	7.0	7.0	7.0	6.9
	JUN 23	JUN 30	JUL 07	JUL 13
0.0	7.3	7.6	7.6	7.4
1.0	7.3	7.5	7.5	7.3
2.0	6.9	7.0	6.9	7.0
	JUL 21	JUL 28	AUG 04	AUG 11
0.0	7.4	7.7	7.7	7.2
1.0	7.4	7.5	7.4	7.4
2.0	7.2	7.1	7.4	7.0

APPENDIX B TABLE 4 CONTINUED

DEPTH (M)	AUG 18	AUG 27	SEP 03	SEP 10
0.0	7.4	7.4	7.4	7.5
1.0	7.4	7.5	7.4	7.4
2.0	7.2	7.4	7.3	7.4

	SEP 22	OCT 06
0.0	7.4	7.4
1.0	7.4	7.4
2.0	7.5	7.4

APPENDIX B TABLE 5

SMITH LAKE 1963

PARTICULATE NITROGEN
(MICROGRAMS / LITER)

DEPTH (M)	MAY 21	MAY 30	JUN 05	JUN 12
0.0	131.1	188.6	199.0	885.6
1.0	.	.	194.7	752.8
2.0	.	.	129.2	158.1
	AUG 27	SEP 03	SEP 10	SEP 25
0.0	063.2	047.6	029.7	023.5
1.0	043.6	039.2	033.4	.
2.0	082.2	.	043.9	035.2
	OCT 09	OCT 25	NOV 06	NOV 11
0.0	026.9	041.9	028.3	037.9
1.0	.	015.3	.	.
2.0	.	065.6	.	.
	DEC 04			
0.0	104.1			
1.0	.			
2.0	075.8			

APPENDIX B TABLE 6

SMITH LAKE 1964

PARTICULATE NITROGEN
(MICROGRAMS / LITER)

DEPTH (M)	JAN 03	FEB 03	FEB 19	FEB 26
0.0		ICE COVER		
1.0	0058.6	0027.1	0055.4	0076.8
2.0	0039.1	0047.0	0061.8	0055.4
	MAR 18	MAR 24	APR 08	APR 14
0.0		ICE COVER		
1.0	0092.9	0108.0	0140.0	0076.4
2.0	0168.0	.	0072.4	0083.1
	APR 20	APR 28	MAY 05	MAY 12
0.0		ICE COVER		
1.0	0104.8	0130.7	0222.6	0256.9
2.0	0098.3	0130.7	.	.
	MAY 18	MAY 23	MAY 27	JUN 02
0.0		ICE COVER		0125.3
1.0	0218.0	0146.2	0211.7	0155.3
2.0	0190.0	0556.0	0092.9	0136.1
3.0			0190.1	.
	JUN 06	JUN 08	JUN 10	JUN 12
0.0	0263.6	0358.8	0405.4	0640.1
1.0	1758.2	0370.4	0389.8	0305.7
2.0	0762.5	0316.8	0198.4	0104.1
	JUN 16	JUN 18	JUN 23	JUN 25
0.0	0753.1	0387.6	.	0071.3
1.0	0602.5	0221.5	0101.5	0094.1
2.0	0126.2	0087.1	0129.6	0079.7

APPENDIX B TABLE 6 CONTINUED

DEPTH (M)	JUN 30	JUL 07	JUL 13	JUL 21
0.0	0324.0	0119.6	0084.2	0050.1
1.0	0047.6	0137.3	0066.4	0046.4
2.0	.	0022.7	0037.7	0044.3

	JUL 28	AUG 04	AUG 11	AUG 18
0.0	0074.4	0067.6	0086.3	0052.5
1.0	.	0076.4	0075.4	0052.5
2.0	.	0051.3	0051.3	0060.1

	AUG 27	SEP 03	SEP 10	SEP 22
0.0	0058.9	0061.1	0093.8	0059.0
1.0	0645.6	0159.3	0101.4	.
2.0	0069.8	0069.8	0050.2	0028.4

	OCT 06
0.0	0063.3
1.0	0103.7
2.0	0084.0

APPENDIX B TABLE 7

SMITH LAKE 1963

CHLOROPHYLL A
(MICROGRAMS / LITER)

DEPTH (M)	JUN 12	JUN 19	JUL 18	JUL 23
0.0	099.3	100.1	007.2	004.3
1.0	060.2	058.4	•	•
2.0	•	016.7	•	•
	JUL 30	AUG 07	AUG 12	AUG 27
0.0	003.6	003.9	002.8	004.2
1.0	•	002.5	003.6	007.7
2.0	•	011.9	002.5	005.4
	SEP 03	SEP 10	SEP 17	SEP 25
0.0	002.5	002.5	002.2	004.0
1.0	002.4	•	•	003.2
2.0	007.4	002.6	•	006.3
	OCT 09	OCT 25	NOV 11	DEC 04
0.0	001.2	001.5	001.2	002.7
1.0	•	001.7	•	001.3
2.0	•	•	•	•
	FEB 26	MAR 18	MAR 24	APR 07
0.0	•	•	•	•
1.0	001.3	005.1	007.9	•
2.0	001.3	004.5		
2.5	•	•	•	010.4
	APR 14	APR 20		
0.0	•	•		
1.0	003.4	007.7		

APPENDIX B TABLE 8

SMITH LAKE 1964

CHLOROPHYLL A
(MICROGRAMS / LITER)

DEPTH (M)	APR 28	MAY 01	MAY 05	MAY 12
0.0	.	.	.	.
1.0	011.8	010.0	012.0	017.0
2.0	.	.	.	.
	MAY 18	MAY 25	MAY 27	JUN 02
0.0	.	.	.	003.3
1.0	016.4	007.2	004.5	006.8
2.0	034.9	020.3	016.5	012.4
2.5	048.4	065.9	027.9	016.7
	JUN 08	JUN 09	JUN 10	JUN 12
0.0	022.6	046.3	017.1	132.3
1.0	010.7	028.6	034.7	019.4
2.0	010.1	015.2	006.8	.
	JUN 15	JUN 16	JUN 18	JUN 23
0.0	062.9	036.9	029.3	005.8
1.0	051.8	032.0	007.4	007.1
2.0	009.2	005.5	006.4	012.2
	JUN 25	JUN 30	JUL 07	JUL 13
0.0	005.3	007.7	003.2	005.7
1.0	005.5	008.2	004.6	002.8
2.0	002.9	003.2	002.2	003.1
	JUL 21	JUL 28	AUG 04	AUG 11
0.0	002.3	003.4	004.5	004.7
1.0	001.9	003.4	002.7	003.8
2.0	001.5	002.8	004.0	002.5
	AUG 18	SEP 10	SEP 22	OCT 06
0.0	004.0	014.0	010.3	011.7
1.0	003.3	013.6	005.0	011.0
2.0	002.2	005.0	003.6	007.0

APPENDIX B TABLE 9

SMITH LAKE

DISSOLVED INORGANIC PHOSPHORUS
(MICROGRAM ATOMS / LITER)

1963

DEPTH (M)	SEP 25	OCT 03	OCT 06	OCT 09
0.0	•	•	1.37	0.24
1.0	4.57	0.63	•	0.75
2.0	1.78	1.09	•	•
	OCT 10	OCT 17	OCT 23	OCT 25
0.0	0.31	0.35	0.41	•
0.5	0.56	•	•	0.52
1.0	0.57	•	•	0.79
	NOV 11	DEC 04		
0.0	1.42	1.17		
1.0	0.74			
2.0	•	1.50		

1964

	JAN 03	FEB 03	FEB 19	FEB 26
0.0	•	•	•	•
1.0	1.86	2.58	3.15	•
1.5	•	2.64	•	•
2.5	3.52	2.74	3.58	4.58
	MAR 18	MAR 24	APR 07	APR 14
0.0	•	•	•	•
1.0	4.22	•	•	3.33
2.0	•	•	4.54	•
2.5	5.60	4.01	•	6.03

APPENDIX B TABLE 9 CONTINUED

DEPTH (M)	APR 21	APR 28	MAY 05	MAY 12
0.0	•	•	•	•
1.0	3.30	3.46	3.53	4.83
2.5	5.24	•	4.07	5.68
	MAY 18	MAY 23	MAY 27	MAY 30
0.0	•	•	•	2.18
0.5	•	•	•	2.10
1.0	3.36	4.40	3.43	1.71
1.5	•	•	•	1.78
2.0	•	2.98	0.93	3.54
2.5	4.24	•	•	3.97
3.0	•	5.24	6.34	•
	JUN 01	JUN 02	JUN 08	JUN 09
0.0	1.91	2.16	1.63	1.84
1.0	2.86	•	1.52	1.32
1.5	2.31	2.76	•	•
2.0	•	2.94	2.48	3.53
2.5	3.30	3.89	•	•
	JUN 10	JUN 12	JUN 15	JUN 16
0.0	2.04	0.30	0.21	0.58
1.0	2.02	1.04	0.64	0.25
2.0	2.84	3.01	2.77	2.15
	JUN 23	JUN 25	JUN 30	JUL 01
0.0	0.72	0.44	0.25	•
1.0	0.95	0.45	0.31	0.20
2.0	2.59	0.37	1.20	2.55
2.5	•	2.12	•	•
	JUL 27	AUG 04		
0.0	•	0.37		
1.0	0.18	0.63		
2.0	•	1.52		

APPENDIX B TABLE 10

SMITH LAKE

DISSOLVED ORGANIC PHOSPHORUS
(MICROGRAM ATOMS / LITER)

1964

DEPTH (M)	FEB 03	FEB 19	FEB 26	MAR 18
0.0	.	.	.	.
1.0	1.87	1.69	.	1.90
1.5	1.76	.	.	.
2.0	.	.	.	.
2.5	2.22	2.32	1.74	2.43
	APR 07	APR 14	APR 21	APR 28
0.0	.	.	.	.
1.0	.	1.15	2.05	1.06
2.0	2.00	.	.	.
2.5	.	1.71	2.79	.
	MAY 05	MAY 12	MAY 18	MAY 23
0.0	.	.	.	.
1.0	.	1.44	1.84	2.04
2.0	.	.	.	2.39
2.5	2.59	.	0.87	.
3.0	.	.	.	2.14
	MAY 27	MAY 30	JUN 01	JUN 02
0.0	.	0.72	.	1.76
0.5	.	1.41	.	.
1.0	2.09	1.63	3.24	.
1.5	.	1.32	1.51	1.79
2.0	.	2.12	.	2.24
2.5	.	6.01	2.70	3.32
3.0	2.81	.	.	.
	JUN 09	JUN 10	JUN 12	JUN 15
0.0	2.08	.	0.76	0.66
1.0	0.40	0.96	1.45	0.47
2.0	8.18	0.91	1.44	1.15

APPENDIX B TABLE 10 CONTINUED

DEPTH (M)	JUN 16	JUN 23	JUN 25	JUN 30
0.0	2.44	0.66	0.82	0.62
1.0	3.50	0.69	0.91	0.56
2.0	.	0.63	0.48	0.49
2.5	.	.	0.64	.

	JUL 07
1.0	0.45

APPENDIX B TABLE 11

SMITH LAKE 1963

ALKALINITY
(MG / LITER CACO3)

DEPTH (M)	JUN 05	JUN 19	JUL 15	JUL 23
0.0	26.5	22.0	31.1	30.9
1.0	26.5	29.7	29.8	30.6
2.0	26.5	28.3	30.7	30.5
	JUL 30	AUG 07	AUG 20	AUG 27
0.0	31.4	33.5	34.5	38.8
1.0	31.6	33.4	34.8	36.4
2.0	34.0	22.0	41.2	36.0
	SEP 25	OCT 25	NOV 13	DEC 09
0.0	44.0	48.0	40.0	.
1.0	44.0	45.0	50.0	54.0
2.0	44.0	44.0	49.0	57.0

APPENDIX B TABLE 12

SMITH LAKE 1964

ALKALINITY
(MG / LITER CACO3)

DEPTH (M)	JAN 03	FEB 03	FEB 19	MAR 18
0.0	.	.	.	.
1.0	65.5	70.0	74.0	38.9
2.0	65.0	72.0	74.0	64.0
	MAR 24	APR 08	APR 13	APR 20
0.0	.	.	.	.
1.0	66.0	60.0	59.0	58.0
2.0	.	.	.	61.0
	APR 28	MAY 05	MAY 12	MAY 18
0.0	.	.	.	.
1.0	54.0	54.0	34.5	26.8
2.0	.	45.0	.	61.5
	MAY 23	MAY 27	JUN 02	JUN 08
0.0	.	.	18.0	27.0
1.0	14.0	13.5	23.0	27.5
2.0	52.0	.	40.0	43.1
	JUN 10	JUN 12	JUN 18	JUN 23
0.0	25.0	35.0	27.0	30.0
1.0	23.0	26.0	27.7	29.0
2.0	42.0	43.0	34.3	40.0
	JUN 30	JUL 07	JUL 13	JUL 02
0.0	28.5	30.0	28.0	30.0
1.0	29.0	.	28.1	31.0
2.0	31.0	34.5	28.0	29.0
	JUL 28	AUG 04	AUG 11	AUG 18
0.0	30.0	37.1	25.0	31.0
1.0	28.5	32.9	28.5	31.0
2.0	32.8	35.0	27.0	31.0

APPENDIX B TABLE 12 CONTINUED

DEPTH (M)	AUG 27	SEP 03	SEP 10	SEP 22
0.0	29.4	33.0	28.2	32.6
1.0	30.8	33.0	26.2	31.8
2.0	30.3	32.0	26.5	32.3

	OCT 06
0.0	36.0
1.0	37.7
2.0	32.9