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Biological Fixation and Transformation of Nitrogen in Small Impoundments

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by

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ABSTRACT

BIOLOGICAL FIXATION AND TRANSFORMATION
OF NITROGEN IN SMALL IMPOUNDMENTS

Biological fixation and transformation of nitrogen was studied in impoundments in central Oklahoma using the acetylene reduction method and ^{15}N techniques. Nitrogenase activity, probably microbial, was detected in a small lake during the winter. This source was calculated to be only 1% of the quantity of N falling directly on the surface in precipitation. Nitrogen fixation was not detected in a highly eutrophic pond, nor was it detected in a large mainstream reservoir. In both heterocystis blue-green algae and nutrient depletion were never observed. Biological N fixation does not appear to be important in these waters. Transformation of nitrogen was studied in the small lake where nitrogen fixation was detected. A Ceratophyllum-periphyton community is large in biomass and dominates the flora. This community was found to be capable of continuous NH_4 assimilation, but could assimilate NO_3 most rapidly only in the light.

An effort was made to learn if NH_4 and NO_3 assimilation by this community could be predicted from a knowledge of concentration. Uptake was linear with concentration but the effect of N starvation on the slope of the curve has not been explored as yet.

One nitrification experiment was performed. Basically, it was a pioneering effort designed to learn if the strategy in the use of ^{15}N isotopes would be analytically feasible. The results will be known when the mass spectrometer used by the author is again functional.

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A) Description of Research Performed

The basic objective of this research was to learn the rates at which nitrogen enters ponds by biological processes and the rate at which it undergoes nitrification and assimilation by organisms. Specific objectives were:

(1) to measure rates of nitrogen fixation in small impoundments

(2) to measure rates of assimilation of ammonia and nitrates by the biota in a prairie pond

(3) To concurrently measure rates of nitrification in the same environment, and

(4) to relate seasonal changes in nitrate and ammonia to the above rates

1) Nitrogen Fixation Studies

An understanding of the nitrogen cycle in aquatic environments is fundamental to understanding the production of the constituent flora and fauna. Current study of the aquatic nitrogen cycle is partly directed toward quantification of the rate at which nitrogen enters an aquatic ecosystem, the rate at which nitrogen is transformed, and the rate at which it is lost from the ecosystem. This paper reports on the occurrence of nitrogen fixation in one type of impoundment, the farm pond.

Farm ponds are usually formed by erecting an earthen dam across a fully or narrow valley. In central Oklahoma, many ponds are continuously turbid due to the suspension of small clay particles (Irwin and Stevenson, 1951). Consequently, phytoplankton and aquatic macrophytes never develop large populations therein. In ponds which are not continuously turbid, however, macrophytes and algae are abundant. Blue-green algae such as Anabaena and Aphanizomenon, which have been associated with

the occurrence of nitrogen fixation in lakes, frequently inhabit clear ponds.

The two impoundments were selected for study because they were relatively clear and because they contained blue-green algae. Pond A has a surface area of 0.21 ha (0.51 acres) and a mean depth of about 50 cm at spillway level (Figure 1). The pond receives runoff from a swine yard. This pond is highly eutrophic.

Pond B is an irregularly shaped impoundment of 5.4 ha (13.4 acres) and a mean depth of about 2 m at spillway level. Two small islands divide the pond into three basins (Figure 2). The pond supports dense growths of aquatic macrophytes, principally species of Najas and Ceratophyllum. The latter was found at all depths throughout the year. Green filamentous algae such as Spirogyra occur along the shore during early spring. American lotus, Nemumbo latea, grows along the east and west shores.

Methods

Water Sampling. Since duckweed, Lemna, frequently occupied the surface of Pond A, a special sampling design was devised to obtain water samples. A map containing sampling cells of known size was used to estimate the area of Pond A occupied by this plant (Figure 1). Then a proportionate number of samples were taken with a non-toxic water sampler at random in the area free of duckweed and in the area covered by duckweed, respectively, and combined to form a composite sample. This water (12 liters) was stored in a carboy and was filtered through cheesecloth to eliminate duckweed before it was used in the experiments described below.

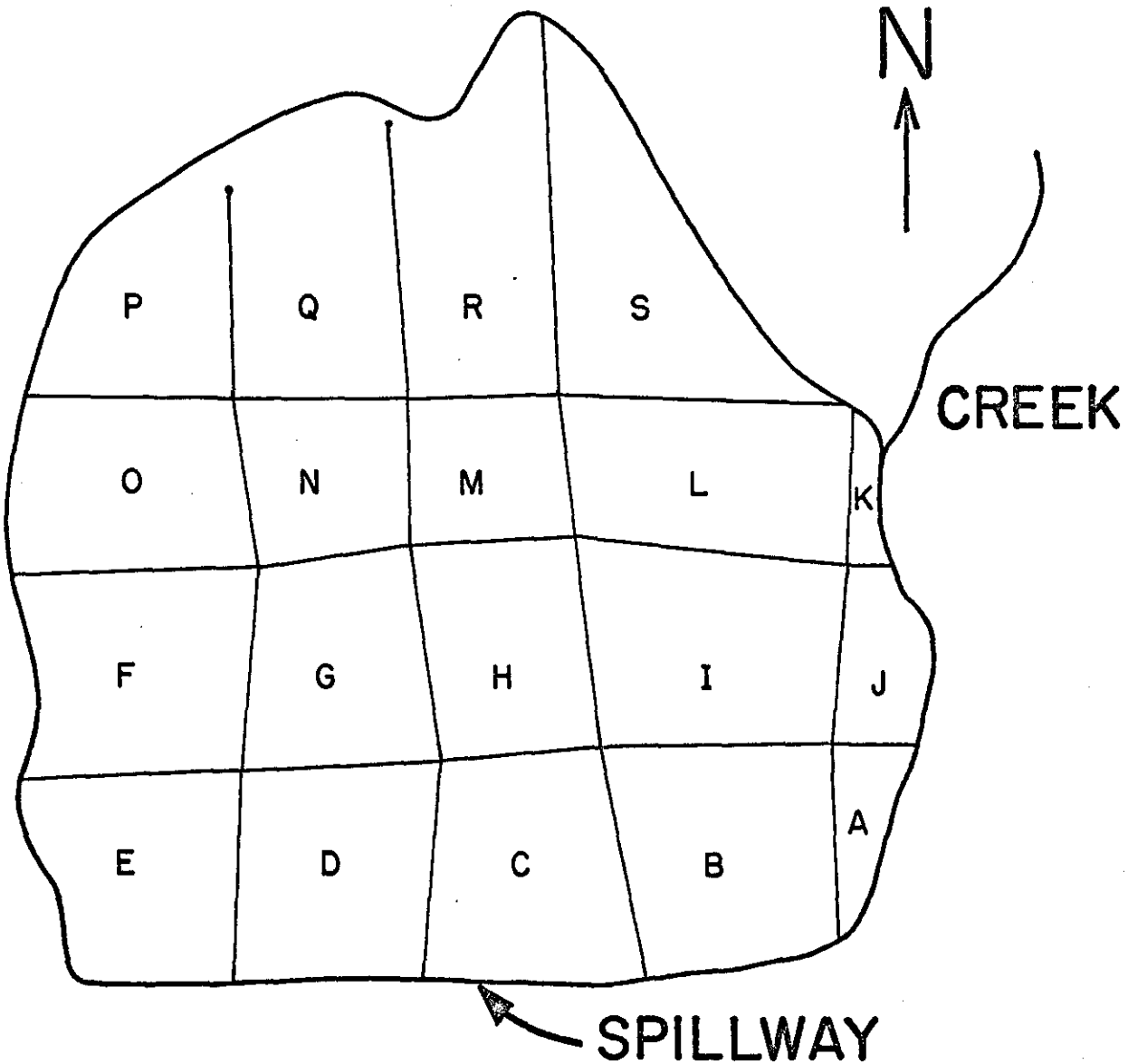

Figure 1. Map of Pond A, located N.E. 1/4, Sec. 6, T 19N, R 2E, Payne County, Oklahoma. The pond was divided into sampling cells of known size (A, B, C, etc.) to estimate the area of the pond covered by Lemna

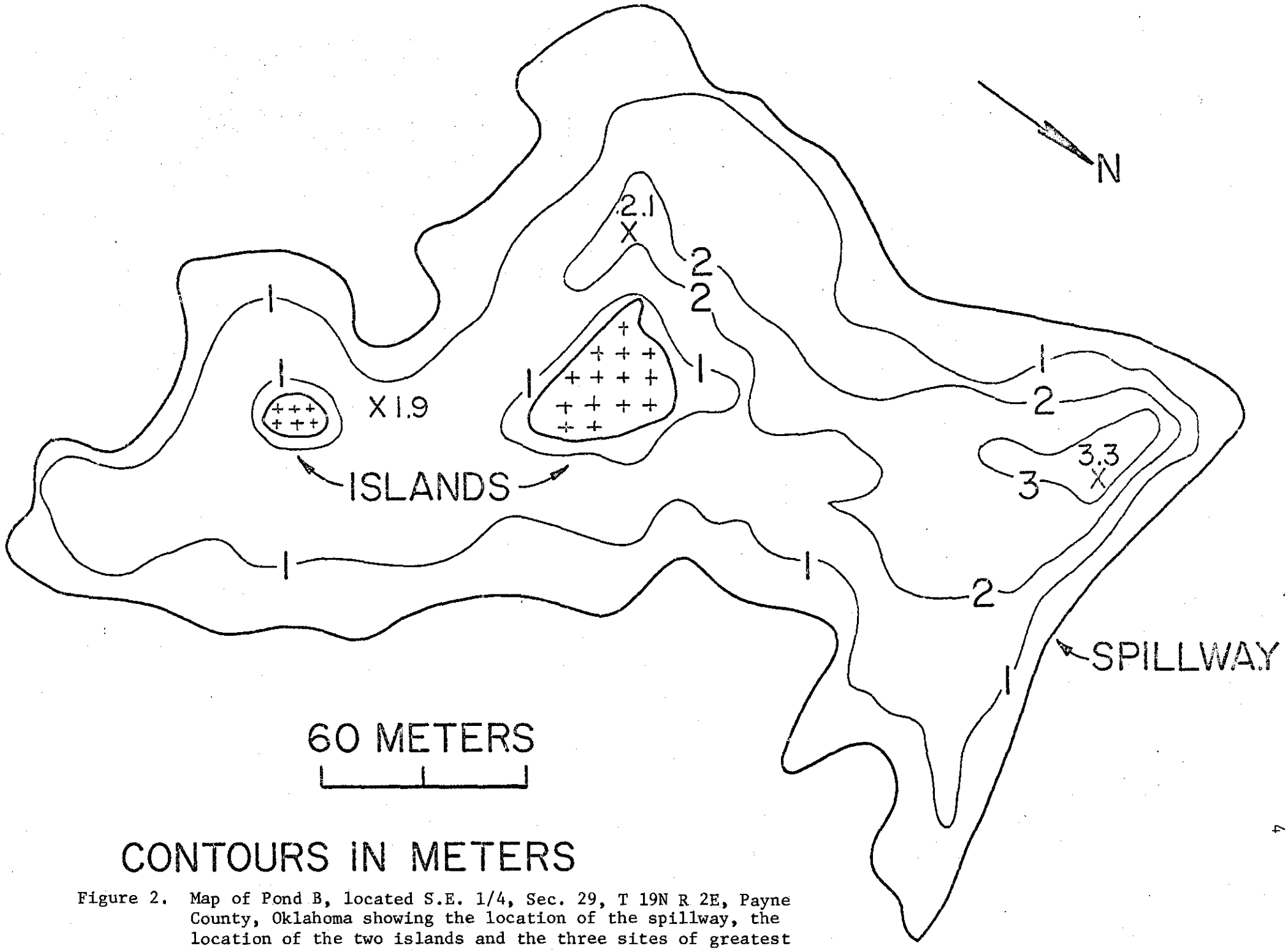

Figure 2. Map of Pond B, located S.E. 1/4, Sec. 29, T 19N R 2E, Payne County, Oklahoma showing the location of the spillway, the location of the two islands and the three sites of greatest depth where water samples were obtained

Three sampling stations were selected on Pond B. Each was located over the greatest depth in the main basin and in the two smaller basins, respectively (Figure 2). At each station about 6 liters of water was obtained from 0.4 and 0.5 m with a non-toxic water sampler. This water was transported to the laboratory in large polypropylene bottles. There a composite sample of 5 to 10 liters was obtained by mixing like quantities of water from each station in a large carboy.

Temperature data were obtained at the station in the main basin of Pond B with thermistor thermometer. Transparency was measured with a secchi disc at all stations and the values reported below are averages of the readings at the three stations.

Precipitation data at Stillwater, Oklahoma, were obtained from the monthly reports of the U. S. Department of Commerce (1968 and 1969). The station where these data were collected is located 5.1 km (3.2 miles) from Pond B.

Nitrogen fixation experiments. Observations on the occurrence of nitrogen fixation were performed using water from Pond A approximately every month between August, 1967 and July, 1968; observations were made using water from Pond B between April, 1968 and May, 1969. The ^{15}N technique was used for all observations on Pond A and for the first five observations on Pond B. The acetylene reduction technique was used for the remainder of the observations on Pond B. Further observations on Pond B were not made, because the owner poisoned the water with diuron during June, 1969.

The ^{15}N technique of Dugdale et. al. (1959), which was used in this research, involved purging a sample of pond water of its dissolved gases with a mixture of 80% He and 20% O_2 under about 150 mm Hg; a small space above the latter was evacuated to about 1 mm Hg. A quantity of N_2 of known ^{15}N enrichment was then admitted to this space to bring the system back to atmospheric pressure. The flask was shaken next to dissolve the $^{15}N_2$ in the water. Samples enriched with $^{15}N_2$ were incubated in duplicate 10 to 15 cm below the surface of Pond A for 8 hours. Sample size was one liter. At the end of incubation the total quantity of organic nitrogen present in the water was determined by a micro-Kjeldahl technique. Some of the NH_4 collected during Kjeldahl distillation was converted to N_2 and this gas was analyzed with a mass spectrometer to learn the final ^{14}N : ^{15}N ratio of the organic matter in the pond water. Untreated pond water or $(NH_4)_2SO_4$ treated similarly served as a control.

Modifications of the technique of Dugdale et al. (1959), were as follows: the Kjeldahl destruction solution was that of Bruel et al. (1947), ethanol was distilled between Kjeldahl distillations of the samples thought to be enriched, and the hypobromite mixture of Bremner (1965a) was used to convert NH_4 to N_2.

Nitrogen gas obtained by following the experimental procedures described above was pumped into breakseals. The latter were sealed with a torch and stored until isotope ratio analyses could be performed. Isotope ratio analyses were accomplished with a mass spectrometer built by Thomas Hoering at the University of Arkansas, Fayetteville.

The acetylene reduction technique described by Stewart et al. (1967) was also used. Two milliliters of concentrated plankton were added to 5 ml serum bottles. Air was removed by flushing each bottle with the gas mixture of He and O_2, then sealed with a rubber serum stopper, and flushed for an additional 1.5 min with the same gas mixture through a No. 22 hypodermic needle, venting it through a second needle. In the field, 0.5 ml high purity acetylene (Matheson Company) was injected from a hypodermic syringe into each serum bottle. Four bottles were prepared. Two were poisoned with 2N HCl or 50% trichloroacetic acid and served as controls. These same poisons were used to end the experiment.

The transparency of the water of Pond B was quite variable. Particulate clay, which was imported after heavy rains, was largely responsible for the low transparency of the water. When turbidity was high, filtration through .45 μ membrane filters was difficult and particulate matter was concentrated by centrifugation with a Foerst centifuge. Blue-green algae are difficult to separate from water by centrifugation and consequently the phytoplankton was concentrated from a large volume of water. Two liters of composite water were slowly centrifuged at the rate of about 6 liters per hour. The concentrated plankton was diluted to 10 ml with pond water. This mixture was stirred and samples of 2 ml were taken to add to the serum bottles.

The depth at which a secchi disc in Pond B was visible varied from 26 to 150 cm so incubation at a predetermined depth on all sampling days was not realistic. All serum bottles were incubated in the pond at about half the secchi disc reading for the day. The

bottles were incubated for 30 minutes, except during the cold season when the incubation period was one hour.

Ethylene formation was detected by gas chromatography using a Hewlett Packard F and M gas chromatographic apparatus. The instrument was fitted with a column containing Porapak R. Analyses were completed at room temperature. High purity N_2 served as the carrier gas.

Chemical Analysis. The micro-Kjeldahl technique described above was used to determine the nitrogen content of particulate matter. Water from which some particulate matter had been removed by centrifugation was filtered through a .45 μ membrane filter. The nitrogen content of filtered and unfiltered water was then determined. The difference in total nitrogen content between filtered and unfiltered water represented the nitrogen content of the particulate matter in the pond water. The micro-Kjeldahl technique was also used to determine the concentration of nitrogen in the serum bottles after the gas phase had been analyzed.

Additional water filtered through a .45 μ membrane filter as above was poisoned with 4 mg/liter $HgCl_2$ and stored at -5 C in acid-washed polypropylene bottles. The NH_4-N concentration was determined by direct nesslerization (APHA, 1960) while the concentration of nitrate was determined by the phenoldisulfonic technique described by Bremner (1965b).

Results

Pond A. Lemna formed an extensive blanket over the surface of Pond A between July, 1967, and the middle of January, 1968. On calm days duckweed covered the pond completely but on windy days patches of open water were observed on the leeward shore.

The subsurface flora of Pond A was dominated by a bloom of Anacystis cyanea, Oscillatoria rubescens and Spirulina sp. during the late summer and autumn of 1967. During February and March, 1968, a Chorella bloom was observed. By the middle of April, however, Anacystis cyanea was observed floating on the surface as well as in the subsurface samples. This alga and Oscillatoria rubescens were the dominant phytoplankton in a bloom that persisted through the summer of 1968. Pond A was seemingly devoid of phytoplankton during December, 1967, and during late April, 1968. Microscopic examination did not reveal the presence of phytoplankton. The failure to observe phytoplankton on 24 April 1968 may be traced to heavy rains during April that diluted the Chlorella bloom and caused the pond to overflow.

The concentration of NH_4-N in Pond A ranged from 0.8 to 20.6 mg/liter on the days of sampling. On nine occasions it was in excess of 3.18 mg NH_4-N/liter. The concentration of nitrate ranged between 3 and 172 μg NO_3-N/liter. Depletion of nitrogen nutrients was never evident.

Uptake of molecular nitrogen could not be detected. Combined nitrogen in untreated pond water and in $(NH_4)_2SO_4$ was converted to N_2 to serve as controls. In each case the isotope ratios of

Table 1. Statistical resume of isotope ratios of nitrogen gas blanks converted from organic matter in pond water and from $(NH_4)_2SO_4$ with alkaline hypobromite.

	Pond Water	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$
N	5	4	3
$\bar{x}$	.3763 atom % ^{15}N	.3823 atom % ^{15}N	.3573 atom % ^{15}N
$S_{\bar{x}}$	.0066	.0111	.0178
95% Confidence interval	.3597 - .3947 atom % ^{15}N	.3712 - .3934 atom % ^{15}N	.3395 - .3751 atom % ^{15}N
Date of analysis	11/30/67	7/15/68	3/24/69

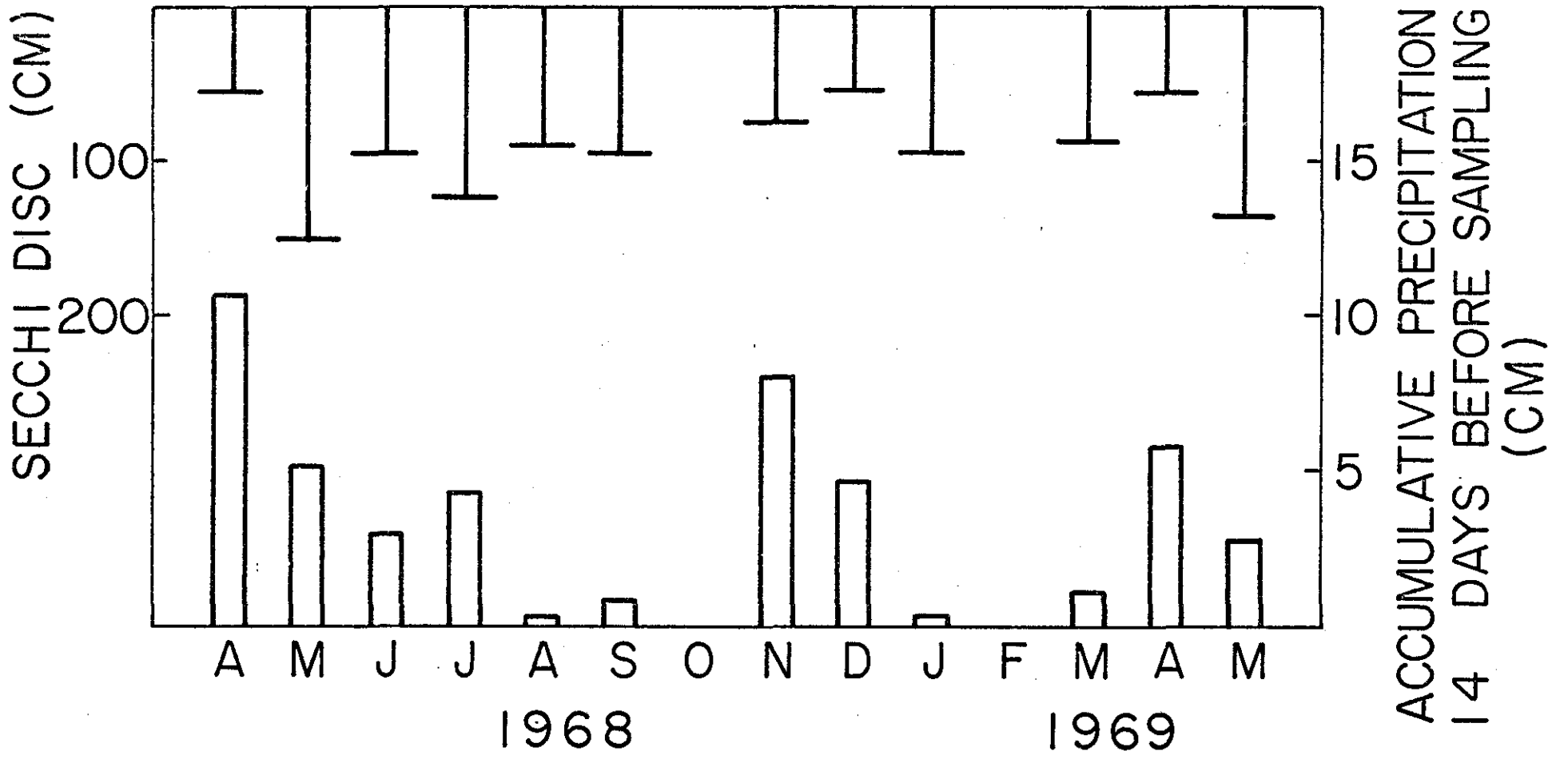

Figure 3. Secchi disc readings for Pond B on the days when nitrogen fixation experiments were performed and total accumulative rainfall 14 days prior to the date of the fixation experiment (Bar graph = rainfall)

nitrogen in organic matter in pond water exposed to 85 to 93% $^{15}N_2$ fell within the 95% confidence intervals for the blanks (Table 1) and thus enrichment was not detected.

Pond B. Aphanizomenon holsaticum was observed in water samples collected during June, September, and December, 1968, and during January, March, and May, 1969. Anacystis cyanea was observed during July and August, 1968, and Anabaena sp. was observed during December, 1968. The planktonic flora at other times consisted of desmids and diatoms. During August, 1968, dinoflagellates were observed. Water samples collected during April and May, 1968, contained large quantities of detritus and few phytoplankton were observed. Heavy rainfall during this period (Figure 3) probably caused a high rate of water exchange, and thus thwarted the growth of the phytoplankton community.

Pond B was relatively clear during the study, compared to other impoundments in central Oklahoma, with secchi disc readings of 26 to 150 cm. In general, there was a positive relationship between turbidity and precipitation (Figure 3), although there were instances where the reverse was true, e. g. during May, 1968.

Surface temperatures ranged from 3.5 to 27.5 C. There was a sharp rise in temperature during March and a sharp decrease during November such that it is possible to delineate two seasons for the pond. A cold season extended from November to March, when surface temperatures on days of sampling ranged from 3.5 to 8.0 C. During the remainder of the year, the warm season,

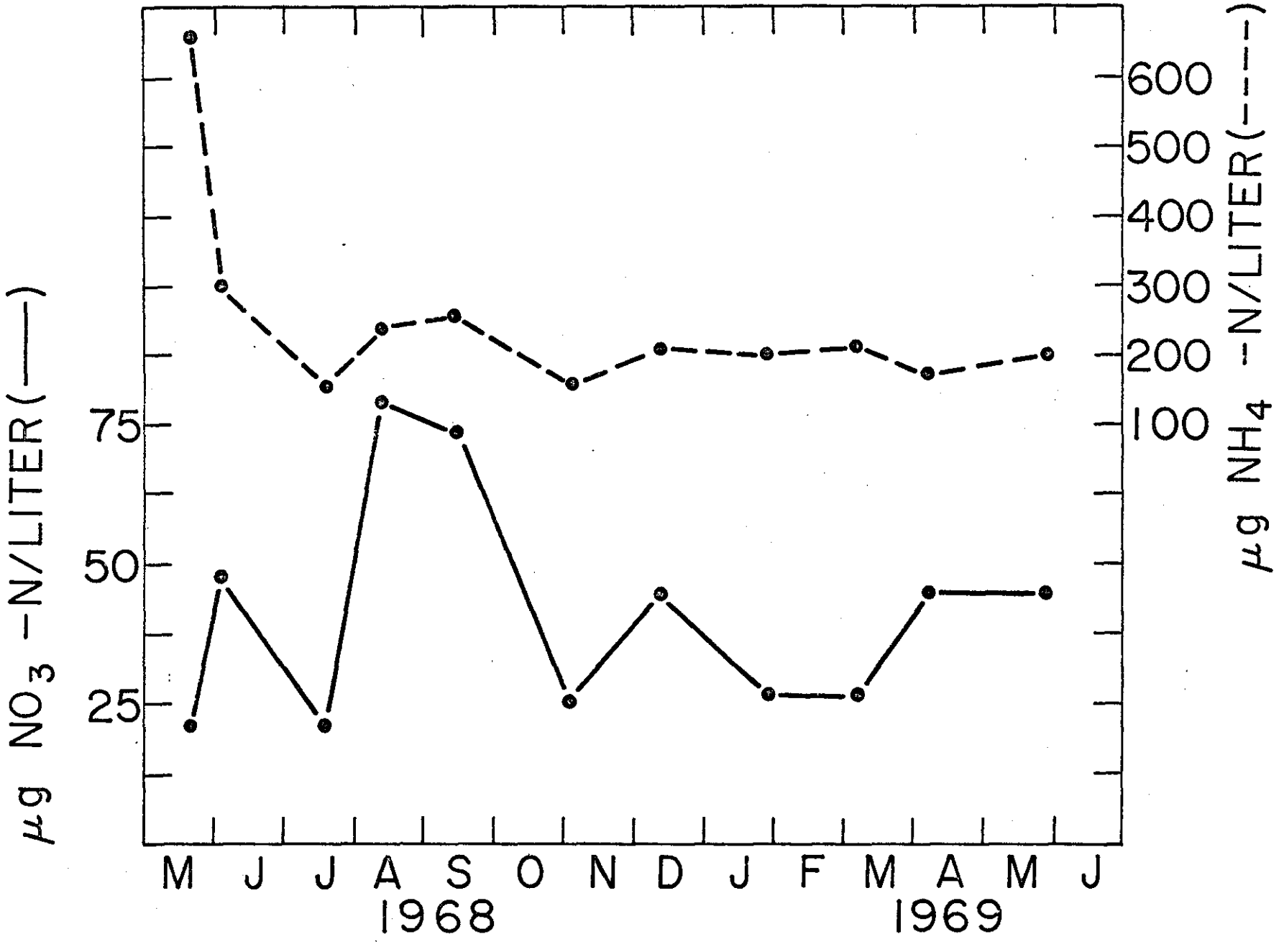

Figure 4. Prevailing concentrations of NO_3-N and NH_4-N in water from Pond B on days when nitorgen fixation experiments were performed

surface temperatures ranged from 17.0 to 27.5 C on days of sampling. Between May and October, surface temperatures at 9000 hours were never less than 20 C on days of sampling.

The concentration of NH_4-N ranged between 160 and 660 µg/liter. However, except for the spring and early summer of 1968, the concentration of NH_4-N was relatively constant (Figure 4). There is a suggestion of a seasonal cycle for NH_4 in 1968 when fairly high concentrations were present at the outset of the growing season and low concentrations were detected during the summer, as might be expected.

The concentration of nitrate varied between 18 and 87 µg NO_3-N/liter. There is a suggestion of nitrate accumulation during the summer of 1968 corresponding to a small increase in the concentration of NH_4-N (Figure 4). The concentrations of NH_4-N and NO_3-N found in Pond B are typical of some natural eutrophic lakes and nutrient depletion was never evident.

Uptake of $^{15}N_2$ could not be detected. Methods of calculation of isotope ratios were the same as those used above for Pond A. The isotope ratios of nitrogen in organic matter in pond water exposed to 90% $^{15}N_2$ fell within the 95% confidence intervals for the blanks (Table 1), and uptake of $^{15}N_2$ was not detected during the spring and summer of 1968.

Between September, 1968 and June, 1969, the acetylene reduction technique was used. On January 27, and on March 3, 1969, the mean rate of acetylene reduction was 0.0236 nmoles C_2H_4/mg N/hr and 0.0285 nmoles C_2H_4/mg N/hr, respectively. The concentration of particulate nitrogen in water from Pond B was 0.09 mg N/liter on January 27,

and 0.18 mg N/liter on March 3. If the rate of acetylene reduction in the serum bottles was the same as the rate in situ, then the mean rate of acetylene reduction on the day of the experiment during January and March, respectively, was 0.0021 and 0.0051 nmoles C_2H_4/liter/hr.

If the assumption is made that acetylene reduction is a valid measure of nitrogen fixation, then a rough conversion can be made from the rate of acetylene reduction to the rate of nitrogen fixation by assuming the molar ratio of ethylene: ammonia equals 1.5. The rate of nitrogen fixation during January was about 0.02 μg N fixed/liter/hr and the March rate was 0.04 μg N fixed/liter/hr.

Discussion

This study reports the first positive evidence of acetylene reduction in an impoundment in Oklahoma during the winter. Blooms of heterocystis blue-green algae belonging to genera which fix nitrogen have been noted by other workers during the winter in Lake Carl Blackwell, Oklahoma (Cooper, 1965) and in Bull Shoals Reservoir, Arkansas (Applegate and Mullan, 1967). The occurrence of heterocystis blue-green algae during the winter does not guarantee that they are fixing nitrogen, however, and fixation reported here may be microbial.

The occurrence of acetylene reduction only during the winter is singular in as much as nitrogen fixation usually occurs during the warm season in natural lakes. During May, 1969, at the height of a bloom of Aphanizomenon in Pond B, phytoplankton were separated from the water by filtration onto a .45 μ membrane filter. Copious

quantities of green material were removed from the filters and assayed for acetylene reduction as usual. No acetylene reduction was detected in spite of the fact that conditions appeared excellent for nitrogenase activity, i.e. high temperatures, high levels of solar radiation and low concentrations of NH_4-N and NO_3-N. It may be that fixation was sporadic and undetected. Goering and Neess (1964) found that the rate of nitrogen fixation in Lake Mendota, Wisconsin, during the ice-free season fluctuated considerably, and sometimes nitrogen fixation was not detected.

Although acetylene reduction (nitrogen fixation) was detected, the quantitative importance of this source of nitrogen seems minor even when generous assumptions are made in calculating the contribution of nitrogen to the pond by the process of nitrogen fixation. The annual contribution of nitrogen by biological fixation of the planktonic flora is roughly 0.95 Kg, if the assumption is made that the annual season during which fixation occurs is 60 days, fixation occurs for 12 hours/day, the fixation rate is 0.03 µg N/liter/day, fixation occurs at the same rate throughout the water column, and the volume of the pond is 44×10^6 liters.

In an earlier study extending over a period of three years, the mean total concentration of NH_4-N, NO_3-N and NO_2-N in precipitation collected about 5 km from Pond B was 2.487 mg N/liter (Heller, 1938). Heller's estimate of the total input of nitrogen in precipitation was 3.7 Kg/ha/yr (3.3 lbs/acre/yr) or 97.9 Kg for the whole pond. Biological nitrogen fixation thus contributes only 1% of the total quantity of nitrogen falling directly on Pond B.

The low contribution is probably a reflection of the paucity of N_2 fixing blue-green algae.

In this study nutrient depletion was never evident. The failure to detect nitrogen fixation in Pond A may be due to the absence of species which can fix molecular nitrogen rather than that the concentrations of inorganic nitrogen compounds were inhibitory. The fixation reported for Pond B may reflect the simultaneous use of small amounts of N_2 along with NH_4 and NO_3. While the total quantity of N_2 fixed may be insignificant compared to the annual contribution from other sources, the feeble nitrogenase activity may have survival value for this population during periods when nitrogen nutrients are exhausted. Further studies are needed to reveal the role of nitrogen fixation by communities in environments where nutrient depletion is uncommon.

Other Related Work

In addition to this work, Mr. Ross Hall, an FWQA trainee, has been using the acetylene reduction technique under my direction to assay for nitrogenase activity in Lake Keystone, a mainstream reservoir on the Cimarron and Arkansas Rivers. No nitrogenase activity, with one exception, could be detected in the surface waters at 2 - 3 week intervals. The water contained high concentrations of NH_4 and NO_3, nutrient depletion of NO_3 never being evident. Blooms of heterocystis blue-green algae never developed and bacterial fixation does not seem to occur.

The advent of the development of the acetylene reduction technique during this study was fortuitous as it allowed the author to study N_2 fixation in other reservoirs.

During October and November, 1969, I detected feeble nitrogenase activity in Beaver Lake and in Bull Shoals Reservoir, Arkansas. This

may have been due to small quantities of heterocystis blue-green algae observed in the lake water. At the present time, however, it appears that the input of N_2 by blue-green algae is negligible in these waters but observations in the above lakes are not extensive enough to be unequivocable.

2) Measure Rates of Assimilation by the Biota

This effort involved two separate tasks, (a) to learn if daily uptake of NO_3 and NH_4 by Ceratophyllum could be predicted from a knowledge of hourly rates and (b) assimilatory capabilities of this plant at various substrate levels were to be observed.

B. Learning the Diel Uptake of NO_3 and NH_4

Little is known about uptake of NH_4 or NO_3 by Ceratophyllum in nature, although NH_4 uptake has been studied in the laboratory by Fitzgerald (1969) in developing bioassay methods to access nitrogen depletion. It seemed likely that Ceratophyllum demersum might play an important role in nitrogen cycling in the Quail Pond because of its large biomass. Boyd (1967) suggested that it might have a profound influence on the standing quantity of NO_3 and NH_4. The work reported here attempted to learn if a knowledge of uptake during short periods of the day could be used to predict daily productivity in terms of NH_4 and NO_3. This knowledge would be of use in planning future experiments of nutrient competition, turnover, and productivity.

Ceratophyllum is a dominant plant in many other waters. It is a non-rooted macrophyte which commonly persists near the bottom during the winter. During spring and early summer it grows rapidly such that the entire community eventually occupies the entire water column in shallow waters. Very often periphyton will be found growing on Ceratophyllum, especially when it is nitrogen replete (Fitzgerald, personal communication). The term Ceratophyllum used below refers to the species Ceratophyllum demersum and associated

periphyton. Examination of the plants used in the experiments described here did not reveal periphyton, but the assumption will not be made that the material was entirely free of periphyton.

Methods

Observations of NO_3 and NH_4 uptake were made during six consecutive periods of four hours duration on 27 and 28 May 1969 (Table 1). Ceratophyllum was obtained from the surface of the pond just prior to incubation. In all cases a stem 20 cm long with one or more apical ends was added to each bottle. Later analyses revealed that 344 to 1131 mg (dry weight) of plant material had been added to each bottle. Water samples were obtained by holding a polypropylene bottle 40 cm below the surface. The water was used to fill four 2 l glass reagent bottles. A subsample for water chemistry was poisoned in the field with 2 mg/l $HgCl_2$, and filtered one hour later through a .45 μ membrane filter. Next 86 μg of 99% $^{15}NH_4$-N as NH_4Cl and 142 μg of 99% NO_3-N as KNO_3 was added to two bottles, respectively. These bottles were incubated in the pond horizontally on a metal frame at 30 cm. Turbidity has a profound effect on light penetration into the water of the pond. The irradiance at 30 cm was about one half of the incident radiation. Irradiance at 30 cm was monitored every 15 minutes with a GM underwater photometer, which had been previously calibrated with an Eppley pyrheliometer. Water temperatures at 30 cm were obtained for each period of incubation with a mixiumum-minimum thermometer.

At the end of incubation, each bottle was poisoned with 2 ml formalin. The Ceratophyllum was placed in a tared crucible and held at -20 C for about one month.

Prior to chemical analysis Ceratophyllum was dried for 24 hours at 105 C and then weighed to determine the dry weight. The material from each crucible was thoroughly cursched with mortar and pestle and about 10 mg was digested for 18 hr in a kjeldahl flask with reagents recommended by Bremner (1965). The NH_4 was distilled into dilute HCl and converted to N_2 with alkaline hypobromite under vacuum. The N_2 was Toepler-pumped into breakseal tubes which were then sealed. Gases were stored in these tubes until isotope ratios could be determined with a mass spectrometer built by Tom Hoering at the University of Arkansas. N_2 converted from Ceratophyllum not exposed to $^{15}NO_3$ or $^{15}NH_4$ was also analyzed to obtain isotope ratio blanks.

Six random samples of Ceratophyllum were secured during the course of obtaining material for incubation. This material was used to obtain the following estimates of biomass: fresh weight, dry weight, ash weight, and kjeldahl nitrogen. After drying in the air at 20 C for one hour, the material was placed in tared crucibles and weighed to determine the fresh weight. Dry weight was determined as above, while ash weight was estimated by burning preweighed samples of dried material at 550 C for 2 hours. The kjeldahl method (Bremner, 1965) was used to determine the N content of the material. The methods of Riley (1953) and Mullin and Riley (1955) were used on the day samples were collected for the analysis of NH_4-N and NO_3-N, respectively.

Table 2 Incubation intervals, irradiance, temperature range and uptake of NO_3 and NH_4 by a community of Ceratophyllum-periphyton over a period of 24 hours at 30 cm.

Time	Irradiance	Temperature Range	Hourly Rate of Uptake	
CDT	gm cal/cm^2/4 hr	C	µg NO_3-N/mg N plant $\times 10^{-1}$	µg NH_4-N/mg N plant $\times 10^{-1}$
0640 to 1040 hrs	22.87	21.1-22.2	6.24	3.63
28 May			3.84	4.25
1040 to 1440 hrs	1923.3	None, 24.4	7.82	3.25
27 May			3.99	1.86
1440 to 1840 hrs	56.89	None, 24.4	3.21	2.61
27 May			4.75	3.21
1840 to 2240 hrs	10.60	21.6-24.4	2.19	3.55
27 May			4.21	2.40
2240 to 0240 hrs	0	None, 21.6	.19	2.25
27 and 28 May	~~.01~~		.10	2.51
0240 to 0640 hrs	.01	21.6-22.2	.16	2.50
28 May			.03	2.23

Results

The % ^{15}N in organic matter was determined by using the expression 100/2R+1, where R is the ratio of the 28 peak to the 29 peak of N determined with a mass spectrometer. The average % ^{15}N in the blanks was .3566 (n = 8) and the percentage in material exposed to $^{15}NO_3$ or $^{15}NH_4$ was in excess of the former.

Uptake per mg N of plant per hour was determined by the following expression: AN/(SNT), where A = atom % excess ^{15}N in the organic matter, N = mg N in the organic matter exposed to the isotope, S = % ^{15}N of the NO_3 or NH_4 in the water at the outset of the experiment and T = 4 hours. N was obtained by multiplying the dry weight of each plant by 2.5%, the percentage of N in dry Ceratophyllum. This approximation is sufficient since the N's in the expression cancel one another. No NO_3 or NH_4 could be detected in the water before the isotopes were added. Therefore, in the determination of uptake, it assumed that S was 99%.

The uptake of NO_3 is strongly related to light, since there is virtually no uptake in the dark (Table 1). Water temperatures were not constant throughout the 24 hour period. However, during the morning of 28 May, water temperatures were relatively constant (Table 1). Between 0240 and 0640 hours there was little uptake of NO_3 and irradiance was nil. Between 0640 and 1040 hours uptake was high and irradiance was substantial. The data strongly suggest that this community requires light energy to accomplish NO_3 assimilation.

Uptake of NH_4 is not strongly correlated with light, but the data do show a small decrease in the rate of uptake at night (Table 1). It is well known that Ceratophyllum can assimilate NH_4 in the dark (Fitzgerald, 1969) but these data suggest that in nature, less NH_4 is assimilated in the dark than in the light.

Tissue analysis revealed that the plant material averaged 86.3% water and that ash and kjeldahl nitrogen averaged 15.0 and 2.5% of the dry plant, respectively.

Almost all the labeled N recovered from Ceratophyllum by the kjeldahl method was probably in reduced form. Labeled NO_3 which may have been merely adsorbed is not sufficiently recovered by the kjeldahl method to be of any consequence in the determination of isotope ratios. In cases where $^{15}NH_4$ was exposed to plants, it is not known if the ^{15}N molecules were adsorbed or incorporated into cellular structure. In all cases, net assimilation was measured, since losses of N were not determined.

Radiation at 30 cm was plotted against time and the area under the curve was integrated for each period of incubation. Mean uptake of NO_3-N during each period was used to estimate the daily rate by multiplying each mean by a factor f, (f = total radiation received/24 hr divided by the total received/4 hr period). The results clearly demonstrate that only estimates of NO_3 uptake made during the period 1440-1840 hrs can be expanded to a realistic daily rate (Table 2). Estimates of NH_4 uptake made during the period 1440-1840 hrs appear to be the most satisfactory to expand to a daily rate (Table 2). However, NH_4 uptake is much less dependant upon light than NO_3 uptake. Simple expansion of the four hour uptake by six yields an estimate of daily uptake of 696 µg NH_4-N/mg plant for this same period, which is very close to the measured uptake of 684 µg NH_4-N/mg plant. Expansion of uptake during other intervals does not yield estimates as satisfactory.

Uptake of NO_3 on the basis of available energy is apparently highest during morning and then rapidly decreases (Table 3). It is not known if this phenomenon is due to light inhibition. Other factors affecting

Table 3 Mean values of measured diurnal assimilation of NO_3 and NH_4 by a Ceratophyllum-periphyton community and daily uptake of these nutrients. Daily uptake was calculated by multiplying the uptake during each period by the f, where f = total radiation received during 24 hrs/radiation received during the period of incubation.

Date	Time	f	Mean Measured NO_3 Uptake μg NO_3-N/mg N plant/4 hr $\times 10^{-1}$	Mean Calculated daily NO_3 Uptake μg NO_3-N/mg N plant/day $\times 10^{-1}$	Mean Measured NH_4 Uptake μg NH_4-N/mg plant/4 hr $\times 10^{-1}$	Mean Calculated NH_4 Uptake Daily μg NH_4-N/mg plant/4 hr $\times 10^{-1}$
28 May	0640-1040	11.95	20.16	240	15.76	188
27 May	1040-1440	1.42	23.60	33	10.22	144
27 May	1440-1840	4.80	15.92	76	11.64	55
27 May	1840-2240	250,000	12.80	32×10^5	13.90	35×10^6
27-28 May	2240-0240	0	.60	0	9.52	0
28 May	0240-0640	25,640	.32	82×10^2	9.46	24×10^4
		Total	73.40		68.40	

photosynthesis may indirectly affect NO_3 uptake. For example, CO_2 could become exhausted by noon, and this event could lead to a depression of NO_3 uptake.

Discussion

The data suggest that measurements of NO_3 and NH_4 uptake made during the afternoon are those which can be expanded to a daily rate most satisfactorily. The failure of Ceratophyllum to assimilate NO_3 in the dark is probably due to lack of (an) operational enzyme(s). The data suggest that some uptake may occur well into the late evening, presumably due to the presence of enzymes synthesized in the light. However, these data indicate that other factors may depress the uptake of NO_3 during that part of the day when light energy is most available.

Some of the differences in the rate of uptake observed here may be due to variability in the age of the material, its nitrogen content or local nutrient depletion in the bottles, all of which could have an effect on the rate of assimilation. The diurnal uptake of ^{14}C by Ceratophyllum demersum is also highly variable such that expansion of rates obtained during one period of measurement to the rate for an entire day is not realistic (Wetzel, 1965).

Continuous NH_4 assimilation by Ceratophyllum probably has a profound role in determining the concentration of NH_4 in the water. Even if the quantity assimilated is much lower than that reported here, continuous uptake by this plant could strip NH_4 from the water as fast as it is released by the processes of decomposition. This may also allow little time for nitrification. If this is true in environments which receive little exogenous NO_3, then the assessment of NH_4 flow into this community may be considerably simplified.

3) Uptake of NO_3 and NH_4 by the Biota

Instead of making monthly estimates of NH_4 and NO_3 assimilation, the author believed far more information on nitrogen cycling in the pond could be gained by measuring the total NH_4 and NO_3 assimilated by the plankton and macrophytes as a function of NO_3 and NH_4 concentration. This would enable one to calculate assimilation coefficients as a function of light, temperature, pigment concentration, dry weight, and could lead to data with more predictive value. With the time available he completed two experiments to learn this relationship for Ceratophyllum.

Methods

Isotopic NO_3 and NH_4 were respectively added in varying concentrations to duplicate 2ℓ bottles containing Ceratophyllum. Incubation was for 4 hours during the afternoon. The same chemical techniques as above were used.

Results

Uptake was linear for both NO_3 and NH_4. However, Ceratophyllum is not able to use concentrations of NO_3-N less than 5 μg/ℓ. The lower limit for NH_4 is not known, but uptake at 20 μg NH_4-N was observed.

Discussion

The data here give promise that assimilation of NO_3 and NH_4 could be predicted from a knowledge of substrate concentration. Project OKLA. A-023 will look into this question in detail.

4) Measurement of Nitrification

One nitrification experiment has been completed. Exactly 6345 μg 15 NH_4-N were added respectively to two autoclaved glass bottles which had been wrapped

with black tape. Two controls were prepared similarly, but poisoned with 2ppm formalin. The bottles were filled with 3ℓ of pond water, covered with a rubber stopper and incubated for four days in the pond described above during April, 1969. Concentrations of NO_3-N, NO_2-N and NH_4-N in each bottle were estimated before and after the experiment. In addition daily measurements were made of the oxygen concentration in each bottle.

Nitrates and NH_4-N were not detected in the water at the outset of the experiment (before the addition of labeled NH_4). However, NO_3-N was present at a concentration of 0.5 μg NO_3-N. After the incubation, the concentration of NO_3-N in the control vessels was 3.25 and 1.10 μg NO_3-N/ℓ was found in the experimental vessels and 0.00 and 0.10 μg NO_2-N/ℓ was found in the control vessels. These values should all be reported merely as traces of NO_3 and NO_2 as even the sensitive spectrophotometric technique employed would not allow better accuracy.

Because NO_3 was almost undetectable at the close of the experiment, it was impossible to carry the experiment further. The idea was to separate NO_3 from all other nitrogen compounds in the water, convert the NO_3 so obtained to N_2 gas and to analyse the N_2 with a mass spectrometer to learn if conversion from $^{15}NH_4$-N to NO_3 had occurred. If it had, the ratio of ^{15}N:^{14}N in the NO_3 could be detected and the quantity of NH_4 moving into NO_3 could be calculated. At least 50 μg N is necessary for this process.

The experiment did demonstrate that nitrification is likely to be very slow in the open water. Of course, if NO_3 was taken up by the plankton as fast as it was formed from NH_4 then little NO_3 would be detected. This is not likely in the dark. Denitrification is not likely as the oxygen concentration in the bottles never fell below 4 mg/l.

This work indicated that an effort had to be made to develop a technique to measure nitrification when NO_3-N is less than 50 μg/ℓ. An experiment was

conducted to learn if a known quantity of $^{15}NO_3$ could be added to the water to accomplish recovery of enough NO_3 for mass spectroscopy. This is theoretically possible as the mass of the NO_3-N added (Ma) and its isotope ratio Ia would be known. The mass of the NO_3 in the water at the end of experiment (Me) would also be known. Finally knowledge of the % ^{15}N in the NO_3(f) recovered would be learned by mass spectroscopy. Thus, the isotope ratio of the (Me) would be:

$\frac{f\ (Ma + Me) - Ia(Ma)}{Me}$. The experiment also sought to correct for carry over of $^{15}NH_4$ and to correct for NO_3 assimilated by the phytoplankton.

This experiment has been partially completed. All that remains to be done are the isotope ratio analyses.

5) Relating Seasonal Changes in Nitrate and Ammonia to the Rates of N_2 Fixation, to NH_4 and NO_3 Assimilation and to Nitrification

No clear cut relationship can be established between the concentration of NH_4 and NO_3 and nitrogenase activity or lack thereof. However, nutrient depletion was never evident in Keystone Reservoir and not even bacterial fixation could be detected. Nutrient depletion was not uncommon in Pond B or at times in Lake Carl Blackwell and sometimes nitrogenase activity was detected in these waters. It is well known that high levels of NO_3 and NH_4 suppress N_2 fixation and this could doubtless be at work here, but the lack of substantial nitrogenase activity is probably due to the paucity of N-fixing algae, more than anything else.

The levels of NO_3 and NH_4 in water from Pond B were usually low. The role of Ceratophyllum and nitrification in this regard has already been discussed.

Other Activities. Two students, Mr. Ross Hall, Jr., and Mr. Bill Cole worked with Dr. Toetz, but were not supported by OKLA. A-012. Mr. Hall will receive his M.S. in August, 1970. He has developed considerable competence in modeling N flow. This should lead to a better conceptual framework to study N cycling. Mr. Cole, a junior college teacher, received first hand experience during the summer of 1970 in use of ^{15}N techniques and other methods. He probably will do a Ph.D. problem in this area.

The lack of a mass spectrometer has hampered this research. The author tried to obtain a surplus instrument recently from AEC, but was not successful.

The author is collaborating with a chemist, Dr. Louis Varga, and they have a grant from AEC to study the biogeochemistry of nitrogen.

The author is one of the few people in the country using ^{15}N to study N cycling. The seed money from OWRR has made it possible to set up such a laboratory to do this kind of research. Expansion of this effort should take place within the next year.

The author has accomplished far less than he had hoped. The chief problem was the lack of a mass spectrometer and the laborious steps in converting organic N to N_2. The latter should no longer be a problem as the purchase of the Coleman N analyzer has increased his efficiency over the kjeldahl method.

PROJECT RELATED PUBLICATIONS

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