NITRIFICATION: A SIGNIFICANT CAUSE OF OXYGEN DEPLETION UNDER WINTER ICE

by

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Changes in concentrations of ammonium, nitrite, nitrate, and oxygen suggested the occurrence of significant nitrification throughout the water column of mesotrophic Lake St. George, Ontario, during the winter months in the years from 1976 to 1984. The existence of nitrapyrin- and acetylene-sensitive ^{14}C-bicarbonate incorporation confirmed that bacterial nitrification occurred. During the period late January to early March (water temperature 2-3°C) nitrification occurred at an average rate of about 13 μg $N \cdot L^{-1} \cdot d^{-1}$ for the years studied. Numbers of detectable nitrifying bacteria appeared too low (by 2 to 4 orders of magnitude) to account for the observed activity. The nitrification activity observed would result in average oxygen consumption amounting to 71% of the observed oxygen depletion. This shows that winter nitrification can be an important factor in promoting oxygen depletion and possibly winter-kill of fish.

Des changements dans les concentrations d'ammonium, de nitrites, de nitrates et d'oxygène suggèrent qu'il s'est produit une importante réaction de nitrification dans toute la colonne d'eau du lac St. George, un lac mésotrophe de l'Ontario, durant les mois d'hiver de 1976 à 1984. La présence d'incorporations de bicarbonate-C_{14} sensibles à la nitrapyrine et à l'acétylène confirme qu'il y a effectivement eu nitrification bactérienne. Durant la période qui s'étend de la fin de janvier au début de mars de chaque année étudiée (température de l'eau: 2 à 3°C), la vitesse moyenne de nitrification était d'environ 13 µg $N \cdot L^{-1} \cdot d^{-1}$. Le nombre de bactéries nitrifiantes énuméré était de deux à quatre fois trop faible pour expliquer l'activité observée. On a calculé que l'épuisement de l'oxygène observé serait attribuable à 71 p. 100 à la consommation moyenne d'oxygène qui résulte de la nitrification. Cela démontre que la nitrification hivernale peut être un important facteur d'épuisement de l'oxygène et pourrait expliquer la mortalité des poissons en hiver.

MANAGEMENT PERSPECTIVES

Changes in concentrations of oxygen and the various nitrogenous forms in Lake St. George from 1976 to 1984 showed that nitrification (conversion of ammonium to nitrite and nitrate) caused an average of 71% of the winter oxygen depletion. This represents the most significant biological processes in the winter months. In fact, it is 30 X greater than plant photosynthesis. Bottle bioassays for nitrification were evaluated to determine how well they would predict rates measured in the lake itself.

PERSPECTIVE-GESTION

Les changements dans les concentrations d'oxygène et des divers composés azotés dans le lac St. George de 1976 à 1984 démontrent qu'en moyenne, 71 p. 100 de l'épuisement en oxygène l'hiver peut être attribué à la nitrification (transformation de l'ammonium en nitrites et en nitrates). Il s'agit là de la plus importante réaction biologique à se produire durant les mois d'hiver. En effet, cette réaction est trente fois plus importante que la photosynthèse des végétaux. On a évalué des essais biologiques sur la nitrification en bouteille afin de déterminer leur efficacité à prévoir les taux mesurés dans le lac.

(Introduction)

Nitrification (the oxidation of ammonium and nitrite to nitrate) has been studied intensively in soils (Schmidt 1982) and in both freshwater and marine sediments (Billen 1976; Jones and Simon 1981). Significant nitrification can also occur in the aerobic hypolimnia of lakes, based on evidence from mass balance of inorganic nitrogen species (Christophi et al. 1981; Verdouw and Dekkers 1982; Takahashi et al. 1982; Hall and Jeffries 1984) and measurement of the incorporation of radioactive bicarbonate in the presence and absence of the nitrification inhibitor nitrapyrin (Billen 1976; Somville 1978; Vincent and Downes 1981; Vincent et al. 1981; Hall 1982). Evidence for nitrification has come also from studies of oxygen depletion rates (Wezernak and Gannon 1967), most probable number determinations of nitrifying bacteria (Christophi et al. 1980; Takahashi et al. 1981), and immunofluorescence studies (Stanley et al. 1979; Ward 1984).

There appears to be little information on nitrification in the water column of lakes in winter under ice, although unpublished data for Wintergreen Lake, Michigan, presented without comment (Wetzel 1975) suggests significant conversion of ammonium to nitrate during the winter months. Such a conversion also occurred during winter in the Bay of Quinte, Ontario, and in untreated limnocorrals in the same Bay (Liao and Lean 1978).

In the present paper we report evidence for significant nitrification under winter ice and suggest that this process can be a major factor in oxygen depletion and possibly winter-kill of fish.

Methods

Lake St. George (43°57'30"N, 79°25'30"W) is 2 km east of Oak Ridges and about 32 km north of Toronto, Ontario. Located on a height of land with no major inflow, its location minimizes pollution from upstream sources. It is a mesotrophic double-basin lake (10.3 ha) and is described by Knowles (1979), Knowles et al. (1981), McQueen and Lean (1983), McQueen et al. (1984), and Wolfe (1979). The present study was carried out in the 5.88-ha eastern basin of the lake which has a maximum depth of 17 m. The lake was selected because of its pronounced seasonal changes in the forms of nitrogen (Knowles et al. 1981). Surface nitrate concentrations range from near zero to 800 $\mu g\ N \cdot L^{-1}$.

Water samples were collected through the ice (0.3 to 0.4 m thick) using a 3-L Van Dorn water sampler or through tygon tubing connected to a peristaltic pump. Temperature and oxygen concentrations were measured using a Yellow Springs Instrument probe (model 54A). The presence of sulfide interfered and in such cases oxygen concentration was assumed to be zero. Samples were processed and analyzed for ammonium, nitrite, nitrate, and nitrous oxide as described by Knowles et al. (1981).

For bottle incubation experiments, 300-mL BOD bottles were filled with lake water from the appropriate depth. To avoid changes in dissolved gas concentrations the sampler tube was kept at the bottom of the BOD bottle which was allowed to overflow for 10-15 seconds. Nitrification was assayed as acetylene-sensitive (Hynes and Knowles 1978) or nitrapyrin-sensitive dark incorporation of ^{14}C-bicarbonate (Billen 1976), and denitrification as the production of nitrous oxide in the presence of acetylene to inhibit reduction of

nitrous oxide (Yoshinari and Knowles 1976; Knowles 1979). Where desired, nitrapyrin [2-chloro-6-(trichloromethyl)pyridine] dissolved in dimethyl sulfoxide (DMSO) was added to give a final concentration of 20 $mg \cdot L^{-1}$ nitrapyrin. Acetylene (25 mL) was added by syringe to each bottle through a rubber stopper, temporarily displacing water into a second syringe until all the acetylene had dissolved. Thus the oxygen concentration was not altered. ^{14}C-bicarbonate (Amersham) was added to a final concentration of 150,000 (1980-82) and 700,000 (1983) $dpm \cdot mL^{-1}$. Drying out of the bottle stoppers was carefully avoided to prevent gas exchange. Bottles were incubated in the dark at close to in situ temperatures.

After appropriate intervals, oxygen was determined using a stirred probe with an Orbisphere oxygen meter. Chemical analyses were done as described above except that ammonium could not be analyzed in nitrapyrin treatments due to DMSO interference. Incorporation of ^{14}C during the first 0.2 to 1.8 days was determined by filtering (0.2 μm Nuclepore, for 1980-82, and 0.45 μm Sartorius 11306, for 1983, membrane filters) 50 mL of sample. Each Nuclepore filter was acidified with 0.05 mL of glacial acetic acid and left to stand overnight in a fume hood, then dissolved with 10:1 methylene chloride:ethanolamine before addition of PCS fluor (Amersham) for liquid scintillation counting. Sartorius filters were acidifed with 1 mL of 1 M H_2SO_4 for 2 hours, then counted as above. Dissolved nitrous oxide was determined as described by Chan and Knowles (1979).

Nitrifying bacteria were enumerated by Most Probable Number (MPN) methods as described by Alexander and Clark (1965) with an incubation period of 6 weeks. Denitrifiers were enumerated by MPN essentially as described by Tiedje (1982) using Difco nutrient broth supplemented with 5 mM nitrate (final concentration).

Nitrapyrin (N-Serve) reagent grade (>99%) was a gift from Dow Chemical of Canada Ltd., Sarnia, Ontario; gases were from Union Carbide Canada, and other chemicals were of reagent grade.

Results

During routine monitoring of Lake St. George in 1975-1976, it seemed that ammonium was being converted to nitrate during the February-March period. Isopleths through the winter period when gas exchange with the atmosphere is eliminated, and into spring overturn, show the period of oxygen depletion in the deep water and changes in chlorophyll, as well as the forms of nitrogen (Fig. 1). Winter began with a concentration of ammonium at about 300 throughout the water column increasing to greater than 500 $\mu g\ N \cdot L^{-1}$. From mid-December to 1 February particulate N (and chlorophyll) declined while nitrate and nitrite increased little. Consistent with the view that dissolved organic nitrogen is refractory, changes in the concentration of this form of N were trivial (data not shown).

FIG. 1

During February and March the process of nitrification became what will be shown to be the most significant metabolic event going on in the lake. Changes in particulate N were small and confined to the uppermost layers of the lake. Nitrite increased in the oxygen-poor deeper waters but ammonium decreased from roughly 500 to 150 $\mu g\ N \cdot L^{-1}$ in 60 days while nitrate concentration showed a corresponding increase. Since changes in the other forms of nitrogen were small during the February period only nitrate and ammonium concentrations need to be considered. (Raw data for this and subsequent years are provided in a series of data reports: Lean, McQueen and Knowles. Water chemistry analysis for Lake St. George, Ontario. CCIW Pub., in preparation.)

This pattern of progressively decreasing concentrations of ammonium and oxygen and a simultaneous increase in nitrate concentrations was observed most years between 1976 and 1984 (Fig. 2). Nitrite levels also generally increased with time to maximum concentrations of 100 to 200 $\mu g\ N \cdot L^{-1}$ (data not shown). Oxygen was low and decreased with depth, and the bottom waters frequently became depleted towards the end of March (Figs. 1 and 2). Possibly due to this oxygen depletion there was evidence of an input of ammonium from the sediment into the bottom of the water column with concentrations decreasing towards the surface (Figs. 1 and 2).

FIG. 2

Mass balances of ammonium, nitrite, and nitrate nitrogen, and oxygen were calculated by trapezoidal integration for the whole lake basin based on data for concentration (Fig. 2) and volume per layer (ice volume was ignored). As exemplified by Table 1 for 1982 data, there was generally a very significant decrease in whole lake total ammonium-nitrogen (121 kg N for 1982) and a very significant increase in nitrate (262 kg N for 1982). For 1982 also, the average rates of decrease in ammonium concentration and of increase in nitrite and nitrate concentrations were 6.55, 1.46, and 22.71 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$, respectively (Table 1, rightmost column).

TABLE 1

For all the years investigated, and based on whole lake mass balance changes (as illustrated in Table 1), the rate of change in concentration of nitrogen oxides ($NO_2^- + NO_3^-$) was much greater than that of ammonium (Table 2) presumably due to an ammonium flux from the sediment or to mineralization of particulate nitrogen. The oxygen consumption associated with the oxidation of ammonium and nitrite to nitrate by nitrifying bacteria is 4.33 $\mu g\ O_2$ per μg of N oxidized through nitrite to nitrate (determined experimentally by Wezernak and

TABLE 2

Gannon 1967). Using this factor it is clear that the oxygen demand of the observed nitrification (BOD_N) is frequently a large percentage of the observed oxygen disappearance in the lake (Table 2), ranging from 30.9 to 98.6% (mean 71.2%). The actual rate of production of nitrite plus nitrate for the whole lake ranged from 8.51 to 23.25 (mean 13.12) $\mu g\ N \cdot L^{-1} \cdot d^{-1}$. The rate of increase in total mass of inorganic nitrogen during the periods studied ranged from 0.24 to 5.19 (mean 2.28) $kg\ N \cdot d^{-1}$ which translates to areal rates of 4.06 to 88.3 (mean 38.81) $mg\ N \cdot m^{-2} \cdot d^{-1}$. Over the total lake basin volume of $2.95 \times 10^5\ m^3$ this represents a mean rate of increase of 7.73 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$, that is, approximately one-half of the observed nitrification rate.

Nitrification was observed in water samples (2-m depth) incubated in bottles in the dark and at in situ temperature (Fig. 3). The accumulation of nitrate was inhibited by either nitrapyrin or acetylene, which also partially inhibited the consumption of oxygen. Acetylene also inhibited the disappearance of ammonium (Fig. 3). The rate of production of nitrate during the period 5 to 40 days was 13.3 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$.

FIG. 3

Nitrapyrin- and acetylene-sensitive dark incorporation of ^{14}C-bicarbonate in samples (2-, 8- and 14-m depth) of water incubated at in situ temperature was converted to nitrification rate using the ratio mg C fixed/mg N oxidized = 0.102 (Christofi et al. 1981). Nitrapyrin-sensitive ^{14}C-bicarbonate incorporation measurements were restricted to incubation periods of less than 2 days. The 12 experiments which were conducted (Table 3) provide estimates of nitrification in the range 1.3 to 27.0 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$ and thus they are similar to those obtained above (Table 2) by mass balance. The observed rates decreased with increasing incubation time over the first 5 days. Acetylene was not as effective as

TABLE 3

nitrapyrin in inhibiting ^{14}C-bicarbonate incorporation (data not shown). Nitrapyrin may inhibit other CO_2-consuming processes (Topp and Knowles 1982; Ward et al. 1982). There are simple problems of very low rates of incorporation particularly in hard water lakes such as Lake St. George (if the filters are not adequately decontaminated, any "incorporation" is obscured). DMSO controls also showed some inhibition over samples with no DMSO. It was, however, noteworthy that the nitrapyrin-sensitive dark ^{14}C-bicarbonate assimilation was only about 25% of total incorporation at 2 m but increased to near 50% at 8 m and 70% at 14 m. This no doubt represents the relative contribution of nitrification to oxygen losses as well.

In view of the appreciable nitrate levels and the frequently low oxygen concentrations, some assays for denitrification were carried out by incubating water samples containing acetylene. Denitrification, indicated by an accumulation of nitrous oxide, occurred only after about 3 to 16 days of incubation when the dissolved oxygen concentrations were in the range 0.3 to 0.9 mg $O_2 \cdot L^{-1}$ (Fig. 4). The potential denitrification rates occurring during the periods of active nitrous oxide production were in the range 1.8 to 28.1 μg N_2O-$N \cdot L^{-1} \cdot d^{-1}$. The data show the possibility for denitrification in the relatively anoxic water which develops during winter stratification. It is noteworthy that no nitrous oxide accumulated in the incubated samples in the absence of acetylene (data not shown), suggesting complete reduction to N_2.

FIG. 4

Enumerations of bacteria at different depths in the water column in 1980, 1981, and 1982 showed denitrifiers in the range 11 to 1700 cells per mL whereas nitrifier numbers were only of the order of 1 or 2 cells per mL (Table 4). Acridine orange direct counts showed roughly 3 X 10^6 total bacteria per mL (J. de Traversay and R. Knowles, unpublished data).

TABLE 4

Discussion

The data provide evidence of significant nitrification during the months of January to March under the ice in Lake St. George. A similar phenomenon appears to have occurred in Wintergreen Lake (Wetzel 1975, p. 244). For Lake St. George, the average in situ rate of production of nitrite and nitrate was 13 μg $N \cdot L^{-1} \cdot d^{-1}$ which was confirmed by bottle incubation experiments in the laboratory at in situ temperature. The occurrence of nitrapyrin- and acetylene-sensitive ^{14}C-bicarbonate incorporation (which according to Hall (1982) may underestimate nitrification) suggests that the nitrification was due to chemolithotrophic CO_2-utilizing nitrifiers (Billen 1976; Hynes and Knowles 1978) rather than heterotrophic organic C-utilizing nitrifiers (Hynes and Knowles 1982). However, only very low numbers of chemolithotrophic nitrifiers were detected. Less than 1 or 2 per mL were enumerated. Assuming a range of specific activities of 1.3 to 81 pg $N \cdot cell^{-1} d^{-1}$ (Schmidt 1982), the observed in situ activity of 13 μg $N \cdot L^{-1} \cdot d^{-1}$ would require a nitrifier population of from 1.6×10^2 to 1.0×10^4 $cells \cdot mL^{-1}$. It appears, therefore, that the MPN enumeration provides an estimate which is 2 to 4 orders of magnitude below the true count as has been reported by others (e.g. Ward et al. 1982) or other types of bacteria are responsible for the observed activity. Methanotrophic bacteria can also oxidize ammonia and are also inhibited by nitrapyrin (Topp and Knowles 1982) and acetylene (de Bont and Mulder 1976). It is unlikely that methanotrophs were significant contributors to the observed nitrification since methane concentrations at all depths were below 0.5 μM in 1980, 1982, and 1983, and only in 1984 did methane levels increase below the 8-m depth to 121 μM in the bottom water (R. Knowles, unpublished data). However a methanotrophic population may have proliferated on the large

amounts of methane made available during fall overturn (R. Knowles, unpublished data).

It is unlikely that the observed increases in nitrite and nitrate could be due to inputs from outside the lake since stream flow begins only at the end of March and then flows immediately under the ice (Wolfe 1979). Furthermore, any nitrogen oxide in ground water could not survive passage through the rich sediments which have considerable denitrification potential (Knowles 1979). Indeed, no nitrate is observed in Lake St. George pore waters (R. Carignan, INRS-Eau, Quebec, unpublished data).

The denitrification assays show that the water column has the potential to reduce nitrate to gaseous products whenever the oxygen is depleted below 0.3 mg $O_2 \cdot L^{-1}$. Our estimate of nitrification rates could be an underestimate if denitrification occurred in the sediment or water column during the periods studied.

The rates of oxygen consumption observed (mean of 75.6 μg $O_2 \cdot L^{-1} \cdot d^{-1}$) are equivalent to 0.38 g $O_2 \cdot m^{-2} \cdot d^{-1}$ and as such are at the upper end of the range observed by Welch et al. (1976) for 16 Ontario lakes in winter. The fact that our observed nitrification must be associated with an oxygen consumption which is a significant percentage of the total observed oxygen depletion indicates that the nitrification process could frequently be a major factor contributing to winter-kill of fish. It is also very important in the nitrogen cycle relative to other processes. For example, the winter uptake of nitrogen by phytoplankton calculated from ^{14}C-bicarbonate uptake data was less than 0.5 μg $N \cdot L^{-1} \cdot d^{-1}$ (D.R.S. Lean, unpublished data); the sedimentation based on sediment trap data was 0.3 μg $N \cdot L^{-1} \cdot d^{-1}$ (M. Charlton, Canada Centre for Inland Waters, unpublished

data); and diffusion of ammonium from the sediment, calculated from pore water concentration profiles and extrapolated to the whole lake, was 2 μg $N \cdot L^{-1} \cdot d^{-1}$ (R. Carignan, INRS-Eau, Quebec, unpublished data). By comparison, a nitrogen oxide production of 13 μg $N \cdot L^{-1} \cdot d^{-1}$ is very significant.

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References

ALEXANDER, M., AND F.E. CLARK. 1965. Nitrifying bacteria. p. 1477-1483. In C.A. Black et al. [ed.] Methods of soil analysis, vol. 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI.

BILLEN, G. 1976. Evaluation of nitrifying activity in sediments by dark ^{14}C-bicarbonate incorporation. Water Res. 10: 51-57.

CHAN, Y.-K., AND R. KNOWLES. 1979. Measurement of denitrification in two freshwater sediments by an in situ acetylene inhibition method. Appl. Environ. Microbiol. 37: 1067-1072.

CHRISTOFI, N., N.J.P. OWENS, AND W.D.P. STEWART. 1979. Studies of nitrifying microorganisms of the Eden estuary, Scotland. p. 259-266. In E. Naylor and R.G. Hartnoll [ed.] Cyclic phenomena in marine plants and animals. Pergamon Press, New York.

CHRISTOFI, N., T. PRESTON, AND W.D.P. STEWART. 1981. Endogenous nitrate production in an experimental enclosure during summer stratification. Water Res. 15: 343-349.

De BONT, J.A.M., AND E.G. MULDER. 1976. Invalidity of the acetylene reduction assay in alkane-utilizing, nitrogen-fixing bacteria. Appl. Environ. Microbiol. 31: 640-647.

HALL, G.H. 1982. Apparent and measured rates of nitrification in the hypolimnion of a mesotrophic lake. Appl. Environ. Microbiol. 43: 542-547.

HALL, G.H., AND C. JEFFRIES. 1984. The contribution of nitrification in the water column and profundal sediments to the total oxygen deficit of the hypolimnion of a mesotrophic lake (Grasmere, English Lake District). Microbial Ecol. 10: 37-46.

HYNES, R.K., AND R. KNOWLES. 1978. Inhibition by acetylene of ammonia oxidation in Nitrosomonas europaea. FEMS Microbiol. Lett. 4: 319-321.

HYNES, R.K., AND R. KNOWLES. 1982. Effect of acetylene on autotrophic and heterotrophic nitrification. Can. J. Microbiol. 28: 334-340.

JONES, J.G., AND B.M. SIMON. 1981. Differences in microbial decomposition processes in profundal and littoral lake sediments, with particular reference to the nitrogen cycle. J. Gen. Microbiol. 123: 297-312.

KNOWLES, R. 1979. Denitrification, acetylene reduction, and methane metabolism in lake sediment exposed to acetylene. Appl. Environ. Microbiol. 38: 486-493.

KNOWLES, R., D.R.S. LEAN, AND Y.K. CHAN. 1981. Nitrous oxide concentrations in lakes: variations with depth and time. Limnol. Oceanogr. 26: 855-866.

LIAO, C.F.-H., AND D.R.S. LEAN. 1978. Seasonal changes in nitrogen compartments of lakes under different loading conditions. J. Fish. Res. Bd. Canada 35: 1095-1101.

McQUEEN, D.J., AND D.R.S. LEAN. 1983. Hypolimnetic aeration and dissolved gas concentrations. Water Res. 17: 1781-1790.

McQUEEN, D.J., S.S. RAO, AND D.R.S. LEAN. 1984. Hypolimnetic aeration: changes in bacterial populations and oxygen demand. Arch. Hydrobiol. 99: 498-514.

SCHMIDT, E.L. 1982. Nitrification in soil. p. 253-288. In F.J. Stevenson [ed.] Nitrogen in agricultural soils. Amer. Soc. Agron., Madison, WI.

SOMVILLE, M. 1978. A method for the measurement of nitrification rates in water. Water Res. 12: 843-848.

STANLEY, P.M., M.A. GAGE, AND E.L. SCHMIDT. 1979. Enumeration of specific populations by immunofluorescence. p. 46-55. In J.W. Costerton and R.R. Colwell [ed.] Native aquatic bacteria: Enumeration, activity and ecology. ASTM, Philadelphia, PA.

TAKAHASHI, M., T. YOSHIOKA, AND Y. SAIJO. 1982. Nitrogen metabolism in Lake Kizaki, Japan. III. Active nitrification in early summer. Arch. Hydrobiol. 93: 272-286.

TIEDJE, J.M. 1982. Denitrification. p. 1011-1026. In A.L. Page, R.H. Miller and D.R. Keeney [ed.] Methods of Soil Analysis, Part 2. Amer. Soc. Agron., Madison, WI.

TOPP, E., AND R. KNOWLES. 1982. Nitrapyrin inhibits the obligate methylotrophs Methylosinus trichosporium and Methylococcus capsulatus. FEMS Microbiol. Lett. 14: 47-49.

VERDOUW, H., AND E.M.J. DEKKERS. 1982. Nitrogen cycle of lake Vechten: concentration patterns and internal mass-balance. Hydrobiologia 95: 191-197.

VINCENT, W.F., AND M.T. DOWNES. 1981. Nitrate accumulation in aerobic hypolimnia: relative importance of benthic and planktonic nitrifiers in an oligotrophic lake. Appl. Environ. Microbiol. 42: 565-573.

VINCENT, W.F., M.T.enthic and planktonic nitrifiers in an oligotrophic lake. Appl. Environ. Microbiol. 42: 565-573.

VINCENT, W.F., M.T. DOWNES, AND C.L. VINCENT. 1981. Nitrous oxide cycling in Lake Vanda, Antarctica. Nature 292: 618-620.

WARD, B.B. 1984. Combined autoradiography and immunofluorescence for estimation of single cell activity by ammonium-oxidizing bacteria. Limnol. Oceanogr. 29: 402-410.

WARD, B.B., R.J. OLSON, AND M.J. PERRY. 1982 Microbial nitrification rates in the primary nitrite maximum off southern California. Deep-Sea Res. 29: 247-255.

WELCH, H.E., P.J. DILLON, and A. SREEDHARAN. 1976. Factors affecting winter respiration in Ontario lakes. J. Fish. Res. Board Can. 33: 1809-1815.

WETZEL, R.G. 1983. Limnology, 2nd edition. Saunders College Publishing, Philadelphia, PA. 767 p.

WEZERNAK, C.T., AND J.J. GANNON. 1967. Oxygen-nitrogen relationships in autotrophic nitrification. Appl. Microbiol. 15: 1211-1215.

WOLFE, B.R. 1979. The role of lake winter cover in the phosphorus budget of a southern Ontario lake. M.Sc. thesis, Trent University, Peterborough. 150 p.

YOSHINARI, T., AND R. KNOWLES. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Commun. 69: 705-710.

TABLE 1. Volumetric information and inorganic nitrogen data for two samplings from Lake St. George during the winter of 1982.

Depth (m)	Volume	Jan. 24		Mar. 18		Rate[b]
Sample (layer)	10^6 L	µg N.L^{-1}	kg N[a]	µg N.L^{-1}	kg N	µg N.L^{-1}.d^{-1}
			Ammonium-N			
1 (0-2)	89.86	503	45.20	40	3.59	11.57
3 (2-4)	70.72	490	34.65	70	4.95	10.50
5 (4-7)	67.91	610	41.25	70	4.75	13.50
8 (7-10)	33.72	600	20.23	110	3.71	12.25
12 (10-13)	21.84	620	13.54	640	13.98	-0.50
14 (13-15)	8.56	640	5.48	960	8.21	-8.00
Mean		577.2		315.0		6.55
Total	292.61		160.53		39.20	
			Nitrite-N			
1 (0-2)	89.86	0	0.00	10	0.90	0.25
3 (2-4)	70.72	0	0.00	40	2.83	1.00
5 (4-7)	67.91	10	0.68	30	2.04	0.50
8 (7-10)	33.72	10	0.34	80	2.70	1.75
12 (10-13)	21.84	10	0.21	60	1.31	1.25
14 (13-15)	8.56	20	0.17	180	1.54	4.00
Mean		8.3		66.7		1.46
Total	292.61		1.41		11.31	
			Nitrate-N			
1 (0-2)	89.86	380	34.15	1090	97.95	17.75
3 (2-4)	70.72	410	28.99	1230	86.99	20.50
5 (4-7)	67.91	350	23.77	1380	93.71	25.75

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TABLE 1 (continued)

Depth (m)	Volume	Jan. 24		Mar. 18		Rate[b]
Sample (layer)	10^6 L	µg N.L^{-1}	kg N[a]	µg N.L^{-1}	kg N	µg N.L^{-1}.d^{-1}
8 (7-10)	33.72	340	11.46	1600	53.95	31.50
12 (10-13)	21.84	380	8.30	1440	31.45	26.50
14 (13-15)	8.56	640	5.48	1210	10.36	14.25
Mean		416.7		1325.0		22.71
Total	292.61		112.15		374.41	

[a] Data for kg N per layer obtained from volume x µg N.L^{-1}.

[b] Rate calculated from the (change in concentration)/(interval in days).

TABLE 2. Rates of consumption of NH_4^+-N and O_2, and of production of (NO_2^- + NO_3^-)-N *in situ* in Lake St. George during winter months (μg N or $O_2.L^{-1}.d^{-1}$)[a].

Measure	1976 Feb-10 to Mar-30	1979 Jan-31 to Mar-20	1980 Jan-24 to Mar-18	1981 Jan-14 to Mar-24	1982 Jan-13 to Feb-22	1983 Jan-09 to Feb-15	1984 Jan-10 to Mar-13	Mean
ΔNH_4^+	7.69	5.92	4.03	5.47	10.37	-0.24	3.56	5.26
$\Delta (NO_2^- + NO_3^-)$	8.51	9.25[b]	10.45	13.31	23.25	17.52	9.57	13.12
Calculated BOD_N[c]	36.80	40.00	45.20	57.60	100.70	75.90	41.40	56.80
Observed ΔO_2	-20.00	50.00	86.00	62.00	140.00	77.00	134.00	75.60
$\frac{BOD_N}{\Delta O_2}$	-	80.00	52.50	92.90	71.90	98.60	30.90	71.20[d]

[a] Mass balances calculated for the whole lake from concentration data of Fig. 1 and volumetric data in the depth range 0 to 15 m, except for 1976 (0 to 14 m) and 1979 (0 to 10 m), as exemplified by Table 1.

[b] NO_2^- data were not available for this year.

[c] Calculated O_2 consumption associated with the production of NO_2^- and NO_3^- assuming the factor of Wezernak and Gannon (1967): BOD_N = 4.33 X nitrified-N.

[d] 1976 data were not included in this calculation.

TABLE 3. Summary of nitrapyrin-sensitive ^{14}C-bicarbonate incorporation experiments (0 to 0.21 or 1.8 day rates) giving initial concentrations of nitrate, nitrite, ammonium and total particulate nitrogen (μg $N \cdot L^{-1}$) as well as oxygen ($mg \cdot L^{-1}$).

Date	Depth	Initial concentrations					Nitrification rate
	(m)	NO_3^-	NO_2^-	NH_4^+	TPN	O_2	(μg $N \cdot L^{-1} \cdot d^{-1}$)
80-Feb-27	2	540	9	467	290	9.3	22.8
	8	1040	14	416	120	4.5	10.8
	14	1560	44	631	216	2.6	27.0
80-Mar-06	2	550	5	390	82	8.1	12.2
	14	1570	7	512	78	2.0	13.1
82-Feb-24	2	1230	36	65[a]	70	1.3	4.5
	8	1680	75	106[a]	67	0.3	7.7
	14	1400	183	858[a]	110	0.3	2.1
82-Mar-03	2	1191	8	39[a]	69	0.8	1.3
	2[b]	1220	7	30[a]	60	6.8	1.7
83-Feb-09	2[b]	510	20	490	110	10.2	10.5
	14[c]	1700	80	760	76	10.3	11.4

[a]Since ammonium was low at some depths, 300 μg $N \cdot L^{-1}$ was added to all samples indicated.

[b]The sample was filtered to remove zooplankton and oxygen was added.

[c]The sample was not filtered but it received oxygen.

TABLE 4. Denitrifying and nitrifying bacteria (ammonium oxidizers and nitrite oxidizers) in Lake St. George water column.

Depth (m)	80-Feb-27			81-Jan-21			82-Feb-25	
	Den.[a]	NH_4^{+b}	NO_2^{-c}	Den.	NH_4^+	NO_2^-	NH_4^+	NO_2^-
0				22	<1	1		
2				24	<1	2	1	1
3	170	1	1					
5	1700	1	1				1	1
8	790	1	1	35	<1	2	1	3
10	130	2	2					
12	79	1	1	11	<1	2		
14	240	1	1					
15				24	<1	2		

[a] Denitrifying bacteria.

[b] Ammonium-oxidizing bacteria.

[c] Nitrite-oxidizing bacteria.

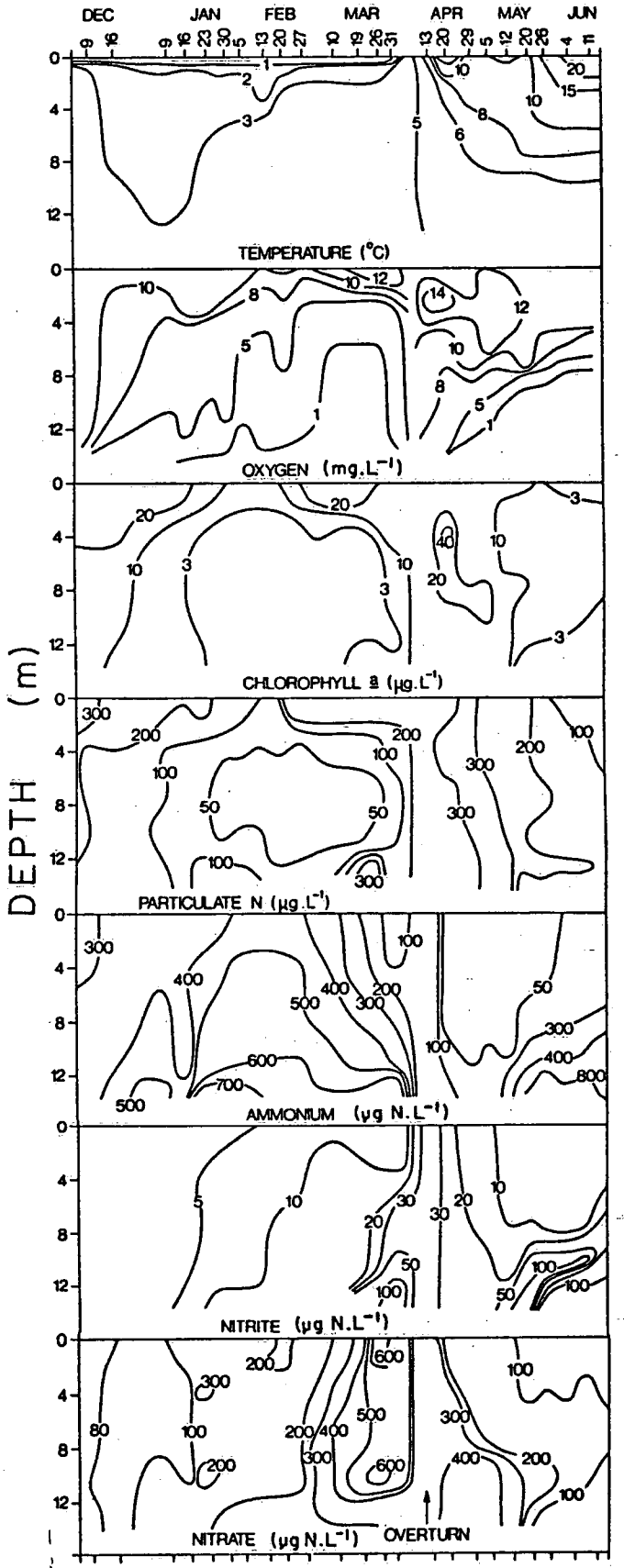
Legends for figures

FIG. 1. Isopleths for temperature, oxygen, chlorophyll, particulate N, ammonium, nitrate, and nitrite for Lake St. George during winter 1975-76.

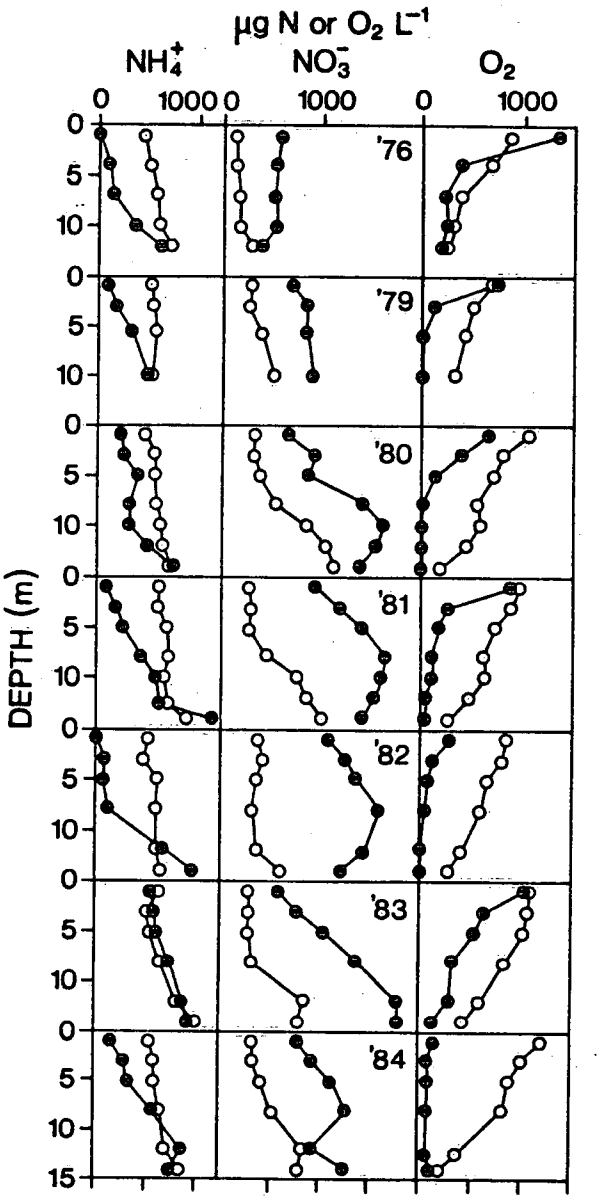
FIG. 2. Concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) nitrogen, and oxygen (O_2) in Lake St. George at the first (O) and second (●) sampling dates (given in Table 2) for winters between 1976 and 1984.

FIG. 3. Changes in concentrations of NH_4^+-N, NO_3^--N, and O_2 in samples of water (2-m depth) incubated in the absence (O) and in the presence of C_2H_2 (●) or nitrapyrin (△). The thick line shows changes in concentrations which occurred in the lake (1980) over the period indicated. Data for NH_4^+-N in the nitrapyrin treatment are not shown since the DMSO solvent interfered with NH_4^+ analysis.

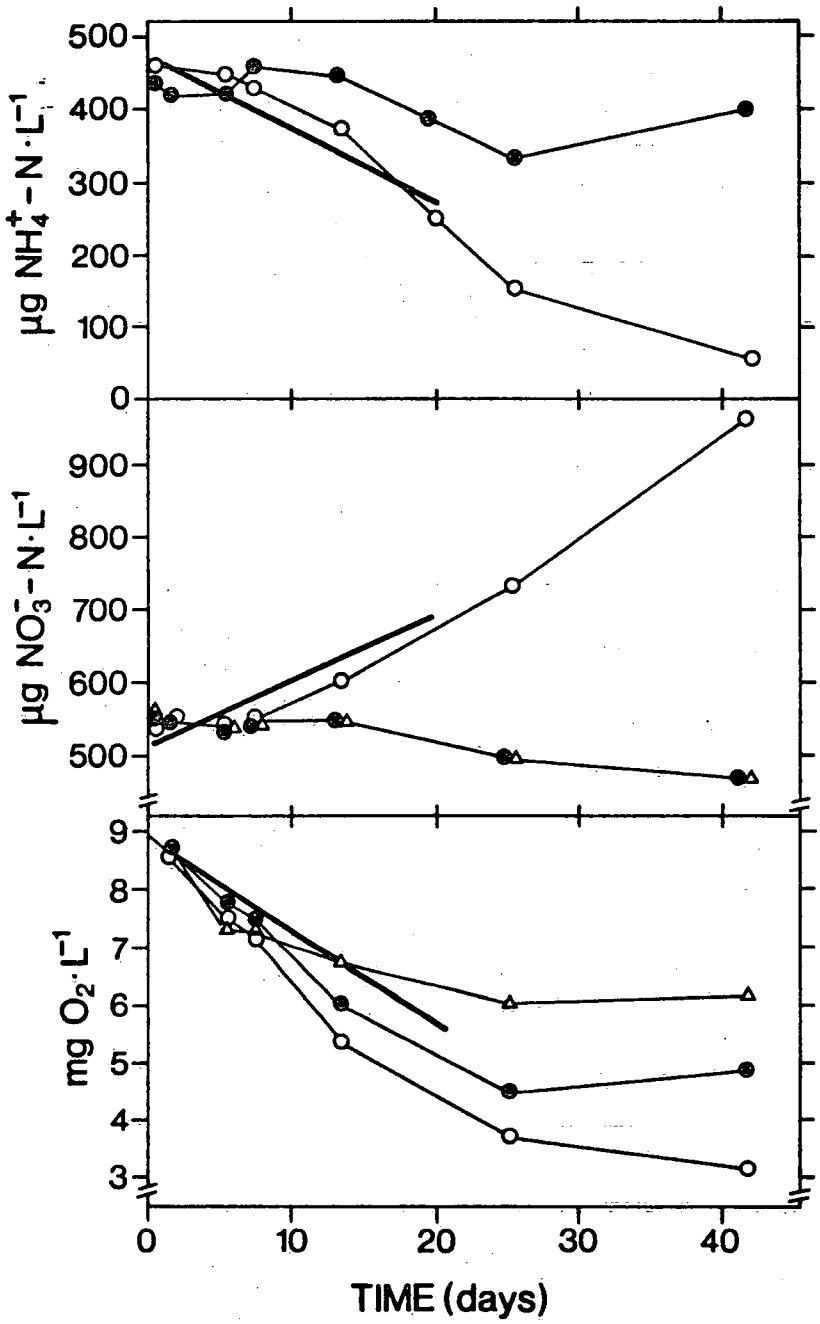
FIG. 4. Production of nitrous oxide (N_2O) and consumption of O_2 in the presence of acetylene at 1.5 (O) and 3.3 (●) mM. There was no N_2O production in the absence of acetylene or in the presence of nitrapyrin.



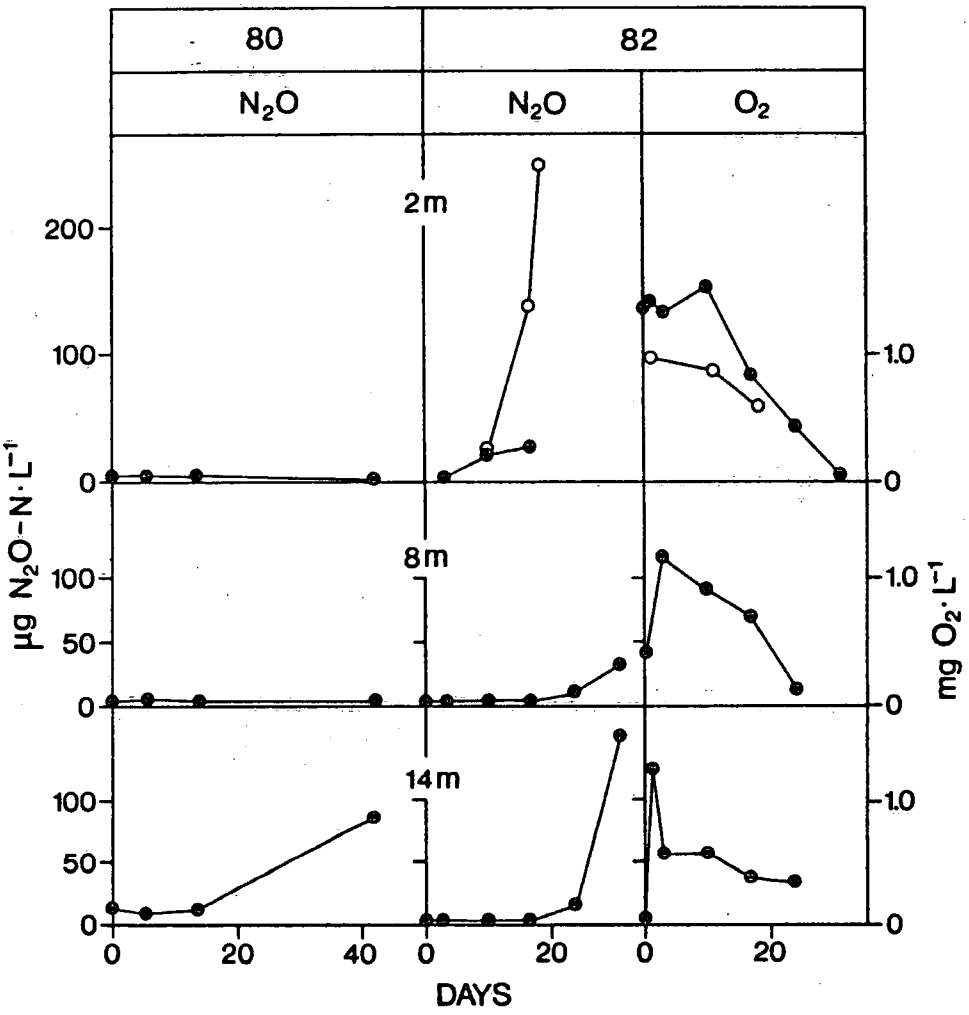
Knowles + Lean Fig 1



Knowles & Lean. fig. 2



Knowles + Lean. Fig. 3.



Knowles + Lean. Fig. 4.