THE IN SITU RATE OF METHANE PRODUCTION IN A SMALL, EUTROPHIC, HARD-WATER LAKE

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ABSTRACT

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By

Richard Floyd Strayer

The formation and subsequent fate of methane in the sediments of lacustrine systems are important processes in the limnetic carbon cycle. Organic matter accumulation in the sediment is moderated by methane leaving the sediment. In order to estimate the methane production rate in eutrophic Wintergreen Lake, the rates at which methane left the sediment by diffusion and bubble evolution were determined.

The rate of diffusion of methane depends upon the concentration gradient and the coefficient of eddy diffusivity. Bi-weekly vertical distributions of dissolved methane were determined throughout the summer in order to estimate the concentration gradient of methane, which was 1.082 mmoles/l/m. The best estimate for the coefficient of eddy diffusivity was 4.3×10^{-3} cm^2/sec and was obtained from the summer data for the vertical distribution of temperature. The resulting rate of diffusion of methane was calculated to be 40.2 mmoles/m^2/day for the period of May 30 to August 21, 1972.

Bubbles evolving from the sediment were collected over the course of the summer and fall by inverted funnels. The gas composition of the bubbles ranged from 55 to 93% methane and 6 to 36% nitrogen. The rate at which methane left the sediment by ebullition in the summer was 21.1 mmoles/m^2/day for the period of May 30 to August 21, 1972.

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Richard Floyd Strayer

A THESIS

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To my mother and father

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INTRODUCTION

The carbon cycle in a lake is comprised of two opposing processes: the synthesis of organic matter by carbon dioxide fixation, and the degradation or mineralization of this fixed organic carbon. Organic matter synthesis by phytoplankton is the predominant source of fixed carbon in a lake's pelagic zone (Ruttner, 1970; Kuznetsov, 1968). Occasionally the production of organic material by chemosynthesis, heterotrophic assimilation of carbon dioxide, or assimilation of allochthonous organic compounds reaches levels that are greater than the productivity of the phytoplankton (Kuznetsov, 1968).

The products of organic matter synthesis are partially decomposed in the water column by the heterotrophic bacteria. The remaining stable particulate organic molecules, which escape significant aerobic decomposition, appear in the sediments (Brock, 1966). Analysis of the ^{13}C to ^{12}C isotopic ratios of phytoplankton and sediments (Deevey *et al.*, 1963; Deevey and Stuiver, 1964) shows that the carbon in the mud is derived from the sedimentation of plankton.

The organic matter in the sediment generally undergoes anaerobic decomposition to form fatty acids and hydrogen. In the anaerobic sewage sludge digester, a well-studied habitat somewhat analogous to anaerobic sediments, a heterogeneous group of facultative and anaerobic bacteria converts proteins, carbohydrates, and fats into saturated fatty acids, carbon dioxide, and ammonia (McCarty, 1963; Jeris and McCarty, 1965). Toerien and Hattingh (1969) and Wolfe (1970) have postulated that hydrogen is also a product of anaerobic decomposition. Hydrogen has been observed to accumulate when methanogenesis was inhibited by the addition of chlorinated methanes to rumen fluid (Bauchop, 1967) and washed cell suspensions from an anaerobic sewage digester (Thiel, 1969).

It is presumed that fatty acids and hydrogen in the sediment are converted to methane by the methane-producing bacteria. However, the physiology of these bacteria is currently in a state of cautious re-examination because of the discovery by Bryant *et al.* (1967) that the described fermentation of ethyl alcohol was not brought about by a pure methanogenic culture. Prior to this discovery, pure culture studies had indicated that primary and secondary alcohols, C_2 to C_6 saturated fatty acids, and one-carbon compounds (formic acid, carbon monoxide, carbon dioxide, and methyl alcohol) were substrates for this group of anaerobic bacteria (Barker, 1956; Stadtman, 1967).

The use of these substrates must now be carefully studied to establish whether they are transformed to methane by a single species or by obligate substrate coupling between two or more species. Wolfe (1970) goes so far as to state that the organisms that use propionate, butyrate, or higher fatty acids and alcohols were not represented in pure culture in 1970. He believes that the methanogenic bacteria are not able to utilize substrates other than formate, acetate, methyl alcohol and carbon dioxide and hydrogen. The link between the alcohols and fatty acids and the methanogenic bacteria is a proposed intermediary population that oxidizes these substrates with concomitant production of hydrogen (Toerien and Hattingh, 1969).

The liberation of methane from the sediment forms the last link in the chain of anaerobic decomposition processes. Dissolved methane can leave the sediment by diffusion and enter the overlying water. Diffusion rates in the sediment are slower than in the overlying water (Mortimer, 1941); therefore, if the rate of methane production exceeds the diffusion rate in the sediment, the concentration of dissolved methane in the sediment will gradually increase until it reaches the critical concentration at which bubbles may form. Thereafter the ebullition of methane controls the maximum concentration of this gas in the sediment (Reeburgh, 1969; Klots, 1961).

Two major fates await the methane that has left the sediment. The organic carbon in the bubbles is lost to the carbon cycle of the lake when the bubble reaches the air-water interface. The second fate, involving the dissolved gas, is its use by methane-oxidizing bacteria as an energy source, converting it to cellular material and carbon dioxide.

The importance of the methane-producing bacteria can be deduced from the foregoing discussion of the carbon cycle.

A decrease in the sediment pH to levels that are below bacterial tolerance is prevented by the transformation of organic acids to methane. The loss of methane from the sediment by diffusion and ebullition represents a loss of organic carbon which otherwise would accumulate. The dissolved methane which is oxidized could contribute to eutrophication because it represents organic carbon which is recycled (Weaver and Dugan, 1972). The fact that molecular oxygen is used by the methane-oxidizing bacteria to oxidize methane means that there is an increase in the rate of oxygen depletion in the deeper waters of a lake (Overbeck and Ohle, 1964; Naguib and Overbeck, 1970; Sorokin, 1961). Thus the formation and oxidation of methane are closely connected with the whole limnic metabolism (Ohle, 1958, cited in Overbeck and Ohle, 1964).

Hungate's (1960, 1962) criteria for a complete ecological analysis of a natural habitat includes the quantitative measurement of microbial activity in the environment. This study attempts to determine the *in situ* rate of methane production in a eutrophic, hard-water lake. In order to estimate this rate the processes of diffusion and ebullition and the rates at which methane leaves the sediments by these two processes were investigated.

MATERIALS AND METHODS

The Lake

These studies were made in Wintergreen Lake, which is located within the W. K. Kellogg Bird Sanctuary, Kalamazoo County, Michigan, R. 9W., T. 2N. Sec. 8. The sampling site was located between the six meter depth contour and the 6.3 m depth maximum. Wintergreen Lake has an area of 0.15 km^2, a maximum depth of 6.3 m, and a mean depth of 3.54 m (Manny, 1971). It is a temperate, dimictic, and second order lake (Hutchinson, 1957; Ruttner, 1970; Manny, 1971; Duong, 1972). Turnover is completed in April and October.

Temperature

Every two weeks the temperature of the water column was measured at one meter depth intervals. The *in situ* temperature was read from the temperature scale on a Model 54 oxygen meter (Yellow Springs Instr. Co., Yellow Springs, Ohio).

Dissolved Oxygen

Water samples were collected for dissolved oxygen analysis on the same dates that temperature was measured. Samples were collected at one meter depth intervals with a 3-liter Van Dorn water sampler. Two sub-samples per Van Dorn were siphoned into 300 ml BOD bottles. The sub-samples were chemically fixed in the field and transported back to the laboratory where the dissolved oxygen concentration was determined by a modified Winkler technique (Mackereth, 1963). Duplicate titrations were made on each sub-sample.

Dissolved Methane

Samples to be analyzed for dissolved methane were collected with the Van Dorn sampler every two weeks. During the summer of 1971 and the winter of 1972 samples were collected at one-half meter intervals (1971 summer samples were collected by T. P. Duong). Sample collections in the summer of 1972 were made at 0 m, 1 m, and at one-half meter intervals from 2 m to 5-1/2 m. Two sub-samples per Van Dorn were siphoned into 26 ml serum bottles and 10 ml of the sub-sample were immediately withdrawn with a syringe in order to create an air headspace. The bottles were then promptly sealed with a rubber serum cap.

The serum bottles were stored overnight at 1 C and were removed two hours prior to methane analysis to allow them to reach equilibrium with the room temperature. Every half hour the bottles were shaken for one minute to facilitate equilibration of the gases between the aqueous and vapor phases. Methane analysis of the headspace gas was made by injecting two 0.2 to 0.6 ml gas samples from each serum bottle into a gas chromatograph equipped with an H_2-flame ionization detector (Model 600-D interfaced with a Model 20 recorder, Varian Aerograph, Walnut Creek, Calif.). A 94 cm, 3 mm diameter stainless-steel column packed with 100-200 mesh Porapak N was used (Waters Assoc., Inc., Framingham, Mass.). The oven temperature was 50 C. The flow rate of the nitrogen carrier gas was 30 ml/min. Methane concentrations in the headspace were determined using a standard curve relating peak heights to known concentrations of methane (Certified Standards of methane in nitrogen were used, Matheson Gas Products, Joliet, Ill.). Samples collected in the summer of 1971 were analyzed by M. Firestone.

The dissolved methane in the original lake-water sample was calculated from the headspace methane concentration and a constant that described the distribution of methane between the aqueous and vapor phases under the conditions of analysis. This constant was determined experimentally using degassed lake water and standard concentrations of methane.

To justify the collection of water samples from only one site, the effects of sampling site location and depth upon the observed vertical distribution of dissolved methane were tested by calculating a two-way analysis of variance (two-way ANOVA). On July 19, 1972, four sampling sites located within the six-meter depth contour were selected. Water samples were obtained from the same depths as in the normal collections and analyzed for dissolved methane.

Sediment-Generated Gas Bubbles

Bubbles evolving from the sediment were collected by inverted funnels (Nalgene, 11 inches diameter, Cole-Parmer Instr. Co., Chicago, Ill.) that were suspended at depths of

1 and 5 m. From May 30 to August 21, 1972, three funnels were suspended at each depth, and from August 21 to October 31, 1972, six funnels were suspended at each depth. Each funnel had a 250 ml glass graduated cylinder attached over the funnel stem in order to collect bubbles by the displacement of water from the cylinder. Once a week the cylinders were removed, sealed with a butyl rubber stopper, and replaced with another cylinder.

Methane and nitrogen analyses were made by injecting 0.3 ml of the trapped gas into a gas chromatograph equipped with a micro-thermistor detector (Basic Model 8000, Carle Instr., Inc., Fullerton, Calif.). Two columns connected in a series were used for gas separation. The first column in the series (94 cm, 3 mm diameter, stainless steel) was packed with Porapak Q (80-100 mesh, Waters Assoc., Inc., Framingham, Mass.) and the second column (189 cm, 3 mm diameter, stainless steel) was packed with molecular sieve 5A (80-100 mesh, Coast Engineering Lab., Redondo Beach, Calif.). Separation was made at a temperature of 55 C. Helium gas at a flow rate of 25 ml/min was used as the carrier gas. The methane concentration and percent volume of the bubble gas were determined by comparison of the bubble's methane peaks with the peaks of a methane standard (C. P. grade, Matheson Gas Products, Joliet, Ill.). Nitrogen percent volume was determined by comparison of the nitrogen peaks with the nitrogen peaks of standard injections of air samples. Bubble volumes were measured after the completion of gas analysis.

An experiment was run in the fall of 1972 to determine whether the differences in the amount of methane that was collected at the two depths were caused by methane diffusing out of the graduated cylinders after the bubbles were trapped. An inverted funnel with attached graduated cylinder was suspended in a 55 gallon drum that had been filled with tap water. The water in the graduated cylinder was displaced with 209 ml of a 5% methane standard (Matheson Gas Products, Joliet, Ill.). After eight days at a temperature of 20 C the volume and methane percent volume of the gas in the cylinder were measured.

RESULTS

Figure 1 is a depth-time diagram of the distribution of temperature in Wintergreen Lake in the summer of 1972. The diagram provides a picture of the development of vertical temperature distribution with time. The vertical temperature distribution for June 26, 1972 (Figure 5) is typical for this lake throughout the summer stratification period.

Data for the dissolved oxygen concentration in Wintergreen Lake during the summer, 1972, is condensed in Figure 2. The vertical dissolved oxygen distribution on June 26, 1972, is also plotted in Figure 5.

The distribution of dissolved methane with depth and time for the summer of 1971 is shown in Figure 3, and for the summer of 1972 in Figure 4. Only those depths below the thermocline (2.5 m) are shown. The distribution of dissolved methane during the winter of 1972 is presented in Table 1. The dissolved methane vertical distribution on June 26, 1972, is exhibited in Figure 5.

Results of the two-way ANOVA testing the effects of sampling site location and depth on the dissolved methane distribution indicate the differences in dissolved methane between the four sites are not significant but the differences between depths are highly significant ($P<0.001$).

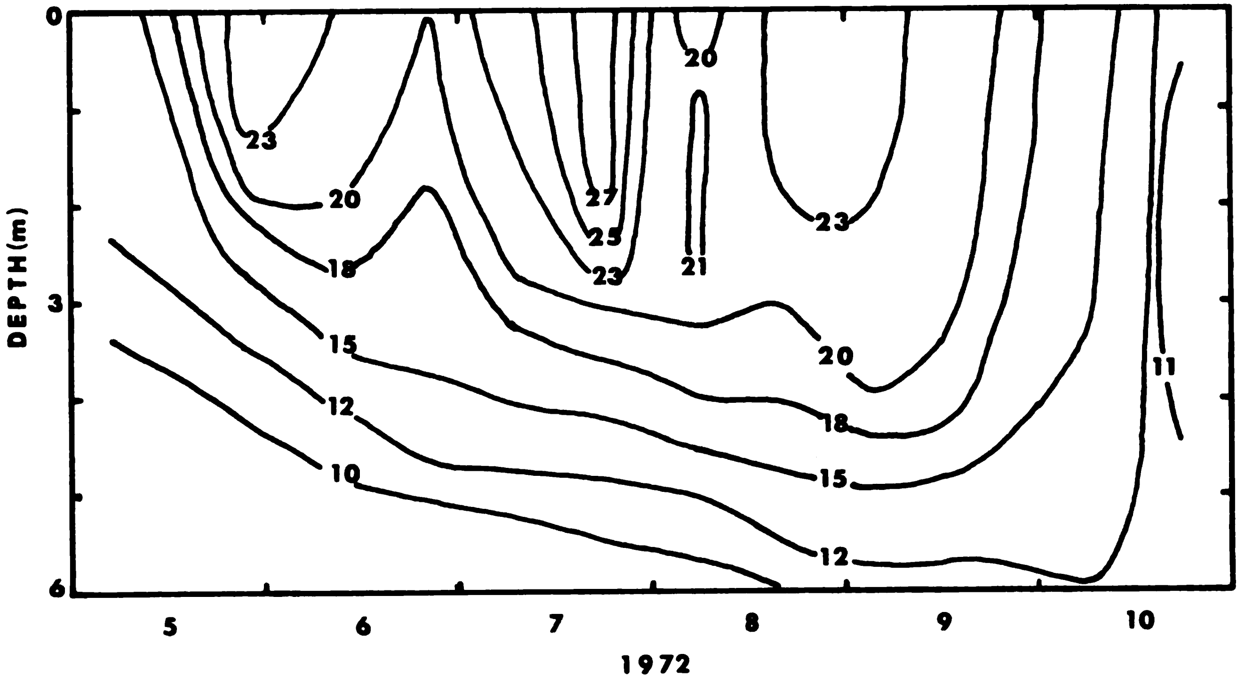

Figure 1. Depth-time diagram of the distribution of temperature (C) in Wintergreen Lake in the summer of 1972.

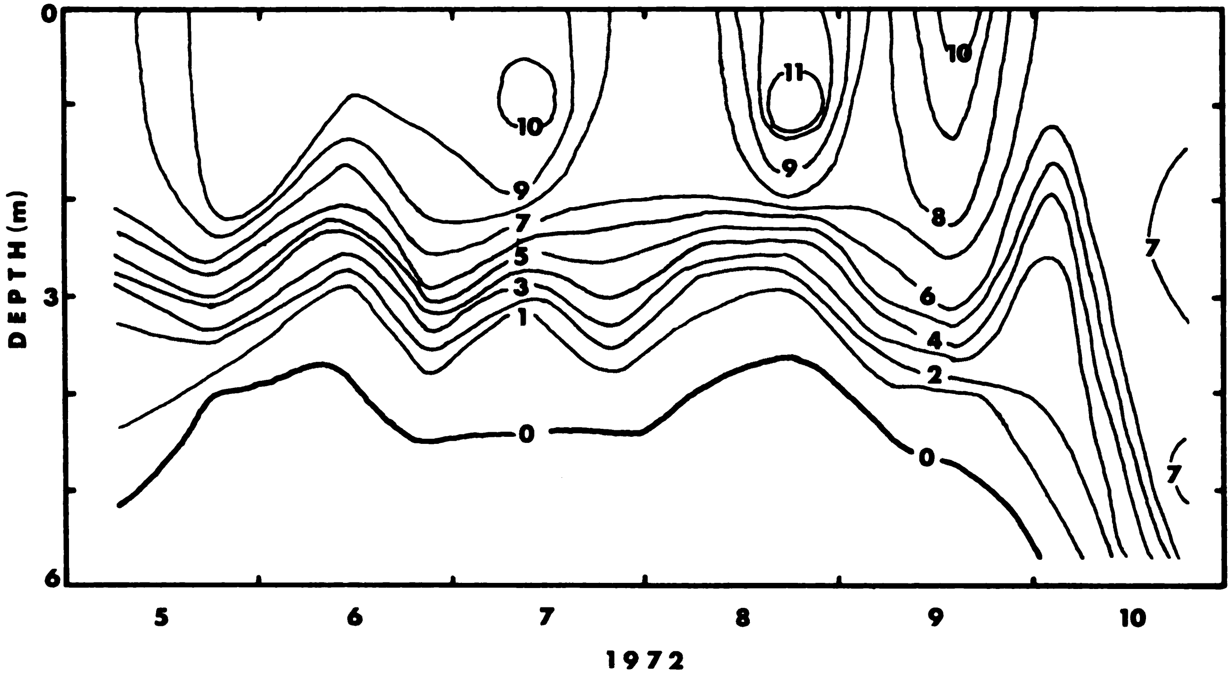

Figure 2. Depth-time diagram of the distribution of dissolved oxygen (mg $O_2/1$) in Wintergreen Lake in the summer of 1972.

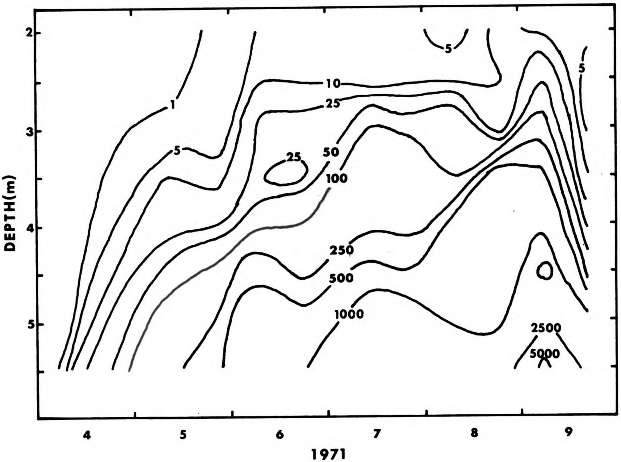

Figure 3. Depth-time diagram of the distribution of dissolved methane (µmoles $CH_4/1$) in Wintergreen Lake in the summer of 1971.

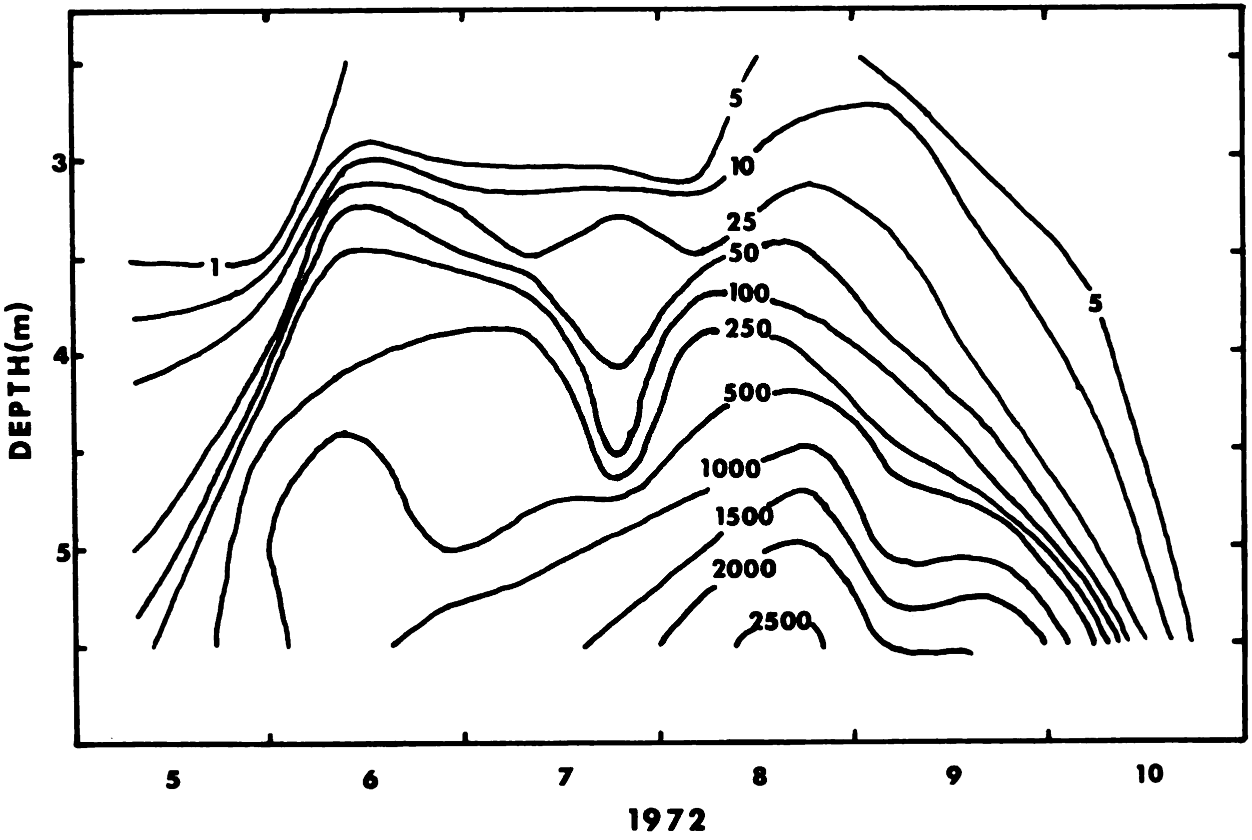

Figure 4. Depth-time diagram of the distribution of dissolved methane (μmoles $CH_4/1$) in Wintergreen Lake in the summer of 1972.

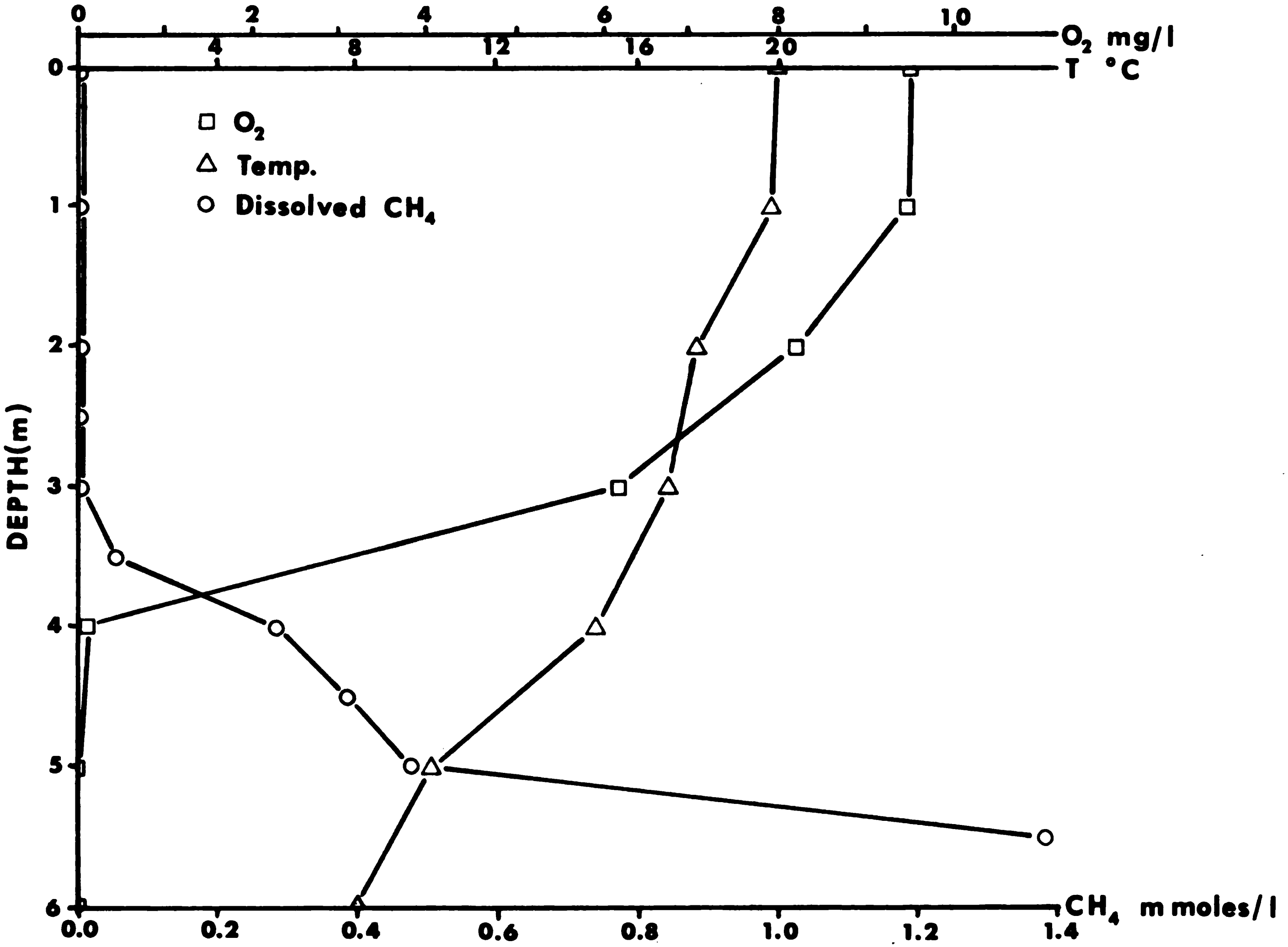

Figure 5. Vertical distribution of temperature -△- (C), dissolved oxygen -□- (mg O_2/1), and dissolved methane -o- (mmoles CH_4/1) in Wintergreen Lake on June 26, 1972.

Table 1. Distribution of dissolved methane (μmoles/l) in Wintergreen Lake during the winter of 1972

Depth, z (m)	Jan 24	Feb 8	Feb 22	Mar 7
0.0	6.1	5.6	14.0	21.6
0.5	1.4	4.8	1.7	1.1
1.0	0.4	0.3	0.7	0.6
1.5	0.3	tr.	0.6	tr.
2.0	0.4	tr.	0.4	tr.
2.5	0.2	tr.	tr.	tr.
3.0	0.3	tr.	tr.	0.8
3.5	0.3	tr.	tr.	tr.
4.0	0.3	tr.	tr.	tr.
4.5	0.3	tr.	3.4	0.5
5.0	1.6	0.5	4.5	15.9
5.5		24.6	113.6	252.5
6.0		49.2		

tr., trace, methane was detected but at levels too low to make an accurate quantitative estimation.

There is an absence of interaction between depths and sampling sites.

The equation used by limnologists to describe the rate of diffusion of a substance across a plane of unit area (Mortimer, 1941, 1942; Hutchinson, 1957) is comparable to Fick's law (Jacobs, 1967):

$$dS/dt = -A(ds/dx) \tag{1}$$

The term dS/dt is the rate of passage of a substance across a plane of unit area (g/sec/cm^2), A is the coefficient of eddy diffusivity (cm^2/sec), and dS/dx is the concentration gradient of the substance at the plane (g/cm^3/cm).

In order to determine the coefficient of eddy diffusivity in the hypolimnion of Wintergreen Lake, two methods of analysis were employed. Mortimer's (1941) method makes use of a rearrangement of equation (1) so that

$$A = \frac{-dS}{(dt)(ds/dx)} \tag{2}$$

Since A is solely an index of the rate of exchange of the water masses, it should be the same if computed from any conservative property of the water. Equation (2) was applied to three sets of data: (1) the conductivity data of Duong (1972) for the period of July 6 to August 24, 1971; (2) the temperature data (Figure 1) for the period of May 23 to August 21, 1972; and (3) the methane data (Figure 4) for the period of May 30 to August 21, 1972. The results of these calculations are presented in Table 2.

Table 2. Estimates of the eddy diffusion coefficient at two levels in the hypolimnion of Wintergreen Lake

Data employed	Period	Year	Depth of estimation	Mean values of $A \times 10^3$ for period
Conductivity	Jul 6 to Aug 24	1971	4 m	0.77
Temperature	May 23 to Aug 21	1972	5 m	1.0[a]
Methane concentration	May 30 to Aug 21	1972	5 m	1.2

[a] Corrected for the coefficient of molecular conductivity (1.2×10^{-3} cm^2/sec).

The second method that was used for calculating A involves applying the principles of Hutchinson (1941) to the expressions of McEwen (1929, cited in Hutchinson, 1941, 1957) to analyze the changes in temperature at a series of depths during a complete heating period. The assumption is made that throughout the hypolimnion the rate of change of temperature with depth is exponential. By fitting the observed vertical temperature gradient to an arbitrary function which can be simply differentiated, mean values of A for different levels can be estimated. Details of these mathematical manipulations are given by Hutchinson (1941). The mean value of A for the 5 m level for the period of May 23 to August 21, 1972, as calculated by Hutchinson's procedure, is 4.3×10^{-3} cm^2/sec after deducting the coefficient of molecular conductivity.

The daily mean dissolved methane concentration gradient for the 5 m plane in Wintergreen Lake over the period of May 30 to August 21, 1972, is 1.082 mmoles/l/m. This gradient multiplied by the 4.3×10^{-3} cm^2/sec value that was obtained for A is used to calculate the mean daily methane diffusion rate across the 5 m plane. The diffusion rate for the period of May 30 to August 21, 1972, is 40.2 mmoles CH_4/m^2/day.

The amount of methane leaving the sediment through ebullition depends on the solubility of methane in water. Combining the formulae for gas saturation in water (Daniels and Alberty, 1967; Stumm and Morgan, 1970; Klots, 1961) and adopting the symbols used by Hutchinson (1957), the following equation is obtained:

$$[CH_4]_s = (760 \text{ mm Hg})(55.56)/K_{CH_4} \qquad (3)$$

$[CH_4]_s$ is the concentration of the pure methane gas in water in equilibrium with one atmosphere of the pure methane at temperature θ_z C for depth z. The 760 mm Hg (one atmosphere) term is the partial pressure of the pure methane gas, and K_{CH_4} is the Henry's law constant for methane at temperature θ_z (Washburn, 1928).

The critical value of the dissolved methane concentration at which, under appropriate conditions, bubbles can form ($[CH_4]_c$, Hutchinson, 1957; Klots, 1961) is calculated from

$$[CH_4]_c = [CH_4]_s\ (0.209 + 0.0967\ z) \qquad (4)$$

if the water is assumed to be approximately saturated with nitrogen (Hutchinson, 1957; Reeburgh, 1969). The atmospheric pressure at the lake's surface is assumed to be one atmosphere.

The calculated values of $[CH_4]_s$, $[CH_4]_c$, and the observed methane concentration at depth z ($[CH_4]_z$) for the 5.5 m depth of Wintergreen Lake, summer, 1972, are given in Table 3.

The theoretical percent volume of the gases in a bubble that has originated in the sediment is given by the following formulae (Hutchinson, 1957):

$$\begin{aligned} \%\ \text{volume } CH_4 &= ([CH_4]_c/[CH_4]_s)/(1.0 + 0.0967\ z) \times 100 \qquad (5) \\ &= (0.209 + 0.0967\ z)/(1.0 + 0.0967\ z) \times 100 \end{aligned}$$

$$\begin{aligned} \%\ \text{volume } N_2 &= ([N_2]_z/[N_2]_s)/(1.0 + 0.0967\ z) \times 100 \qquad (6) \\ &= (0.791)/(1.0 + 0.0967\ z) \times 100 \end{aligned}$$

These two gases are assumed to be the major constituents of the bubbles and the water is assumed to be approximately saturated with nitrogen (Hutchinson, 1957). Table 4 shows the theoretical and observed percent volumes of methane and nitrogen in bubbles that were collected at the 1 m and 5 m depths in the summer of 1972.

The amount of methane that was collected by the inverted funnels over the summer of 1972 is plotted in Figure 6. The experiment that was performed in the 55 gallon drum showed that after eight days both the volume and methane percent volume of the gas in the cylinder were unchanged.

Table 3. Calculated concentrations of methane saturated water ($[CH_4]_s$), calculated critical concentrations of methane at which bubbles may form ($[CH_4]_c$), and observed concentrations of methane ($[CH_4]_z$) for the depth z 5.5 m of Wintergreen Lake during the summer of 1972

Date	θ_z (C)	$[CH_4]_s$ (μmoles/l)	$[CH_4]_c$ (μmoles/l)	$[CH_4]_z$ (μmoles/l)
May 30	8.0	1970	1459	410
Jun 12	8.7	1932	1431	950
Jun 26	9.0	1914	1418	1390
Jul 10	9.4	1895	1404	1300
Jul 24	10.2	1862	1379	1640
Aug 7	10.5	1844	1366	2420
Aug 21	12.0	1772	1313	2800
Sep 5	13.1	1726	1279	1890
Sep 18	12.6	1747	1294	1930
Oct 3	13.2	1720	1274	1020

Table 4. Theoretical percent volume of methane and nitrogen in bubbles formed at a depth of 6 m, and observed percent volume of methane and nitrogen in bubbles collected at 1 m and 5 m in Wintergreen Lake during the summer of 1972

Date	Theoretical % volume CH_4	Observed % volume CH_4 1 m	Observed % volume CH_4 5 m	Theoretical % volume N_2	Observed % volume N_2 1 m	Observed % volume N_2 5 m
Jun 6	49.9	64	85	50.1	39	20
Jun 12	49.9	69	93	50.1	24	22
Jun 26	49.9	82	67	50.1	22	30
Jul 3	49.9	51	83	50.1	14	14
Jul 24	49.9	52	73	50.1	48	22
Aug 21	49.9	53	79	50.1	34	18
Sep 25	49.9	54	59	50.1	38	24
Oct 3	49.9	56	55	50.1	33	35
Oct 10	49.9	52	66	50.1	39	30
Oct 17	49.9	59	58	50.1	29	36

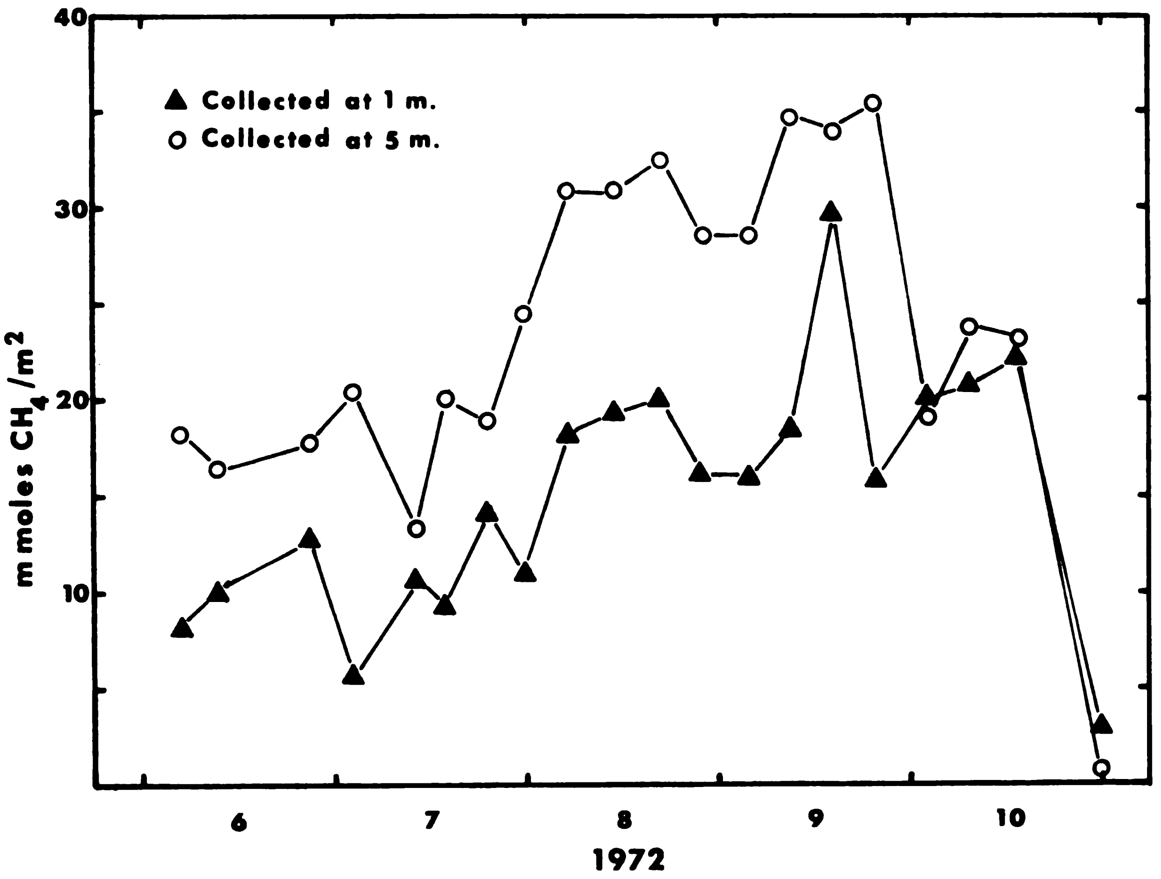

Figure 6. Amount of methane gas (mmoles/m^2) collected by inverted funnels suspended 1 m and 5 m from the surface of Wintergreen Lake in the summer of 1972.

DISCUSSION

Immediately following the break-up of the ice cover in Late March, the lake had a uniform vertical distribution of temperature and dissolved oxygen. As the air temperature increased, thermal stratification began and the dissolved oxygen concentration in the hypolimnion diminished. By early May, thermal stratification was established (Figure 1) and the dissolved oxygen at the bottom was depleted (Figure 2). The methane-producing bacteria are among the most fastidious anaerobes (Barker, 1956); therefore, significant quantities of dissolved methane were not detected in the water column until after the oxygen at the bottom had been depleted (Figures 3 and 4).

Throughout the summer, periods of wind-generated turbulence greatly affected the temperature and oxygen distributions in the epilimnion (Figures 1 and 2). However, a large water-density gradient existed in the thermocline due to the rapid decrease in temperature in that layer. This density gradient prevented the waters of the hypolimnion from mixing with those of the epilimnion. Throughout the summer there were only minor fluctuations in dissolved oxygen and temperature in the hypolimnion (Figures 1 and 2). During this summer stagnation period the dissolved methane continually built up in the hypolimnion, reaching a maximum in the first week of September, 1971 (Figure 3) and in mid-August in 1972 (Figure 4).

The vertical distribution of temperature, oxygen, and methane on June 26, 1972 (Figure 5) indicated that although thermal stratification was not clearly defined, there appeared to be an interaction between the dissolved oxygen and dissolved methane concentrations. This relationship may be explained by Sorokin's (1961) observation that the maximum methane-oxidizing activity in Kuibyshev Water Reservoir occurred at the boundary between the aerobic and anaerobic zones.

As the air temperature declined in the fall, Wintergreen Lake became less stable. The sediments were exposed to oxygen near the beginning of October (Figure 2), but dissolved methane concentrations in the water column started to decline in the last week in August (Figures 3 and 4). This decline was probably due to an increase in turbulence and bacterial methane-oxidation. With the fall turnover, the entire water column was mixed and the distribution of dissolved oxygen and temperature was again uniform. The dissolved methane throughout the water column was essentially nil.

Dissolved methane levels in Wintergreen Lake reached 5.6 mmoles/l at 5.5 m in 1971 and 2.8 mmoles/l at the same depth in 1972. The raw data indicated that the 1971 methane maximum may have been incorrect. The 5.5 m dissolved methane concentrations on the sampling dates just before and after this maximum were from two to three times smaller.

The 1972 data showed the increase in concentration up to the maximum was much more gradual.

Sorokin (1961) observed somewhat lower dissolved methane concentrations in August at two sites in Kuibyshev Reservoir. A maximum of 0.28 mmoles/l (my conversion from Sorokin's data, 6.2 ml/l) occurred at the 18 m bottom of one site and a maximum of 0.32 mmoles/l (converted from 7.3 ml/l) two meters above the bottom (13 m) of the second site. Kuibyshev Reservoir was not thermally stratified and Sorokin believed that some kind of exchange occurred between the bottom layers and surface layers of water. A maximum dissolved methane concentration that is comparable with the Wintergreen values was determined in August by Howard *et al.* (1971) for Lake Erie. They measured 3.2 mmoles/l (my conversion from 51 parts per million) in a sample taken at a depth of 4 m, which was just above the bottom.

The dissolved methane concentrations immediately under the ice (Table 1) showed an increase over the sampling period. Methane production in the winter is presumed to be slower than in other seasons, but the accumulation of methane under the ice implied that the methane concentration in the sediment was high enough for bubble formation. A preliminary, single bubble trap that was suspended at 5 m from February 22 to March 7, 1972, collected 20 ml of gas. The bubbles arising from the sediment were trapped under the ice and the gases probably redissolved, forming a concentration gradient at the water surface. The maximum methane concentration in the winter was 0.25 mmoles/l on March 7

(Table 1). Also on March 7, Kuznetsov (1959) measured a maximum methane concentration of 0.36 mmoles/l (converted from 8.0 ml/l) at the bottom, 12 m depth in Lake Beloje. However, these lower winter values of dissolved methane do not imply that methane production by the sediments is similarly reduced.

Several studies have estimated the methane production rate in a lake by measuring the volume and percent composition of sediment generated bubbles (Rossolimo, 1935; Howard *et al.*, 1971). The total methane production rate may have been underestimated by these studies because the loss of methane from the sediments by diffusion was not measured. This exclusion may have been due to a belief that the low solubility of methane in water made diffusion unimportant, especially since a low solubility means a low critical concentration for bubble formation.

A reliable estimate of methane diffusion depends upon a reliable estimate for the coefficient of eddy diffusivity and a measurable gradient. The value for the coefficient varies inversely with stability (Mortimer, 1941) and the thermocline region is the most stable portion of the lake. Below the thermocline stability decreases with increasing depth and thus, the coefficient of eddy diffusivity should increase with depth. The value for A at 4 m obtained from Duong's (1972) 1971 conductivity data was less than the other values for A (Table 2), which were calculated from the 5 m depth in 1972. The 4 m value for A was included to establish that A increased with depth.

The value for A that was obtained from the 1972 dissolved methane data (Table 2) is underestimated because methane is not a conservative property of the water (Mortimer, 1941). To calculate this coefficient of eddy diffusivity, the increase in methane above the 5 m plane had to be determined. This increase in methane (11.1 mmoles/m^2/day) is the minimum methane diffusion rate because it is exclusive of the methane that was oxidized. The value for A obtained from the methane data can be considered the minimum value for A. But the value for A that was calculated by Mortimer's (1941) method from the temperature data (Table 2) is smaller. Mortimer also noticed this fact for his values of A obtained from temperature data and attributed it to the uptake of heat by the sediment.

The coefficient of eddy diffusivity for Wintergreen Lake that was calculated by Hutchinson's (1941) procedure is the only coefficient that is greater than the minimum value for A calculated from the methane data. The assumption that the rate of temperature change with depth is exponential must be valid if this coefficient can be used in calculating the rate of methane diffusion. Hypolimnetic values for the temperature term of McEwen's (1929, cited in Hutchinson, 1941, 1957) expression were plotted logarithmically against depth and fell on a straight line, justifying the use of McEwen's equations in the calculation of the coefficient of eddy diffusivity.

Hutchinson (1941) obtained values for A of 2.1 x 10^{-3} cm^2/sec for the clinolimnion of Linsley Pond and 3.0 x 10^{-3}

cm^2/sec for Lake Quassapaug. Both of these lakes are deeper than Wintergreen Lake. Mortimer (1941) and Hutchinson (1957) noted that the larger and deeper lakes had a larger coefficient of eddy diffusivity. But the Wintergreen Lake value for A of 4.3×10^{-3} cm^2/sec is larger than the values for Linsley Pond and Lake Quassapaug. Several other quite small lakes also do not fit this pattern. Newcombe and Dwyer (1949) calculated a value for A of 5.7×10^{-3} cm^2/sec for Sodon Lake, Michigan. This lake is smaller than Wintergreen Lake (0.023 km^2 versus 0.15 km^2) and although it is deeper, Sodon Lake is meromictic and has a 2 m thermocline. Newcombe and Dwyer (1949) also stated that the lake was relatively sheltered from direct winds. These facts would lead to the conclusion that their A should be smaller than the coefficient in larger lakes. For Abbot's Pool, which has an area of approximately 0.004 km^2, and a maximum depth of 3.5 to 4.0 m, Happey (1970a) calculated the mean coefficient of eddy diffusivity to be a very large 115.0×10^{-3} cm^2/sec. This value for A was calculated by Hutchinson's (1941) procedure. The coefficient of eddy diffusivity was also calculated using Mortimer's (1941) method as applied to phosphate, silicate and ammonia data. These values of A for Abbot's Pool were 18.3×10^{-3}, 18.3×10^{-3} and 7.4×10^{-3} cm^2/sec (Happey, 1970b).

The rates of diffusion of dissolved methane from the sediment into the overlying water could not be calculated because it was not possible to determine either the coefficient of eddy diffusivity or the concentration gradient for this interface. The rate that was determined (40.2 mmoles/m^2/day) was for the 5 m depth, but the methane flux through both this depth and the sediment interface should be similar as long as no methane is produced or oxidized in between.

Supersaturation of the water at 5.5 m with methane apparently occurred over the approximate period of July 28 to September 24, 1972 (Table 3). The period of possible bubble formation was longer, extending from July 17 to near the end of September. Again, these data are not for the sediment but for the water column. Methane concentrations in the sediment are most certainly higher, and in fact, bubbles were collected from late May to the fall overturn. The possibility of bubble formation in the water column complicated the interpretation of the vertical distribution of dissolved methane.

The differences between expected and observed percent volume of nitrogen and methane in bubbles collected at the 5 m depth (Table 4) could be the result of a false assumption. The theoretical expected percent volume assumes nitrogen is at approximate saturation. In his study on the vertical distribution of gases in Chesapeake Bay sediments, Reeburgh (1969) attributed the decreasing nitrogen and argon concentrations with depth to a gas stripping action of methane bubbles. Kuznetsov (1959) attributes a similar sharp decrease in dissolved nitrogen just above the sediment of Lake Beloje in the winter to nitrogen fixation. Unfortunately dissolved nitrogen was not measured in Wintergreen Lake, but it is probable that the observed percent volumes are due to dissolved nitrogen not being at saturation levels in the sediment.

The gas composition of bubbles collected at 1 m was not the same as the gas composition of bubbles collected at 5 m (Table 4 and Figure 6). Wyman *et al.* (1952) investigated the disappearance of bubbles in seawater and found that the rate of solution of gases in the bubbles decreased with decreasing pressure. This implies that less and less gas will diffuse out of a bubble as it rises from the sediment to the surface, with the bubble undergoing a decrease in hydrostatic pressure along the way. The rate of solution of a bubble was also found to decrease with time (Wyman *et al.*, 1952). But the main factor influencing the rate of solution of gases in a bubble was the concentration gradient of the gas in the water surrounding the bubble. As a bubble in Wintergreen Lake rises to the surface, it goes from an area nearly saturated with dissolved methane and possibly low in dissolved nitrogen to an area nearly saturated in dissolved nitrogen and very low in dissolved methane (Figure 5). The observed differences in percent volume between bubbles collected at 1 m and 5 m could be due to the diffusion of methane out of the bubble and nitrogen into the bubble as it goes from the sediment towards the lake surface.

No loss of methane was observed from the funnels which contained a known amount of a standard methane mixture and placed in the 55-gallon drum. However, this experimental system was not an open one. The water under a funnel suspended in a lake would have higher diffusion coefficients than the water under a funnel suspended in a barrel. The possible presence of methane-oxidizing bacteria in the dissolved methane-dissolved oxygen gradient under the 1 m funnels cannot be excluded as a cause of the observed differences.

The percent volume of methane in bubbles collected at the 5 m depth of Wintergreen Lake ranged from 55 to 93% and from 6 to 36% for nitrogen. The percent volume of the gases in bubbles collected 0.5 to 1.0 m above the bottom (13 m) of Lake Beloje ranged from 74.1 to 87.2% methane, 0.2 to 19.0% nitrogen and 5.1 to 18.4% hydrogen (Rossolimo, 1935). The maximum amount of carbon dioxide detected was 2.9% but it was usually less than 1.5%. No increasing or decreasing trends of the three major gases were evident. Bubbles collected 15 cm above the bottom (4 m) of Lake Erie during the months of August and September were found to have a composition of 95% methane, 3% nitrogen and 2% carbon dioxide (Howard *et al.*, 1971). The composition was the same whether the collection period was 10 min, 6 hr, or 5 days. The bubbles collected in Wintergreen Lake were not analyzed for hydrogen. Carbon dioxide was present, but in such low quantities that an estimate was not attempted.

During the first half of October the amounts of methane that were collected at 1 m and 5 m were nearly the same (Figure 6). The nitrogen percent volume of the bubbles collected at 5 m showed an increase in this period, while the methane percent volume at this depth showed a decrease

(Table 4). The lake was turning over at this time so the funnels at both depths would experience similar physical and chemical conditions. The possibility of diffusion of methane out of the funnels and the presence of methane-oxidizing bacteria under the funnels would be the same at both depths.

The maximum amount of methane leaving the Wintergreen sediment through ebullition took place in August and September (Figure 6) and corresponded to the rapid increase in dissolved methane in the hypolimnion over the same period (Figure 4). The daily rate of methane leaving the sediment by ebullition barely exceeded 35 mmoles/m^2 during this maximum and averaged 21.1 mmoles/m^2/day over the period of May 30 to August 21, 1972. Rossolimo (1935) collected 443 ml of gas/m^2/day in May. The gas was 78.5% methane, so the rate of methane leaving the sediment was 15.5 mmoles/m^2/day. Gas volumes were measured by Rossolimo throughout the summer and reached 1420 ml/m^2/day in July, but unfortunately the gas composition of these bubbles was not determined. Rates of gas evolution from the sediments of Lake Erie were determined by Howard *et al.* (1971) to be 1.8 cc/min/cm^2 and the rate of methane production 1.71 cc/min/m^2 or 110 mmoles/day/m^2.

Attempts at determining the amount of methane leaving the sediments by diffusion were not made in the studies by Rossolimo (1935) and Howard *et al.* (1971). This omission could considerably underestimate the total amount of methane produced. The diffusion rate of methane at 5 m in Wintergreen

Lake was 40.2 mmoles/m^2/day, which amounts to 191% of the total methane leaving the sediment by ebullition. Even the minimum methane diffusion rate of 11.1 mmoles/m^2/day determined from the increase in methane exclusive of oxidized methane makes diffusion an important process in ridding the sediment of organic carbon.

The total net productivity in Wintergreen Lake in 1971 was estimated by Duong (1972), for the period of June 21 to August 24, to be 2190 mg C/m^2/day. This figure includes net primary productivity, dark fixation and excretion. The amount of this carbon reaching the sediment was not determined. For approximately the same period in 1972, 250 mg C/m^2/day left the sediment by ebullition and 480 mg C/m^2/day left by diffusion, for a total of 730 mg C/m^2/day leaving the sediments. The remaining 1460 mg C/m^2/day represents the sum of that carbon which was mineralized in the water column plus refractile organic carbon such as humus and lignin that was left to accumulate in the sediment. Using the ebullition data from the 5 m funnels it can be estimated that less than 11.5% of the total carbon produced left the lake's carbon cycle and entered the atmosphere as methane. The remainder was either recycled, lost to the sediment, or converted to carbon dioxide. The above estimates for only one season probably underestimate the role of methane production in an annual cycle since methane production would be expected to exceed organic matter production during the winter months.

> "The measurement of the quantitative nature and flux of *in situ* activities is perhaps the most important problem in microbial ecology."
>
> (Wiebe in Odum, 1971)

> "The rates of exchanges or transfers from one place to another are more important in determining the structure and function of an ecosystem than the amounts present at any one time in any one place. Cycling rates as well as standing states must be quantitated."
>
> (Odum, 1971)

This study has attempted to measure the quantitative nature and flux of the *in situ* activity of the methane-producing bacteria.

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