Hydrobiologia **210**: 191–201, 1991.

Methane production in meromictic Ace Lake, Antarctica

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Received 18 October 1989; in revised form 18 October 1989; accepted 20 April 1990

Key words: methane, methanogenesis, meromictic lake, Antarctica

Abstract

Methane occurred in the monimolimnion, at depths greater than 11 m, of an antarctic meromictic lake, Ace Lake (depth 24.7 m). Although the water of the lake was of approximate marine salinity, bottom waters were depleted in sulfate (less than 1 mmol l^{-1}). The temperature of the bottom waters of the lake were constantly between 1 °C and 2 °C. Rates of methanogenesis from ^{14}C-labelled precursors (bicarbonate, formate and acetate) were determined in time course experiments with the detection of $^{14}CH_4$ produced by a gas chromatography-gas proportional counting system. Rates of $^{14}CH_4$ production were difficult to determine as the reactions were always near our limit of detection.

Reliable determinations of rates of methanogenesis at some depths using some precursors were obtained, the fastest rate being 2.5 μmol kg^{-1} day^{-1} at depth 20 m. Assuming constant rates of methanogenesis with time, this would equate to a turnover of methane in the lake every two years.

The slow rate of methanogenesis suggests that the methanogens in Ace Lake may be working at well below their optimum temperature although definitive statements regarding the presence of psychrophilic methanogens in this antarctic lake must await isolation attempts or longer field studies using alternative methodologies.

Introduction

Ace Lake (68° 24′ S, 78° 11′ E) is a meromictic lake in the Vestfold Hills, Antarctica (Burton, 1980). The lake has a maximal depth of 24.7 m and is covered by ice for greater than 9 months of each year (Burton & Barker, 1979). The ratios of the concentrations of major ions (except for sulfate concentrations (Burton & Barker, 1978)) in water drawn from Ace Lake are similar to the ratios found in sea water (Burton, 1981) but Ace Lake exhibits a density stratification due to salinity ranging from about 6‰ at the surface to 43‰ in its bottom waters (P. P. Deprez, Personal Communication). Total S is about 24% of that found in seawater, the S is considered to have been lost through sulfate reduction in earlier phases of meromixis and lake mixing in the development of the lake prior to the establishment of the current meromixis (Burton & Barker, 1978) as current sulfate in the surface waters is highly enriched in ^{34}S (+41.2‰, Burton & Barker, 1978) when compared with seawater (+20.3‰ to +20.7‰, Nakai & Jensen, 1967). Although

chlorosity of the bottom water is high, 24.5‰, sulfate in the bottom water has been almost totally reduced to sulfide (Burton & Barker, 1979) and current sulfate reduction rates were very slow and below the limit of detection (Franzmann *et al.*, 1988). Stable water temperatures at depths below the oxycline in Ace Lake over a period of 12 months show that no mixing occurs below a depth of 10 m although mixing does occur in the surface 10 m (Hand & Burton, 1981). The presence of a large pool of sulfide in the monimolimnion (where there is little sulfate for its replacement if lost to the system) and the constant mean ^{34}S values for total sulfur-containing chemical species throughout the monimolimnion indicate that the monimolimnion is a closed system with respect to sulfur and very little mixing occurs between depths within the monimolimnion and between the monimolimnion and the mixolimnion (Barker & Burton, 1979).

Methane is present in the bottom waters at saturated concentrations (Burton, 1980). Methane is produced in anoxic marine sediments by methanogenic bacteria. The majority of known species of methanogenic bacteria obtain their energy from acetate or from the reduction of CO_2 by molecular hydrogen or formate (Boone & Whitman, 1988). In some environments the methanogens obtain hydrogen for the reduction of CO_2 from acetate and higher fatty acids through interspecies hydrogen transfer (Conrad *et al.*, 1985; Bryant *et al.*, 1977; Zinder & Koch, 1984; Ahring & Westermann, 1987). Trimethylamine (King *et al.*, 1983) or dimethyl sulfide (Kiene *et al.*, 1986) can act as precursors for methanogenesis. Where sulfate is depleted in marine environments, such as occurs in Ace Lake (Burton, 1980), methanogenesis usually occurs through CO_2 reduction (Claypool & Kaplan, 1974). After the exhaustion of sulfate in lake sediments CO_2 is the next favorable electron acceptor remaining for the oxidation of organic matter (Hanselmann, 1986).

The temperatures of the bottom waters in which methane concentrations are highest (Burton, 1980) and in which sulfate concentrations would limit sulfate reduction (Franzmann *et al.*, 1988) never exceed 5 °C (Hand & Burton, 1981). Ace Lake represents a stable environment where psychrophilic methanogens, which as far as the authors are aware have hitherto not been reported, could potentially develop. Antarctic bacteria with a reduced optimum and minimum temperature for growth when compared with tropical and temperate taxonomic counterparts have been reported, although many antarctic bacteria inhabit environments with *in situ* temperatures well below their optima (McMeekin & Franzmann, 1988). Rates of methanogenesis have not been reported for environments on continental Antarctica, although rates have been published for some of the freshwater lakes of Signey Island in maritime Antarctica (Ellis-Evans, 1984).

Ace Lake was visited in the summer of 1987–88 and rates of methanogenesis at near *in situ* temperatures were determined by radiometric means. In addition, other physico-chemical parameters were measured in Ace Lake in order to determine their effects on the rates determined. This study is herein reported.

Materials and methods

Water samples were collected at 1 m intervals with a 2 litre Kemmerer bottle through a 22.5 cm diameter whole drilled through the ice. Sediment samples were collected with a remotely activated sampler (King & Everitt, 1980). Samples taken for gas analyses were over-flowed into ground-glass stoppered Winkler titration bottles. Samples were flown by helicopter from the sample site to the laboratory at Davis station.

Physico-chemical parameters

In situ temperature was measured with a Yeo-Mal Model 606 conductivity and temperature detector (CSIRO). Salinity ($\pm$ 1‰) was measured with a hand refractometer (ATAGO, Japan). Eh and pH were measured in the laboratory by electrode. The following parameters were measured as cited:

sulfate concentration (Tabatabai, 1974), sulfide concentration (Ellman, 1959) and oxygen concentration (Strickland & Parsons, 1972). Samples collected for methane and CO_2 concentration determinations had 5.0 ml of 2.0% $CdCl_2$ (to precipitate sulfide) added to the Winkler bottles prior to sealing at the sample site. Methane was extracted from water samples by the syringe technique of Martens & Val Klump (1980) and CO_2 was extracted from water and sediment samples by the method of Culbertson *et al.* (1981). Methane and CO_2 were quantified by gas chromatography (12′ Haysep Q® column; column isothermal at 50 °C; detection by thermal conductivity with detector temperature 200 °C and a current of 250 mA, helium carrier gas flow rate 30 ml min^{-1}, Varian 3700 gas chromatograph). The concentrations of low molecular weight volatile fatty acids in water and sediment porewaters (collected by centrifugation of sediments) were determined by ion exchange chromatography (Burton & Xu Lu-Qiang). Pore water content in sediments was determined by the method of Skyring *et al.* (1983).

Radiometric experiments

Methane production rates were estimated by the measurement of $^{14}CH_4$ formed from Na $H^{14}CO_3$, [^{14}C]-formate and [^{14}C-2]-acetate in time course experiments using gas chromatography-gas proportional counting (GC-GPC) procedures (Nelson & Zeikus, 1974).

Prior to the collection of water samples 120 ml serum bottles were crimp-sealed with butyl-rubber septa and flushed with high purity nitrogen. Immediately after a water sample was obtained from a desired depth, a subsample of 20 ml was collected in a syringe and injected into a prepared bottle through the septum into which a bleed needle was also inserted to allow the escape of displaced nitrogen. For sediment samples, about 20 g of sediment was spooned into 120 ml serum bottles on the lake ice surface; 20 g samples were never accurately obtained in the field. The bottles were immediately crimp-sealed and the headspace above the mud was flushed for 5 min with high-purity nitrogen. All bottles were stored in insulated containers during transport to the laboratory to prevent temperature fluctuations. At the laboratory, samples were injected through the septum with the appropriate radioisotope solution. Triplicate analyses were performed at each depth for water and sediment samples.

Radioisotopes were diluted in a degassed and sterile 0.002% resazurin solution, with pH adjusted to 9.3, so that inoculation of a sample with 0.1 ml of radioisotope solution delivered either one of the following: 16 μCi $NaH^{14}CO_3$ (53.0 mCi/mmol, Amersham), 3.2 μCi [^{14}C] sodium formate (56 mCi/ml, Amersham), or 3.2 μCi [2-^{14}C] sodium acetate (57 mCi/ml, Amersham). After addition of the radioisotope each sample was shaken vigorously for 10 seconds and incubated at 1 °C; the temperature of the bottom water of Ace Lake as reported by Hand (1980). Initially, 0.5 ml of the headspace was sampled every 24 hr and tested for [^{14}C] methane by GC-GPC, but due to low activity, this was later changed to every 5 days.

The GC-GPC counting system employed the gas chromatography system previously mentioned. Radioactivity in the gas was measured with a Canberra Industries (Connecticut, USA) GPC attached to the temperature conductivity detector with no oxidation step (Culbertson *et al.*, 1981). The signal was processed by a CI-10B integrator (LCD/Milton Roy, Florida, USA). The linear relationship between peak area and DPM was determined using $^{14}CO_2$ standards made by preparing different dilutions of $H^{14}CO_3$ (Culbertson *et al.*, 1981) in sealed serum bottles and acidifying the samples with 6 N HCl. For each standard, 0.5 ml was injected into the GC-GPC system and another 0.5 ml was injected into a scintillation vial through a septum seal and which contained 0.5 ml ethanolamine. After the vial containing ethanolamine was left at room temperature for 18 hr to absorb the $^{14}CO_2$, 1.5 ml ethanol and 5.0 ml of cocktail (Instagel, Packard) was added and the DPM determined by the channels ratio method in a 1215 Rackbeta II scintillation counter (LKB). The limit of detection

for the GPC was 160 DPM. Given the sample and container volumes and the time period over which the experiment could be maintained during our visit to Antarctica (16 or 24 days depending on date of sample collection) and the detection limit of 160 DPM, the limit of detection of $^{14}CH_4$ production was between *ca.* 0.3 nM kg^{-1} day^{-1} and *ca.* 0.7 nM kg^{-1} day^{-1} depending on which isotope was used, the incubation time and the accurate sample weight. Samples which had been autoclaved at 121 °C for 20 min prior to the addition of isotopes were used as controls.

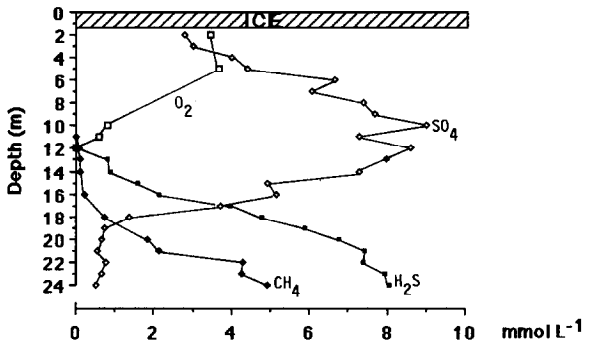

Fig. 1. Profiles of the concentrations of oxygen $\times 10^1$ (□), sulfate (◇), sulfide (■), and methane (◆) with depth in Ace Lake, December 1987.

Results

Since 1979, the maximum depth of Ace Lake has increased from 23 m (Burton, 1980) to 24.7 m. The thickness of the ice cover was 1.7 m at the time of our first field trip (12 December, 1987) and this had decreased to 1.0 m by our last (31 January, 1988) by which time the ice plug was surrounded by a moat of free water. The physico-chemical parameters measured through the water column are presented in Table 1 and Fig. 1. The lake was stratified with respect to salinity ranging from about 15% of seawater salinity at the surface to 120% of seawater salinity for bottom waters. The salinity stratification was reinforced with a temperature stratification below 10 m (Table 1). Oxygen was present in the upper 11 meters but was not detected below that depth (Fig. 1). Total CO_2 concentration increased with depth (Table 1). Sediment, at the surface and at a depth of 12 cm, contained total CO_2 concentrations of 70.13 and 67.41 mmol kg^{-1} wet weight respectively. Through the first 12 cm depth the sediment was a greenish black paste with a porewater content of 74.9% at the surface and 73.3% at 12 cm sediment depth. Hydrogen sulfide was present in the water column at a depth of 12 m and its presence coincided with a redox shift from +279 mV at 11 m to −25 mV at 12 m (Table 1). The concentration of hydrogen sulfide increased with depth to the bottom where a maximum concentration of 8 mmol l^{-1} was reached. Sulfate concentration and salinity increased with depth through the mixolimnion (Fig. 1, Table 1) but sul-

Table 1. Physical and chemical data for Ace Lake.

Depth m	Temp. °C	Methane mmol l^{-1}	Eh	Salinity ‰	Total CO_2 mmol l^{-1}	Formate mmol l^{-1}	Acetate mmol l^{-1}	Propionate mmol l^{-1}	Butyrate mmol l^{-1}
2	0.2	BLD[a]	+287	5	4.99	ND[b]	ND	ND	ND
5	4.8	BLD	+283	14	7.44	ND	ND	ND	ND
10	11.4	BLD	+280	30	ND	ND	ND	ND	ND
11	9.3	0.02	+279	30	ND	ND	ND	ND	ND
12	8.4	0.06	− 25	30	13.41	0.07	BLD	BLD	BLD
17	4.5	ND	−176	32	21.16	0.06	0.12	BLD	BLD
20	3.0	1.84	−167	37	47.56	BLD	BLD	BLD	BLD
22	2.3	4.29	−167	40	55.31	0.21	0.32	0.19	0.11
24	1.9	4.93	−167	42	49.82	0.45	0.61	0.33	0.33

[a] BLD, Below limits of detection.
[b] ND, Not determined.

fate concentration decreased steadily below a depth of 12 m (8.6 mmol l^{-1}) to a depth of 19 m (0.7 mmol l^{-1}) (Fig. 1). Below a depth of 19 m the decrease in sulfate concentration with depth was less pronounced and the concentration dropped to 0.5 mmol l^{-1} at 24 m depth. Throughout 1984, sediment porewaters had been found to contain 0.04 to 0.00 mmol sulfate kg^{-1} wet weight (unpublished data).

Methane concentration was below the limit of detection (0.9 μmol l^{-1}) at a depth of 10 m but peaked in the bottom waters at 4.9 mmol l^{-1}. Above depth 16 m inclusive, the concentration of methane versus depth can be fitted to a straight line; depth (m) = 10.530 + 1.145 mmol CH_4 l^{-1} (R^2 = 1.0). At 18 m and below, a plot of methane concentration versus depth does not fit a straight line equation as closely; depth (m) = 17.585 − 0.055 mmol CH_4 l^{-1} (R^2 = 0.92). The relationship between depth (below 18 m) and methane concentration is better described by the trinomial equation; depth (m) = 14.758 + 5.404 (mmol CH_4 l^{-1}) − 1.631 (mmol CH_4 l^{-1})^2 + 0.185 (mmol CH_4 l^{-1})^3 (R^2 = 0.96). This curve is concave down for depths below 20 m and concave up for depths 18 m to 20 m.

Volatile organic acids were present in the monimolimnion, generally increasing in concentration with depth (Table 1). The concentrations of organic acids at a depth of 12 cm and at the sediment surface were respectively (in mmol kg^{-1} wet weight): formate, 2.21, 3.66; acetate, 2.69, 5.09; propionate, 2.44, 4.16; butyrate, 4.18, 1.75.

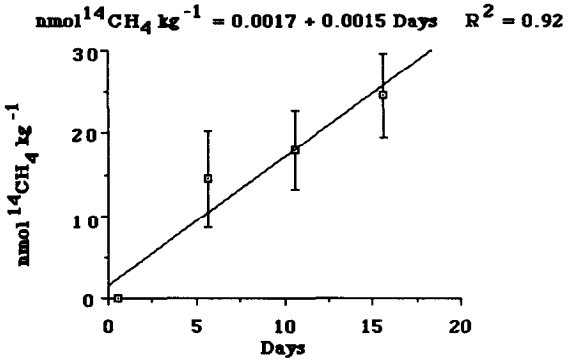

Fig. 2. Production of $^{14}CH_4$ per kg of anoxic water collected from the monimolimnion of Ace Lake at a depth of 7 m and inoculated with [^{14}C] formate. Results represent the means of three samples with bars indicating ± standard error.

Rates of production of $^{14}CH_4$ from different radioisotope precursors ($NaH^{14}CO_3$, [^{14}C] formate, and [2-^{14}C] acetate at different depths within the water column and sediment are given in Table 2. Most of the rates determined are at the approximate limit of detection for the experiments conducted (between *ca.* 0.7 and *ca.* 0.3 mmol kg^{-1} day^{-1}) and many of the data gave a poor fit to straight line correlations of methane produced per kg versus time. Many correlations had probability values greater than 0.05, an indication of the

Table 2. Rates of $^{14}CH_4$ produced from the radioisotopes, $NaH^{14}CO_3$, [^{14}C] formate and [2-^{14}C] acetate, in triplicate samples from the sediments and monimolimnion of Ace Lake, with incubation at 1 °C for 24 days. Probability values (p) are given for the regression of a fitted straight line of $^{14}CH_4$ production versus time.

Sample	$^{14}CH_4$ production rate nmol kg^{-1} day^{-1} from:		
	$NaH^{14}CO_3$	[^{14}C] Formate	[2-^{14}C] Acetate
Water, 12 m depth[a]	–[b]	–	–
Water, 17 m depth	0.5 ($p > 0.25$)	15.0 ($0.0001 < p \leq 0.005$)	0.6 ($p > 0.25$)
Water, 20 m depth	1.0 ($p < 0.005$)	0.6 ($0.1 < p \leq 0.25$)	0.8 ($p \leq 0.1$)
Water, 22 m depth	0.6 ($p \leq 0.025$)	0.9 ($0.005 < p \leq 0.01$)	0.1 ($p > 0.25$)
Water, 24 m depth	0.6 ($p \leq 0.25$)	0.9 ($0.1 < p < 0.25$)	1.3 ($p \leq 0.025$)
Sediment, surface	–	0.1 ($p > 0.25$)	0.8 ($p > 0.25$)
Sediment, 12 cm depth	0.2 ($0.1 < p < 0.25$)	–	0.3 ($p = 0.005$)

[a] All sample from 12 m depth turned red on incubation due to oxidation of reazurin.
[b] – = did not show production of $^{14}CH_4$ with time.

variance within data obtained from working at or near the limit of detection. An example of one of the better correlations obtained is given in Fig. 2.

Of the radioisotopic precursors and samples used, formate showed the highest production rates at 15.0 nmol kg^{-1} day^{-1} in water drawn from a depth of 17 m. The specific activity of the labelled formate used at this depth was high as the concentration of the natural formate pool was only 0.06 mmol l^{-1}. Rates of production of labelled methane from labelled bicarbonate were very low and the data were generally unreliable ($P > 0.05$). A combination of natural slow rates of methanogenesis in an environment with a large pool of CO_2 would be expected to result in poor reduction of $^{14}CO_2$ to [^{14}C] methane. Methane production rates, determined from the [^{14}C] methane production rates and specific activity of radioisotopes, were calculated for experiments in which the correlation of [^{14}C] methane produced and time gave p values ≤ 0.05. This occurred for five of the determined rates, and the rates for methanogenesis for these sites were: water from 20 m, 2500 nmol kg^{-1} day^{-1} from total CO_2; water from 22 m, 1758 nmol kg^{-1} day^{-1} from total CO_2; water from 17 m, 367 nmol kg^{-1} day^{-1} from formate; water from 24 m, 122 nmol kg^{-1} day^{-1} from acetate; sediment from a depth of 12 cm, 459 nmol kg^{-1} day^{-1} from acetate. Although rates of similar magnitudes may occur in other regions of the monimolimnion, unreliable radioisotope methane production data makes its calculation inconsequential. The fastest measure of methanogenesis recorded was 2.5 μmol kg^{-1} day^{-1} (= 56 μl of methane at STP kg^{-1} day^{-1}) from total CO_2 at 20 m depth. The concentration of methane at that depth is 1.84 mmol l^{-1}. If all methane at the depth originated from production at that same depth the methane pool would turn over every two years.

Gas was not observed bubbling at the surface of the lake through our holes in the ice on any of our visits. When the sediments were disturbed by sediment sampling a quantity of gas bubbles reached the surface, an indication that gas pockets were trapped within the sediment matrix. The percentage methane in the gas was not determined.

Discussion

The waters of Ace Lake are extremely stable. The lake is not affected by currents, and the ice cover, which is present for at least 9 months of the year (and in some years for 12 months (Hand, 1980), shelters the lake from wind induced turbulence in the water column. The water column exhibits increasing density with depth, due to increasing salt concentration (Table 1). This density increase below the chemocline is reinforced by a temperature stratification in the summer period (Table 1) which is the only period in which the ice cover can be absent. The temperature data of Hand (1980) showed there was virtually no eddy diffusion in the monimolimnion of Ace Lake; the movement of ions and heat is largely restricted to molecular diffusion as occurs in some other antarctic lakes (Yusa, 1979). This observation for Ace Lake was also suggested by the data of Burton & Barker (1979).

Ace Lake has several vertical zones which can be defined by the electron acceptors available for bacterial respiration. Zonation of electron acceptors in marine systems is usually limited to sediments as the water column is well mixed and oxygen is dispersed throughout it. However, due to the stagnation of the bottom waters of Ace Lake and the activities of the microbiota within them, oxygen has been completely consumed from the monimolimnion (Fig. 1) and the sulfate concentration has been depleted to a level that is rate limiting to further sulfate reduction; < 1 mmol l^{-1} as determined in pure cultures of sulfate reducers (Postgate, 1951). The accumulation of hydrogen sulfide, the endproduct from sulfate reduction, produces a drop in Eh to -167 in the monimolimnion (Table 1). Respiration by sulfate reduction is energetically more favorable than methanogenesis (King, 1984) although very slow rates of methanogenesis can still occur in marine sediments with sulfate concentrations of 15 mmol l^{-1} (Senior *et al.*, 1982). Where sulfate is not rate limiting (at depths above 19 m in Ace Lake, Fig. 1) the sulfate reducing bacteria may outcompete the methanogens for substrates.

Within the monimolimnion the concentration

of organic acids reach minimal concentrations at 20 m depth (Table 1); the depth at which the reliable measurements of the rate of methanogenesis using CO_2 was greatest. The concentration of organic acids in the water column and sediment are very high when compared with concentrations determined in other lakes (usually in the range 20–70 μmole l^{-1}; Jones *et al.*, 1982; Conrad *et al.*, 1986). Ace Lake is of marine salinity but lacks sulphate in its sediments. In the absence of sulfate, marine sediments accumulate CH_4 and acetate and propionate concentrations can reach as high as 2 mM and 0.5 mM respectively (Sorensen *et al.*, 1981). The high concentrations of total lipids in Ace Lake sediments (20 mg g^{-1}; Volkman *et al.*, 1988) would be expected to be reflected in high concentrations of volatile fatty acids as 'high concentrations of long-chain fatty acids are generally paralleled by high concentrations of the product acetate' (Conrad *et al.*, 1986). Hydrogen gas was not detected in Ace Lake (Burton, 1980). Hydrogen is usually not detected in anaerobic lake environments by gas chromatography as, for thermodynamic reasons, anaerobic degeneration of organic acids will not occur unless the hydrogen partial pressure is very low (Conrad *et al.*, 1986).

As a result of the detection limits in the methodology used, and the distribution of sulfate and total carbonate in the lake, only two of the depths gave reliable readings for the production of $^{14}CH_4$ from $NaH^{14}CO_3$ ($p \leq 0.05$, Table 2). For depths above 20 m, the sulfate reducers would outcompete methanogens for substrates, the concentration of sulfate being greater than 1.0 mmol l^{-1}. If low rates of methanogenesis occurred at 17 m from the reduction of CO_2, they would not have been measured due to the high concentration of total carbonate. At a depth of 17 m, methanogenesis utilizing formate as a precursor was measured due to the much higher specific activity of this radioisotope in a natural formate pool of low concentration (0.06 mmol l^{-1}, Table 1). At 20 and 22 m depths, sulfate reduction was substrate limited, and values of 2.5 and 1.8 μmol l^{-1} day^{-1} was measured using radiolabelled carbonate.

Higher values would be expected in the sediments where organic matter accumulates. In the sediment more substrate would be available for methanogenesis from the fermentation of accumulated organic matter and subsequent hydrogen transfer from fermentative and acetogenic bacteria. Methanogenesis from H_2/CO_2 would not be measured in sediments with any reliability, because of the extremely high concentration of the natural pool of total carbonate; about 70 mmol kg^{-1} sediment wet weight. King (1984) noted the difficulties of radioisotope tracer experiments in environments which contained high concentrations of the precursor, which can give negative results at low activities.

Table 3. Rates of methanogenesis in anaerobic aquatic environments.

Site	Approximate rate μmol kg^{-1} day^{-1}	Conditions	Ref.
Wintergreen Lake	1152–1240[a]	*in situ* temperature	Lovely & Klug, 1987
Wintergreen Lake	580[a]	10 °C	Smith & Klug, 1987
Beltam Tarn	381–552[b]	10 °C	Jones *et al.*, 1982
Lake Mendota	10.5[a]	10 °C	Winfrey *et al.*, 1977
Lake Black Kichier	23	4 °C	Ivanov *et al.*, 1976
Knaack Lake	1.4–18.2[a]	4 °C	Winfrey & Zeikus, 1979
Sombre Lake	135[b]	4–10 °C	Ellis-Evans, 1984
Big Soda Lake	0.002–0.01[a]	12 °C (high sulfate)	Iversen *et al.*, 1987
Saltmarsh	24 x 10^{-6}[a]	*in situ* temperature (high sulfate)	Senior *et al.*, 1982

[a] Assumes 1 ml ≈ 1 g.
[b] Assumes pore water content ≈ 74%, as occurs in Ace Lake.

Reliable measures of methanogenesis ($p < 0.05$) from acetate were determined in bottom water and in sediment at 12 cm depth. Rates at these depths could not be compared to rates of methanogenesis by CO_2 reduction. In sediments, greater viable counts for acetate utilizing methanogenic bacteria usually occur below the hydrogen-oxidizing methanogens (Mah *et al.*, 1977).

From the sites at which methanogenesis was measured in Ace Lake, maximal rates were 2.5 μmol kg^{-1} day^{-1} at 1 °C at depth 20 m. The rate in Ace Lake is slow when compared with rates of methanogenesis from CO_2 reduction in eutrophic lakes of the northern hemisphere, and even the permanently cold Sombre Lake on Signey Is in maritime Antarctica (Table 3). The rate is comparable to the rate determined for the meromictic lake, Knaack Lake (Table 3) but greater than the rates from environments of high sulfate concentrations such as Big Soda Lake and Saltmarsh (Table 3). The differences in rates of methanogenesis between different sites is difficult to interpret given the variability of physicochemical parameters between sites. Nonetheless, Ace Lake would seem to be a productive lake as it has high concentrations of organic acids in the monimolimnion and sediments (Table 1), supports a large population (12200–32100 m^{-3} at 2 m depth; 70–12800 at 10–10.5 m depth) of the copepod *Paralapidocera antarctica*, which has no predators (Bayly & Burton, 1987), and contains 20 mg lipid g^{-1} sediment in the top 5 cm of the sediment (Volkman *et al.*, 1988). Methanogenesis is the route by which carbon is oxidized below the zone of sulfate reduction in the monimolimnion. Why are reduction rates so slow?

Rates of methanogenesis in sediments which reach 23 °C have optimum temperatures between 35 to 42 °C (Zeikus & Winfrey, 1976). The mesophilic population of methanogens from these sediments were still active at 4 °C but at slow production rates (Zeikus & Winfrey, 1976). Methanogenesis in the freshwater and temperature stable lakes (0–6 °C) of antarctic maritime islands also have temperature optima well above (>30 °C) *in situ* temperatures (Ellis-Evans, 1984). Unless psychrophilic methanogens inhabit Ace Lake, and to date no species of psychrophilic methanogens have been described (Boon & Whitman, 1988), the most likely parameter limiting methanogenesis in Ace Lake is temperature.

The potential for interspecies transfer of hydrogen is great as the concentrations of organic acids in the monimolimnion is high. It is possible that acetogenesis is inhibited by the high sulfide concentration in Ace Lake (8 mmol l^{-1}; Fig. 1) as although some methanogenic bacteria can tolerate 10 mmol l^{-1} sulfide (Rajagopals & Daniels, 1986), anaerobic respiration of acetate in lake sediments can be inhibited up to 90% by 10 μg ml^{-1} (0.29 mmol l^{-1}) sulfide (Winfrey & Zeikus, 1977). In Lake Mendota, methanogenesis is inhibited by 3.2 mmol l^{-1} sulfide in the porewater (Winfrey & Zeikus, 1979) and in Knaack Lake, methanogenesis from acetate is inhibited by 5 μg ml^{-1} sulfide (0.15 mmol l^{-1}) (Winfrey & Zeikus, 1979).

Given the high concentration of methane in the bottom waters of Ace Lake (Fig. 1) rates of methanogenesis from precursors other than those tested may occur. Dimethyl sulfide which acts as a substrate for methanogens (Keine *et al.*, 1986) is present in the mixolimnion but only in very low concentrations (1 nmol l^{-1}) in the monimolimnion of Ace Lake (J. Gibson, pers. com.) Trimethylamine can contribute significantly to methane production in marine sediments (King *et al.*, 1983) and in salt marsh sediments (Oremland *et al.*, 1982). Trimethylamine is not utilized by sulfate reducers and production of methane from trimethylamine in sediments can occur in the presence of 18 mmol l^{-1} sulfate. If trimethylamine was a significant precursor of methane in Ace Lake methanogenesis would not necessarily show the requirement for low sulfate concentrations as suggested by Fig. 1. Also, methanogenesis does not occur in other non-sulfate depleted, anoxic lake monimolimnia which have been examined throughout the Vestfold Hills (H.R. Burton, Personal communication).

The distribution of methane concentrations with depth within Ace Lake was best described by two equations (Fig. 3). The concentration versus

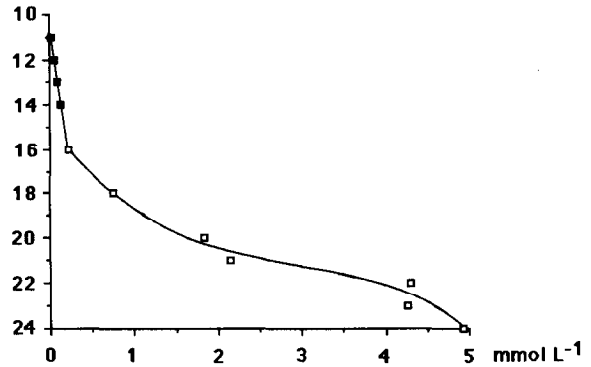

Fig. 3. Regression analysis of the distribution of methane concentrations in Ace Lake; concentration of methane versus depth for depths 16 m to 11 m described by a straight line (Depth = 10.53 + 1.145 $[CH_4]$; $R^2 = 1.0$); concentration of methane concentration versus depth for depths 18 m to 24 m described by a trinomial equation (◇ Depth = 14.76 + 5.04 $[CH_4]^2$ + 0.19 $[CH_4]^3$; $R^2 = 0.96$).

depth from 16 m to the oxycline (11 m) fits a straight line and suggests removal of methane by diffusion without biological production or depletion. A plot of temperature through the same region also gives a straight line (Depth = 22.61 − 1.25 [°C]: $R^2 = 1.0$). The distribution of methane concentrations versus depth from 18 m and below was best represented by a trinomial relationship ($R^2 = 0.96$; Fig. 3). This distribution shows a 'concave up' curve for depths between 16 and 20 m (Fig. 3). A 'concave up' curve suggests a nett removal of gas by oxidative processes. Anaerobic oxidation of methane occurring in zones dominated by sulfate reducing bacteria has been recorded although evidence to date suggests sulfate reducing bacteria are not involved in this process (Zehnder & Brock, 1980; Iversen *et al.*, 1987). The concave up region of the methane distribution curve coincides with a zone of continuing depletion of sulfate with depth (Fig. 1) and stops at about 20 m where the concentration of sulfate becomes rate limiting to sulfate reduction rates. The convex up region of the curve (20 m and below) coincides with maximal measurements for the rates of methanogenesis and confirms that methane is produced in the water column and its presence is not only a result of diffusion from the sediments.

In conclusion, methanogenesis in Ace Lake is slow. The environment is permanently cold but there is no evidence that psychrophilic methanogenic bacteria inhabit the lake. Nonetheless, antarctic methanogens may show lower optima and minima temperatures for growth when compared to their taxonomic counterparts from temperate environments, as has been shown for halobacteria (McMeekin & Franzmann, 1988). Although methane is produced slowly in Ace Lake, the very stable waters of the monimolimnion trap the gas at saturation concentrations.

Acknowledgements

We thank Peter D. Nichols and referees for their comments on the manuscript and Andrew McTaggart for assistance with the determinations of organic acid concentrations. This work was supported by grants from the Antarctic Science Advisory Committee and the Australian Research Council.

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