LIMNOLOGY
AND
OCEANOGRAPHY

July 1987
Volume 32
Number 4

Limnol. Oceanogr., 32(4), 1987, 781–793

Big Soda Lake (Nevada). 1. Pelagic bacterial heterotrophy and biomass[1]

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Abstract

Bacterial activities and abundance were measured seasonally in the water column of meromictic Big Soda Lake which is divided into three chemically distinct zones: aerobic mixolimnion, anaerobic mixolimnion, and anaerobic monimolimnion. Bacterial abundance ranged between 5 and 52 × 10^6 cells ml^{-1}, with highest biomass at the interfaces between these zones: 2–4 mg C $liter^{-1}$ in the photosynthetic bacterial layer (oxycline) and 0.8–2.0 mg C $liter^{-1}$ in the chemocline. Bacterial cell size and morphology also varied with depth: small coccoid cells were dominant in the aerobic mixolimnion, whereas the monimolimnion had a more diverse population that included cocci, rods, and large filaments. Heterotrophic activity was measured by [methyl-^{3}H]thymidine incorporation and [^{14}C]glutamate uptake. Highest uptake rates were at or just below the photosynthetic bacterial layer and were attributable to small (< 1 μm) heterotrophs rather than the larger photosynthetic bacteria. These high rates of heterotrophic uptake were apparently linked with fermentation; rates of other mineralization processes (e.g. sulfate reduction, methanogenesis, denitrification) in the anoxic mixolimnion were insignificant. Heterotrophic activity in the highly reduced monimolimnion was generally much lower than elsewhere in the water column. Therefore, although the monimolimnion contained most of the bacterial abundance and biomass (~60%), most of the cells there were inactive.

Big Soda Lake, Nevada, is a meromictic lake which represents environmental extremes of pH, salinity, and sulfide concentration. It has sharp and predictable vertical gradients in temperature, oxygen, and water density that make it ideal for the study of pelagic microbial processes. In this series of papers we report our seasonal studies of bacterial processes within the chemically stratified water column of this lake. These studies included measures of bacterial abundance and heterotrophic activity, sulfate reduction, methanogenesis, methane oxidation, and sinking losses of seston from the mixolimnion.

Bacterial activity in aquatic environments has been measured by several methods. The assimilation, respiration, and turnover of radioactive organic substrates (usually amino acids and sugars) have often been used as indices of bacterial heterotrophy. However, these methods measure only a fraction of total bacterial production

[1] Bound reprints of this four-paper series can be obtained from R. S. Oremland.

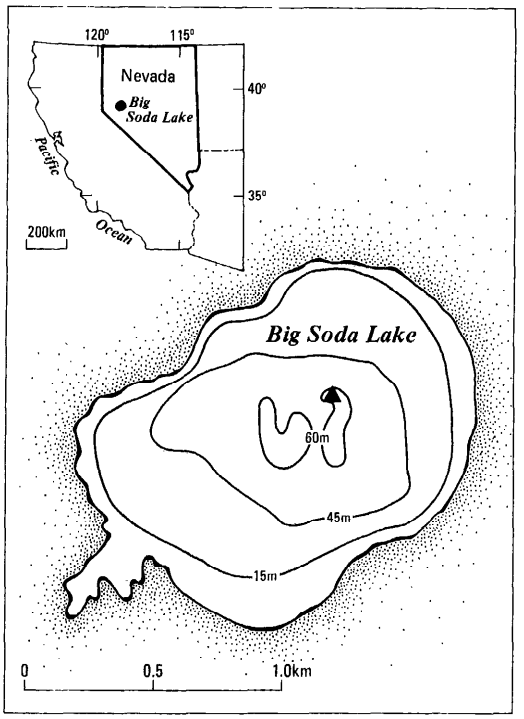

Fig. 1. Location map for Big Soda Lake. Sampling site—▲.

and mineralization. Recently, [methyl-^{3}H]thymidine incorporation has been used to estimate bacterial growth rates (Fuhrman and Azam 1980, 1982; Kirchman et al. 1982; Riemann et al. 1982; Moriarty and Pollard 1981, 1982). Most studies have focused on the assimilation of thymidine in aerobic systems. Recently, Pollard and Moriarty (1984) have shown that some anaerobic bacteria also assimilate thymidine and suggested that the method was applicable to anaerobic systems.

In this study, [^{3}H]thymidine incorporation and [^{14}C]glutamate uptake were used to compare bacterial heterotrophic potential in the aerobic and anaerobic zones of Big Soda Lake and to measure the turnover of a representative amino acid. Results showed that thymidine incorporation and glutamate turnover rates varied between the aerobic and anaerobic zones and that highest rates of incorporation occurred in the anaerobic zone directly below the oxic–anoxic interface. In contrast, extremely low rates were found in the monimolimnion.

This research was supported in part by NSF grant BSR 80-19918 to C. R. Goldman and by the U.S. Geological Survey. Part of this work was conducted while J. Zehr was an NRC-USGS research associate. C. Culbertson, S. Paulsen, and L. Miller provided field support. We thank J. Fuhrman, H. Ducklow, J. T. Hollibaugh, and R. L. Smith for reviewing the manuscript. This series of papers is dedicated to the memory of our friend and colleague Jack Hargis.

Site description

Big Soda Lake is an alkaline, saline, meromictic lake in western Nevada (39°31′N, 118°52′W). It occupies a small volcanic crater with morphometry characterized by a narrow littoral zone and a steep sloping bottom that drops to a central basin with maximum depth of 65 m (Fig. 1). Regional irrigation initiated in 1905 caused a gradual rise of 18 m in the level of this previously hypersaline (TDS = 125 g liter^{-1}: Breese 1968) and holomictic lake. It resulted in a dilution of surface salinity and established a permanent, slowly sinking chemocline (Hutchinson 1937; Kimmel et al. 1978) that is presently at a depth of 34.5 m.

The chemocline is an extremely sharp pycnocline that partitions the water column into two layers having markedly different chemistries (Fig. 2). Major ions are Na^+, Cl^-, SO_4^{2-}, and HCO_3^-. Concentrations of all dissolved constituents (except the divalent cations) are much higher in the monimolimnion. Concentration of total dissolved solids increases from 26 g liter^{-1} in the mixolimnion to 88 g liter^{-1} in the monimolimnion. Alkalinity, as bicarbonate, is sixfold greater below the chemocline than above it, but pH is 9.7 throughout the water column. Sulfate is 58–68 mM, dissolved organic carbon is high (20–60 mg liter^{-1}), and iron concentration is low (0.36 μM: Kharaka et al. 1984). Reduced forms of C, N, and S in the mixolimnion occur at relatively low concentrations and are generally confined below the thermocline. The bottom waters are highly reducing (Priscu et al. 1982) with high concentrations of NH_3 (2.7 mM), reduced sulfur compounds (~14 mM), and elevated methane levels (>50 μM: Oremland and Des Marais 1983; Kharaka et al. 1984).

The mixolimnion has an annual cycle of mixing comparable to a cold monomictic lake, with thermal stratification from spring through autumn and near-complete turnover in winter (Fig. 3). During summer–autumn when the thermocline inhibits vertical mixing, dissolved inorganic nitrogen concentration and phytoplankton biomass are both low in the epilimnion. Dissolved oxygen (DO) disappears at a depth of about 20 m, coincident with the depth of 1% surface irradiation, and a dense layer of purple photosynthetic bacteria is present below the oxycline. During thermal stratification, combined productivity by chemosynthetic bacteria in the oxycline and photosynthetic bacteria (500–800 mg C m^{-2} d^{-1}) exceeds that of phytoplankton (~100 mg C m^{-2} d^{-1}: Cloern et al. 1983*a*,*b*). Phytoplankton productivity appears to be limited by the availability of iron as well as nitrogen (Axler et al. 1978; Priscu et al. 1982). Convective and wind mixing in winter erode the thermocline, and the mixolimnion then becomes nearly isothermal. The winter mixing event redistributes oxygen and ammonia; DO penetrates down to about 30 m and dissolved inorganic nitrogen (DIN) concentration increases to about 15 μM in the surface layer (Fig. 3). During this period phytoplankton biomass increases markedly, but the bacterial layer disappears and autotrophic productivity (~2,800 mg C m^{-2} d^{-1}) is dominated by algal photosynthesis.

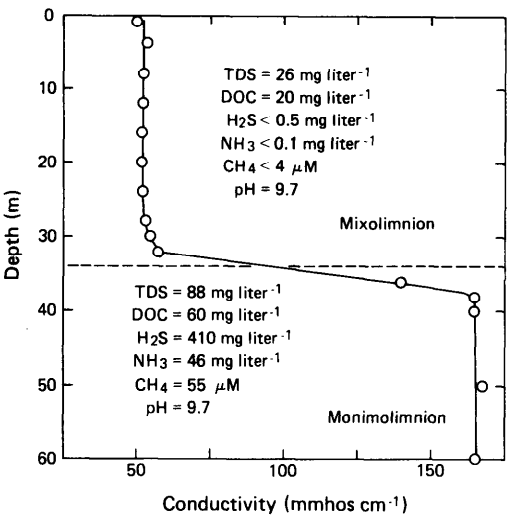

Fig. 2. Depth profile of conductivity in Big Soda Lake and the chemical differences between the mixolimnion and the monimolimnion (data from Cloern et al. 1983*b*; Kharaka et al. 1984).

Methods and materials

Bacterial cell density and biomass—Samples were collected during October 1983, July 1984, October 1984, February 1985, and May 1985 with a 7-liter Niskin bottle. Samples were transferred to sterile 125-ml amber polyethylene bottles (filled completely) and stored on ice until analysis. Samples could be stored this way for up to 48 h without significantly altering counts. Bacterial abundance was determined by acridine orange (AO) direct counting (Hobbie et al. 1977) with epifluorescence microscopy (Harvey et al. 1984). The chemistry of Big Soda Lake necessitated modifications of the AO-staining procedure. They involved isotonic dilution of unfixed samples at pH 9.7, followed by filtration onto black Nuclepore filters (0.2-μm pore size) before AO staining. Organic-AO complexes remaining on the filters were removed by sequential rinses with isotonic 0.1 M citrate (pH 6.6). At least 300 bacteria were counted per sample. A more thorough discussion of the preparative technique is given elsewhere (Harvey 1987).

Bacterial cell size distributions and abundances were used to calculate cell volumes, which were then converted to cell carbon with a conversion factor of 2.2×10^{-13} g C μm^{-3} (Bratbak and Dundas 1984). Dimensions of individual cells were determined from scaled photomicrographs and each bacterium placed into 1 of 12 categories: cocci (diam = 0.2, 0.4, 0.7, 1.0, or 2.0 μm), rods (length = 1.0, 1.5, 2.0, or 3.0 μm), and filaments (length = 5, 10, or 15 μm; width = 0.2 μm). The equations of Palumbo et al. (1984) were used to calculate the width of rods. Spiral-shaped organisms were treated as rods.

Heterotrophic activity—Depth profiles of [^{3}H]thymidine incorporation and [^{14}C]glutamate uptake were obtained in October 1983, July 1984, February 1985, and May 1985. In October 1983, February 1985, and May 1985 incubations were performed at in situ temperatures in ice chests. In July 1984, samples were incubated in situ at their

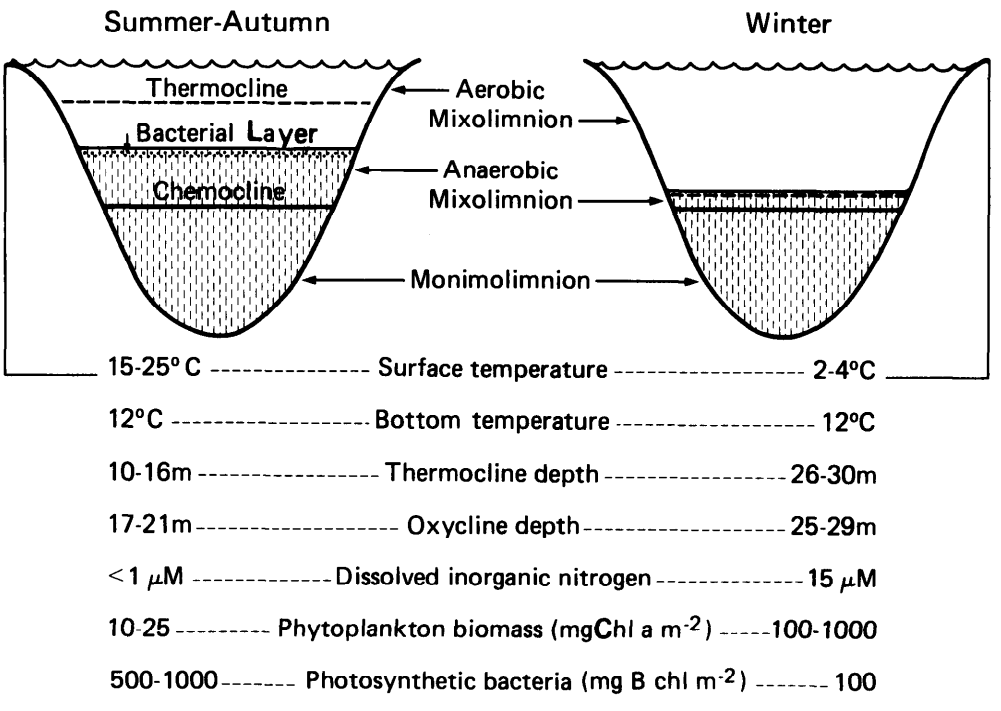

Fig. 3. Seasonal changes in selected physical, chemical, and biological parameters in the mixolimnion of Big Soda Lake (from Cloern et al. 1983*a,b*, 1987; Oremland and Des Marais 1983).

respective depths. Water was collected with an opaque PVC Van Dorn bottle and transferred to sterile plastic or glass syringes. Care was taken to exclude air bubbles. A comparison of incubations with both plastic and glass syringes showed no significant differences between uptake rates. The loaded syringes were capped with plastic needle hubs fitted with rubber septa. Plastic syringes of 10 and 35 ml were used for thymidine incorporation and glutamate uptake experiments. In February 1985, 10-ml glass syringes were used for both glutamate and thymidine experiments.

[Methyl-^{3}H]thymidine (ICN, 50 Ci mmol^{-1}) or uniformly labeled [^{14}C]glutamate (ICN, 225 mCi mmol^{-1}) was added to the syringes. Final concentrations of [^{3}H]thymidine and [^{14}C]glutamate were 20 and 18–29 nM. Glutamate incubations were terminated by filtration onto 0.45-μm Millipore filters. Thymidine incorporation into macromolecules was measured by the cold-trichloroacetic acid (TCA) extraction method of Fuhrman and Azam (1980, 1982). All uptake experiments were corrected for time-zero controls or Formalin kills. On one date (February 1985) thymidine incorporation into both hot- and cold-TCA-insoluble material was measured at 15, 25, and 30 m. The maximum percentage of radioactivity found in hot-TCA-insoluble material was 33% at 25 m.

Radioactivity was determined by standard scintillation techniques with 15 ml of scintillation cocktail. External standard ratios were used for quench correction. Millipore filters were dissolved with 1 ml of ethyl acetate before addition of the scintillation cocktail (Fuhrman and Azam 1982). In October 1983, [^{14}C]glutamate uptake was analyzed with a thin-window Geiger-Mueller counter.

Thymidine incorporation rates were calculated by assuming that the isotope concentration (20 nM) was much higher than

ambient concentrations and saturated bacterial uptake systems. Experiments showed that assimilation rates were saturated at 5–10 nM in the anaerobic zones and at 20 nM in the aerobic mixolimnion. [^{3}H]thymidine is also diluted by internal de novo synthesis of thymidine (Moriarty and Pollard 1981) which may vary among the diverse microbial populations in Big Soda Lake. This problem was not addressed in this study. Glutamate turnover was calculated as the fraction of the pool assimilated per hour and is referred to as turnover rate. Since the glutamate pool was not known and may have been significantly increased by the isotope addition, glutamate turnover rates cannot be interpreted as absolute rates. On the assumption that there were no major differences in glutamate pool sizes, then they do indicate relative differences in heterotrophic activity between samples. Glutamate turnover rates were not corrected for mineralization.

We performed light-vs.-dark and size-fractionation experiments with water from the photosynthetic bacterial layer under conditions approximating in situ temperature (10°C) and light intensity (<75 μEinst m^{-2} s^{-1}), using syringes in ice chests covered with neutral-density screening. Light-vs.-dark uptake of [^{14}C]glutamate and [^{3}H]thymidine in the bacterial layer was size-fractioned by sequential filtration (Lane and Goldman 1984). After filtration of thymidine samples, ice-cold 5% TCA was added to the filter towers. The filters were extracted for 3 min and then were rinsed four times with 5% cold TCA. The mineralization of [^{14}C]glutamate to $^{14}CO_2$ was measured by acidification of a 40-ml subsample after incubation. Erlenmeyer flasks (125 ml) were fitted with a plastic adapter which held a scintillation vial at right angles to the top of the flask. Acid was injected into the flask (18 N H_2SO_4, amount determined by titration to pH 2) to stop the reaction and liberate CO_2. Phenethylamine (0.4 ml) was injected onto a Whatman No. 1 filter-paper wick in the scintillation vial. The flasks with the CO_2-trapping apparatus were then agitated on a shaker table for 2 h. The filter paper with the absorbed $^{14}CO_2$ was collapsed into the bottom of the vial and 15 ml of PCS (Amersham) scintillation cocktail was added. Recovery of $H^{14}CO_3^-$ was 72±6% (n = 4).

Results

Bacterial abundance and biomass—Bacterial abundance in the water column ranged between ~5 and 52 × 10^6 cells ml^{-1}. In general, bacterial abundance and biomass were lowest in the aerobic mixolimnion, increased in the anoxic mixolimnion, and reached highest values at the chemocline (Fig. 4). The monimolimnion had about 60% of the total bacterial abundance of the water column, the anoxic mixolimnion accounted for 24–33%, and the aerobic mixolimnion 15–17% (Table 1). Similar proportions were also calculated for biomass. Bacterial abundance was highest in the chemocline (irrespective of season) where cell counts ranged between 2.4±0.3 × 10^6 cells ml^{-1} in February 1985 and 52.4±0.6 × 10^6 in May 1985 (Fig. 4). High cell counts in the chemocline coincided with elevated biomass, reaching values as high as ~2 mg C $liter^{-1}$. Surprisingly, bacterial abundances in the photosynthetic bacterial layer were comparable to abundances found elsewhere in the mixolimnion.

The dominant phototroph appeared to be an exceptionally large (4–14 μm^3; diam = 1.5–3 μm) *Chromatium* sp. which was present at a density of ~2 × 10^6 cells ml^{-1} (about a third of the total counts). *Ectothiorhodospira vacuolata* was also observed, but at much lower abundances. The highest values of bacterial biomass in the water column were associated with the photosynthetic layer and occurred during July (2.2 mg C $liter^{-1}$) and October 1984 (4.1 mg C $liter^{-1}$). These peaks were absent during February and May 1985. These results are consistent with seasonal measures of turbidity, bacteriochlorophyll *a*, adenosine triphosphate, and protein (Cloern et al. 1983*a*,*b*; Oremland et al. 1985, 1987).

The average cell volume and size distributions varied with depth (Fig. 5). Average cell size was largest (>1.7 μm^3) in the bacterial layer at 19 m. The most numerous bacteria in the layer were large photosyn-

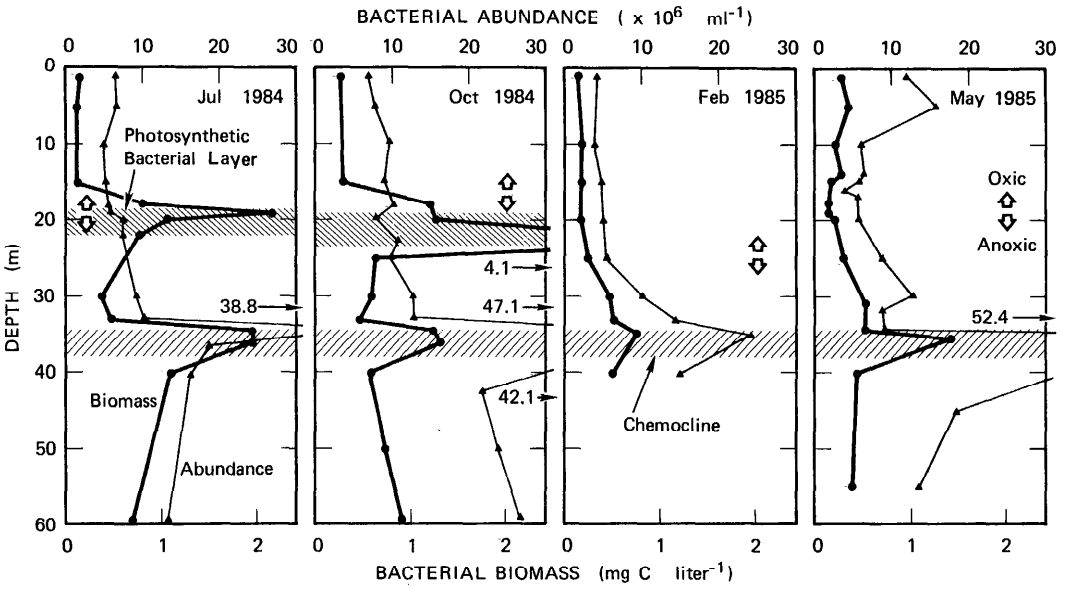

Fig. 4. Depth profiles of bacterial numbers and biomass in Big Soda Lake. Arrows indicate locations of an oxycline. Hatched bars—approximate locations of bacterial layers.

thetic bacteria, which accounted for ~90% of the biomass. With the exception of the photosynthetic bacterial layer, bacteria in the monimolimnion were generally larger than those in the mixolimnion. The epilimnion was dominated by small cells (75% were <0.3 μm). In contrast, a majority of bacteria at 18 m (below the oxycline) had cell lengths >0.7 μm. Filamentous forms were evident in the lower depths of the mixolimnion, the chemocline, and in the monimolimnion, but not in the upper mixolimnion. Relative abundance of small bacteria (cell length <0.3 μm) decreased with depth and accounted for only 18% of total bacterial abundance at 60 m.

Heterotrophic activity—Thymidine incorporation was generally linear during 6-h incubations. Incorporation by monimolimnion samples was measurable, but much lower than in the mixolimnion. Two-hour incubations were used on all other sampling dates. In the photosynthetic layer, a large fraction of the thymidine incorporation and glutamate uptake was due to organisms <1 μm (Fig. 6). Mineralization of glutamate and its uptake by cells <3 μm was greater in the light. Thymidine incorporation was not

Table 1. Mean glutamate turnover (h^{-1}), thymidine assimilation (pmol $liter^{-1}$ h^{-1}), bacterial abundance ($\times 10^6$ ml^{-1}) and bacterial biomass (mg C $liter^{-1}$) in the acrobic mixolimnion, anaerobic mixolimnion, and anaerobic monimolimnion of Big Soda Lake during the four seasons. (Not determined—ND.)

	Oct 83		Jul 84		Feb 85		May 85	
	Glutamate	Thymidine	Glutamate	Thymidine	Glutamate	Thymidine	Glutamate	Thymidine
Aerobic mixolimnion	0.01	2.7	0.01	14.2*	0.01	6.8	0.03*	ND
Anaerobic mixolimnion	0.03*	4.4	0.03	36.1*	0.01	12.0	0.01	ND
Anaerobic monimolimnion	0.00	1.2	0.02*	1.0	0.01	2.1*	0.00	ND
	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass
Aerobic mixolimnion	4.07	ND	5.81	0.29	4.79	0.16	7.60	0.22
Anaerobic mixolimnion	6.63	ND	8.52	0.94	10.10	0.41	10.93	0.33
Anaerobic monimolimnion	16.20	ND	21.91	1.39	19.50	0.62	25.31	0.68

* Highest annual rate.

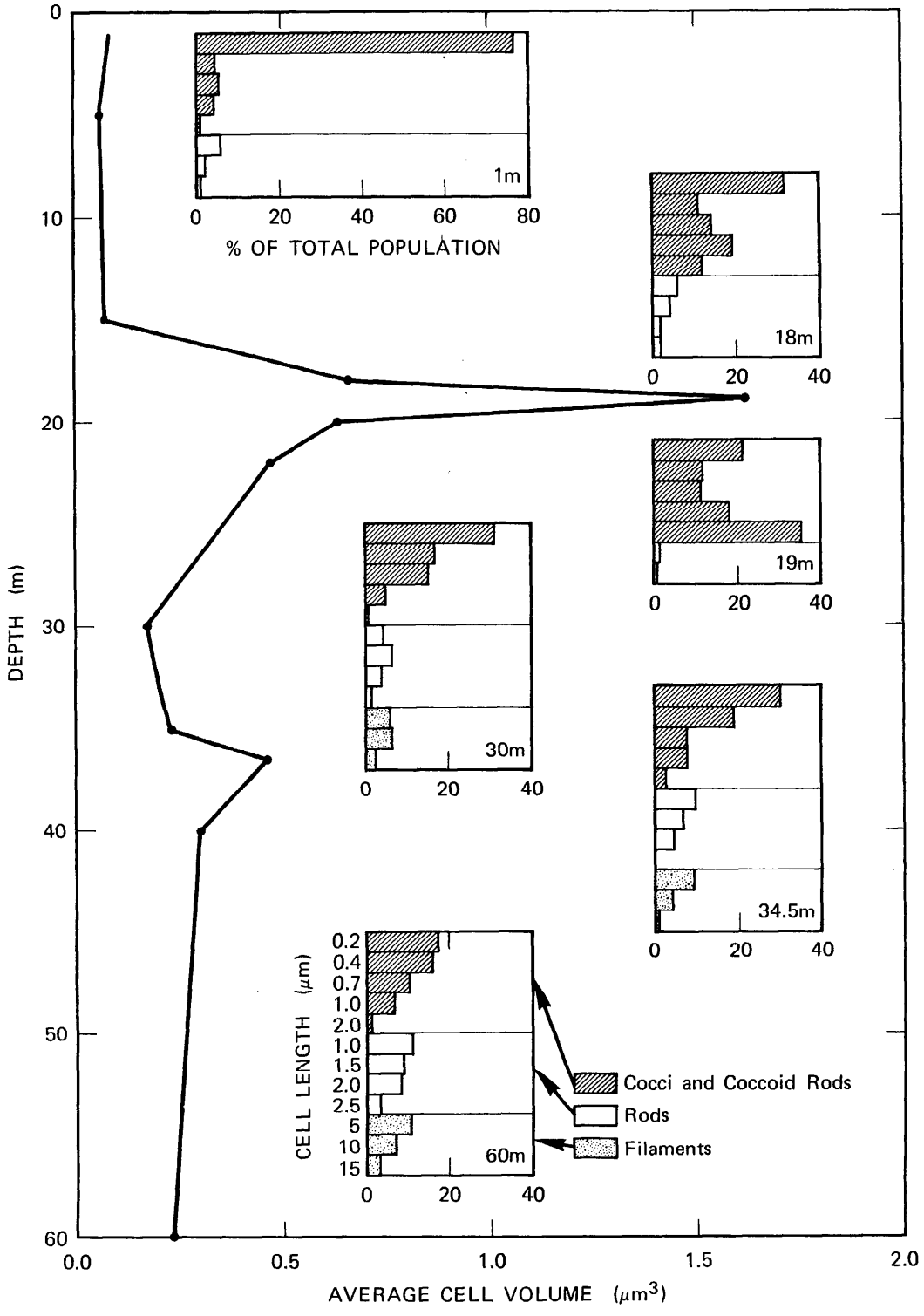

Fig. 5. Depth profile of the bacterial average cell volume in Big Soda Lake, July 1984. Insets—distribution of population cell sizes for cocci, rods, and filaments determined at six depths.

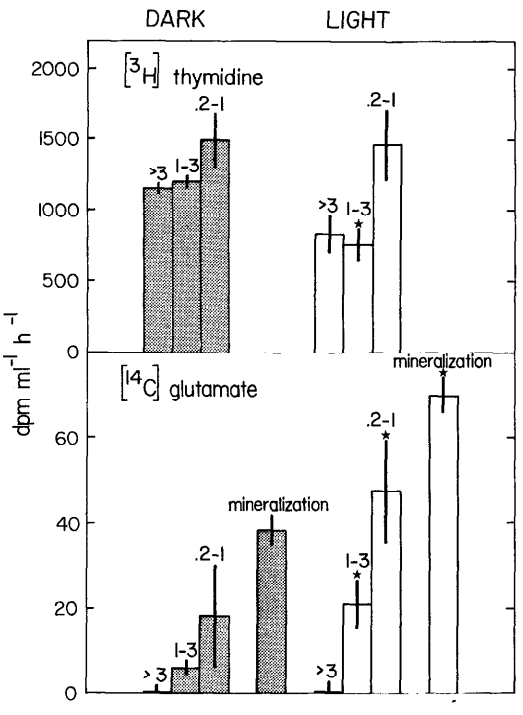

Fig. 6. Size fractionation of [methyl-^{3}H]thymidine and [^{14}C]glutamate assimilation in the photosynthetic layer, October 1984. Error bars are standard deviations of means of three replicates. Stars—significant ($P < 0.05$) difference between light and dark rates.

greater in the light in any size class, and incorporation in the 1–3-μm size class was lower in the light.

In October and July, depth profiles of [^{3}H]thymidine incorporation and [^{14}C]glutamate turnover showed distinct maxima at or just below the photosynthetic bacterial layer (Table 1; Fig. 7). Glutamate turnover rates in the mixolimnion (aerobic and anaerobic) were similar in July and October. In contrast, rates of thymidine incorporation were nearly 10-fold higher in July vs. October (Fig. 7). However, in winter when the photosynthetic bacterial layer was absent, depth profiles of glutamate turnover differed from thymidine incorporation. Rates of thymidine incorporation were still maximal below the oxycline in February, but rates of glutamate turnover varied little with depth and showed no peak at the oxycline (Fig. 7). Small peaks in glutamate turnover and thymidine incorporation were observed at the chemocline. In May, rates of glutamate turnover were high in the aerobic mixolimnion and decreased with depth (Fig. 7).

Seasonal maxima for glutamate turnover and thymidine incorporation in a specific zone usually occurred on different dates (Table 1). For example, maximal thymidine incorporation in the monimolimnion was in February, whereas maximal rates of glutamate turnover in this zone were observed in July. In general, uptake of both substrates was lower in the monimolimnion than in the mixolimnion (Table 1; Fig. 7).

Total thymidine incorporation (Fig. 7) correlated well with thymidine incorporation per cell ($P < 0.05$). Rates of cellular thymidine incorporation in the monimolimnion were always low, but seasonal differences were observed in the mixolimnion (Fig. 8). Rates in the mixolimnion were maximal in July, whereas they were intermediate in February and low in October.

Discussion

Aerobic mixolimnion—Glutamate turnover was seasonally constant at 0.01 h^{-1} from summer through winter and showed a threefold increase during May 1985. The increased surface-layer glutamate turnover and bacterial abundance during May could have been a residual response to the winter-spring phytoplankton bloom (Cloern et al. 1987). Thymidine incorporation rates were highest in summer (Table 1), but unfortunately no measurements were made during the spring period of elevated cell densities and glutamate turnover. The seasonality in thymidine incorporation cannot be explained by differences in temperature alone because intermediate growth rates were measured during winter (e.g. February 1985; Table 1). The summer peak in thymidine incorporation is also not explained by phytoplankton productivity, which is low during summer (Cloern et al. 1983*a*).

In general, rates of thymidine incorporation were low compared to other aerobic environments. Highest rates of thymidine incorporation per cell were equivalent to the lowest found in the Southern California Bight (Fuhrman and Azam 1982) but were within the range of values found in the ant-

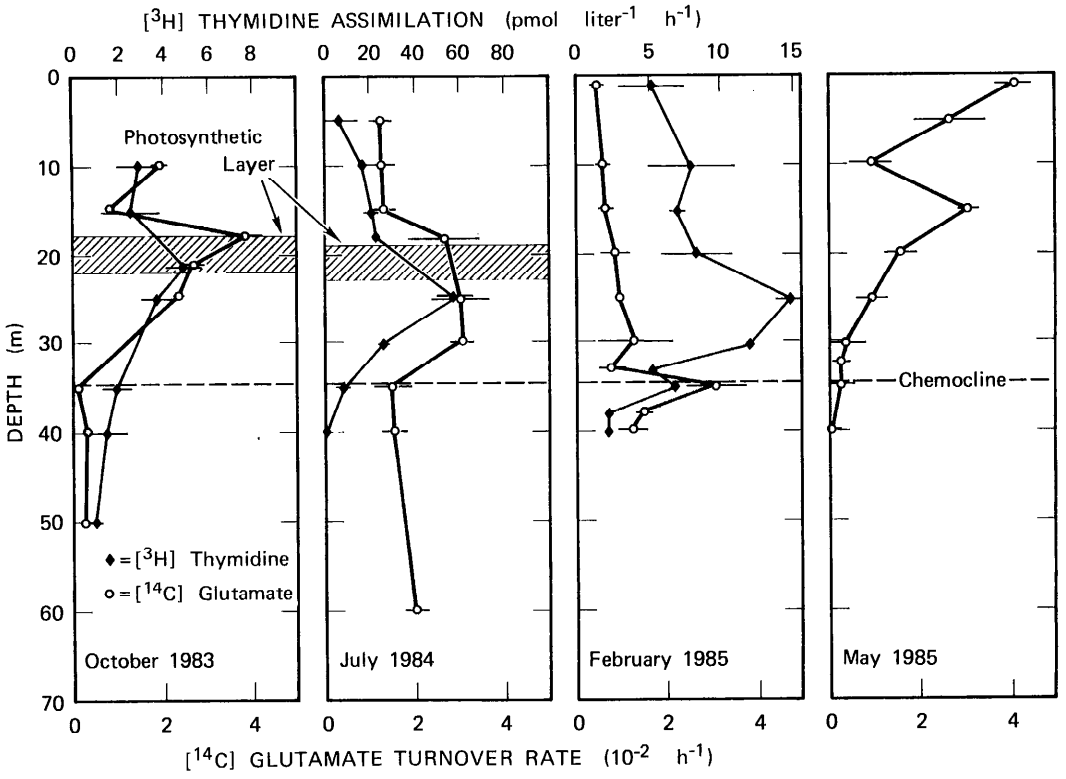

Fig. 7. Depth profiles of [methyl-^{3}H]thymidine and [^{14}C]glutamate uptake in Big Soda Lake. Error bars are standard deviations of means of three replicates. Note scale differences in thymidine assimilation.

arctic (Fuhrman and Azam 1980). The low rates are presumably due to low availability of nutrients and extreme conditions for bacterial growth found in the aerobic waters of Big Soda Lake.

Bacterial growth rates can be calculated from rates of thymidine incorporation with a conversion factor for number of cells produced per mole of thymidine incorporated. Fuhrman and Azam (1980) calculated the factor to be 0.2–1.3 $\times$ 10^{18} cells mol^{-1} thymidine. However, McDonough et al. (1986) used a value of 2 $\times$ 10^{18} (from Fuhrman and Azam 1982) for Lake Oglethorpe, which is seasonally stratified and anoxic in the hypolimnion. McDonough et al. (1986) also estimated the conversion factor, using the Kirchman et al. (1982) method, and found that it was about 1 $\times$ 10^{19} in Lake Oglethorpe. We estimated bacterial growth rates in the aerobic mixolimnion of Big Soda Lake using both 2 $\times$ 10^{18} and 1 $\times$ 10^{19} to get ranges for the conversion factor. Growth rates were 0.12–0.59 d^{-1} in July and 0.07–0.34 d^{-1} in February. These rates are similar to those found by McDonough et al. (1986) in the aerobic epilimnion of Lake Oglethorpe in July and February.

For comparison, growth rates were also calculated from the turnover of [^{14}C]glutamate. The amount of ^{14}C in particulate material was assumed to reflect the turnover of glutamate in bacterial cells. The bacterial glutamate pool was estimated from bacterial biomass data and the bacterial amino acid composition of *Escherichia coli* (Ingraham et al. 1983) as follows:

$$\text{Growth rate (d}^{-1}\text{)} = \frac{\text{[glutamate uptake (nmol liter}^{-1}\text{ d}^{-1}\text{)]}}{\text{[bacterial glutamate pool (nmol liter}^{-1}\text{)]}}$$

where glutamate uptake equals the product of turnover rate (d^{-1}) times the concentra-

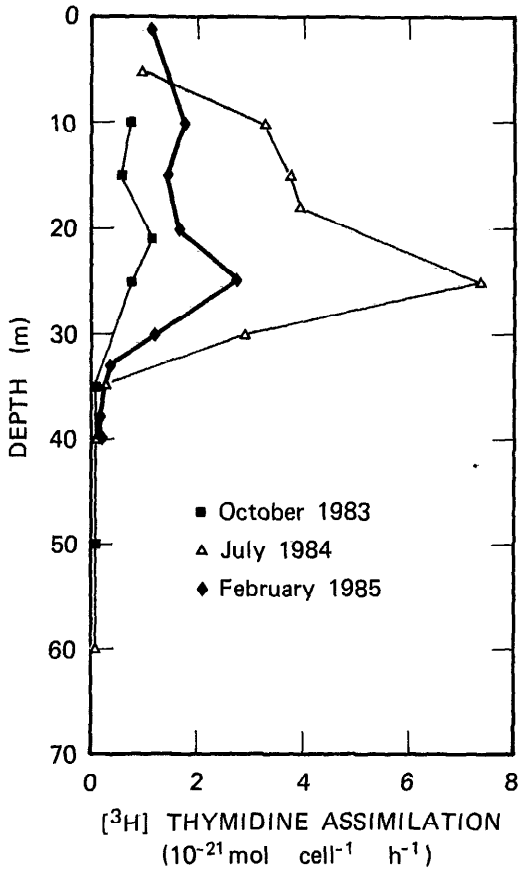

Fig. 8. Depth profiles of thymidine assimilation per cell.

tion added, and the bacterial glutamate pool is derived from bacterial biomass data assuming 0.55 mg C (mg dry wt)$^{-1}$ and 250 nmol glutamate (mg dry wt)$^{-1}$. Estimated growth rates for July and February were 0.04 d^{-1} and 0.08 d^{-1}. Even though the turnover rates of glutamate appear high relative to the thymidine incorporation rates, the growth rates calculated from those data are lower, possibly because the thymidine conversion factor was too high for this bacterial community.

Anaerobic mixolimnion—The highest rates of thymidine incorporation and glutamate uptake were in July, when the photosynthetic bacterial layer was present (Fig. 7). Rates of glutamate turnover were low during February and May, but high rates of thymidine incorporation persisted in the anaerobic mixolimnion during February. Apparently, glutamate turnover is more closely linked to the presence of the photosynthetic layer than is thymidine incorporation. This disparity suggests that glutamate and thymidine are assimilated by different components of the anaerobic bacterial community.

Seasonal changes in thymidine incorporation and glutamate turnover were not correlated. In February and May, mean glutamate turnover rates were comparable (0.01 h^{-1}), but lower than in July or October (0.03 h^{-1}). However, rates of thymidine incorporation in February were intermediate between those in October and July (Table 1). This discrepancy between thymidine incorporation and glutamate turnover may be due to higher ambient glutamate concentrations in February and May. However, the difference between depth profiles of thymidine incorporation and rates of glutamate turnover in February could also be due to a change in the metabolic character of the microbial community. Rates of thymidine incorporation in February could indicate that heterotrophic bacteria were growing at the expense of sinking photosynthetic bacteria. This hypothesis is supported by the high sedimentation rates of photosynthetic bacteria in February (Cloern et al. 1987).

Results of size-fractionation experiments in the photosynthetic bacterial layer indicated that small (<1-μm diam), unattached heterotrophic bacteria assimilated a significant fraction of both glutamate and thymidine (>30 and 50% respectively, Fig. 6). Incorporation of thymidine and uptake of glutamate by cells >1 μm could be due to the purple sulfur bacteria or to associated epibiontic bacteria (Esteve et al. 1983). The light-associated increase in turnover of glutamate in the <1-μm size class was unexpected, as was the enhancement of rates of glutamate mineralization in the light (Fig. 6). These enhancements were most likely due to the stimulation of heterotrophic activity by photosynthetic bacteria. The dependence of heterotrophic bacteria on release of photosynthate conforms to the "phycosphere" theory (Bell and Mitchell 1972). Parkin and Brock (1981) suggested that green sulfur bacteria may provide elec-

tron donors for sulfate reduction in Knaack Lake. However, Smith and Oremland (1987) could not detect sulfate reduction in the photosynthetic bacterial layer of Big Soda Lake. Nonetheless, a similar relationship could exist between the purple sulfur bacteria and other anaerobes (e.g. fermenters or denitrifiers).

The anoxic mixolimnion is a transition zone between the highly reduced monimolimnion and the oxygenated epilimnion. Profiles of [^{14}C]glutamate turnover and [^{3}H]thymidine incorporation indicate substantial microbial activity in this zone. Other researchers have found peaks in ATP (Karl et al. 1977) or dark CO_2 assimilation (Sorokin 1964; Tuttle and Jannasch 1979) near oxic–anoxic interfaces, which is consistent with the findings of this study. Cloern et al. (1983*a*) found a peak in dark CO_2 assimilation in the region of the oxic–anoxic interface in Big Soda Lake, which they demonstrated was due to chemoautotrophic activity. It is not clear whether chemoautotrophs incorporate exogenous [^{3}H]thymidine or [^{14}C]glutamate, but the maximum in [^{3}H]thymidine incorporation and [^{14}C]glutamate turnover extended well into the anoxic zone and thus could not have been due to chemoautotrophs.

Purple sulfur bacteria were present in the anoxic mixolimnion below the photosynthetic layer, but at greatly reduced abundances (0.1×10^6 ml^{-1} at 30 m, 2.1×10^6 ml^{-1} at 19 m). Therefore, the high rates of uptake found below the layer were not due to these microbes. Rates of methane production and sulfate reduction were both very low in the anoxic mixolimnion (Smith and Oremland 1987; Iversen et al. 1987). In addition, no significant denitrification activity could be detected in the water column of Big Soda Lake (Oremland et al. 1987). Therefore, it is unlikely that the observed heterotrophic activity was linked to any of these processes. Since other possible electron acceptors (such as Fe^{3+}) are present in low concentrations (Kharaka et al. 1984), we interpret the high rates of glutamate turnover and thymidine incorporation found in the anaerobic mixolimnion to be due to fermentative organisms. Cloern et al. (1987) found that organic carbon is efficiently mineralized in the mixolimnion of Big Soda Lake. The high rates of glutamate turnover and thymidine incorporation apparently associated with fermentative processes imply that this pathway may be extremely important in decomposition and nutrient regeneration.

Growth rates were higher than in the aerobic mixolimnion. Estimates from thymidine incorporation (as calculated above) were 0.2–1.0 d^{-1} in July. The estimate of growth rate from glutamate turnover (as calculated for the aerobic mixolimnion) was 0.04 d^{-1}. McDonough et al. (1986) found that production rates were highest at the oxic–anoxic interface, in good agreement with the findings of our study.

McDonough et al. (1986) calculated similar growth rates in the epilimnion of Lake Ogelthorpe during July to those we calculated for Big Soda Lake in July. They found that the amount of thymidine incorporated into protein was high (30% into DNA + protein) in the metalimnion. We also found that about 35% (*see methods*) of the thymidine incorporation was in the protein fraction. The similarity between the findings of these two studies indicates that the high incorporation rates found in the anoxic mixolimnion of Big Soda Lake are not due to the unique biology and chemistry of this lake, but to the presence of an oxic–anoxic interface.

Chemocline and monimolimnion—These bacterial communities were characterized by high densities and biomass (Fig. 4) and a more diverse microbial assemblage of smaller cell size than found elsewhere in the water column (Fig. 5). In contrast to the photosynthetic layer, the chemocline and monimolimnion did not exhibit high bacterial activities (Figs. 7 and 8). Rates of thymidine incorporation per cell and glutamate turnover were far lower than elsewhere in the water column (Fig. 8, Table 1). In addition, uptake of [^{3}H]glucose by monimolimnion waters was 10-fold to 30-fold less than in the mixolimnion (R. L. Smith pers. comm.; Oremland et al. 1987). Growth rates estimated from thymidine incorporation were very low (0.002–0.01 d^{-1}). Growth rates calculated from glutamate turnover were also low (0.009 d^{-1}) and agreed well with

the rates calculated from thymidine incorporation.

Although isotope dilution with higher ambient pool sizes could conceivably account for lower uptake rates of these substrates, the fact that three compounds all showed greatly diminished uptake makes this explanation unlikely. This agreement is reinforced by the observation that rates of anaerobic decomposition of oxalate in monimolimnion sediments were 50 times slower than in the littoral zone despite the fact that sediment pool sizes (~ 100 μM) in both locations were equivalent (Smith and Oremland 1983). Therefore, the low rates of thymidine uptake at the chemocline and in the monimolimnion (Figs. 7 and 8) could be due to one or more of the following factors: low growth rates of the indigenous microorganisms, predominance of inactive cells, or inability of the indigenous microbes to incorporate thymidine (i.e. lack of thymidine kinase). Isolates from the monimolimnion were found to incorporate thymidine into cold-TCA-insoluble material (J. Zehr and A. Catena unpubl. data). If we assume that these cells were representative of the in situ population, then the low rates of thymidine incorporation were due to low growth rates of indigenous microbes or the predominance of inactive cells. The implication is that the high bacterial abundance in the monimolimnion was dominated by relatively inactive, slow-growing cells. Although it would suggest that the low rates of incorporation in the monimolimnion were due to low growth rates, the extrapolation of culture data to in situ populations is somewhat tenuous.

The monimolimnion of Big Soda Lake represents an extreme environment for microbial growth. In addition to being highly alkaline, the waters are hypersaline and have high concentrations of free sulfide and other reduced sulfur compounds (Smith and Oremland 1987; Kharaka et al. 1984). This extreme environment may inhibit microbial metabolism and growth. For example, sulfide can inhibit the growth of methanogenic bacteria (Cappenberg 1975; Mountfort and Asher 1979) and the uptake of [^{14}C]acetate by hot-spring microbial communities (Brock et al. 1971). Furthermore, the high pH (9.7) and alkalinity of the monimolimnion may limit the availability of divalent cations which are required to maintain cell wall integrity. Concentrations of Mg^{2+} and Ca^{2+} were much lower in the monimolimnion (0.15 and 0.04 mM) than in the mixolimnion (3.25 and 2.17 mM) (Kharaka et al. 1984) and considerably lower than in seawater. Another possibility is an inhibitory effect of the 2.7 mM ammonia (Deal et al. 1975). Regardless of the actual cause of inhibition, cells entering the monimolimnion from the mixolimnion may become moribund. Microbial activities, however, are measurable in the monimolimnion. Thus the observed sulfate reduction (Smith and Oremland 1987), anaerobic methane oxidation, and methanogenesis (Iversen et al. 1987) were probably due to a flora that was adapted to the chemistry of this zone. Therefore, it is likely that the monimolimnetic flora is composed of a mixture of moribund mixolimnetic cells and indigenous, slow-growing bacteria.

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Submitted: 21 January 1986
Accepted: 14 January 1987