Purple Sulfur Bacteria Control the Growth of Aerobic Heterotrophic Bacterioplankton in a Meromictic Salt Lake

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In meromictic Mahoney Lake, British Columbia, Canada, the heterotrophic bacterial production in the mixolimnion exceeded concomitant primary production by a factor of 7. Bacterial growth rates were correlated neither to primary production nor to the amount of chlorophyll *a*. Both results indicate an uncoupling of bacteria and phytoplankton. In the chemocline of the lake, an extremely dense population of the purple sulfur bacterium *Amoebobacter purpureus* is present year round. We investigated whether anoxygenic phototrophs are significant for the growth of aerobic bacterioplankton in the overlying water. Bacterial growth rates in the mixolimnion were limited by inorganic phosphorus or nitrogen most of the time, and the biomass of heterotrophic bacteria did not increase until, in autumn, 86% of the cells of *A. purpureus* appeared in the mixolimnion because of their reduced buoyant density. The increase in heterotrophic bacterial biomass, soluble phosphorus concentrations below the detection limit, and an extraordinarily high activity of alkaline phosphatase in the mixolimnion indicate a rapid liberation of organically bound phosphorus from *A. purpureus* cells accompanied by a simultaneous incorporation into heterotrophic bacterioplankton. High concentrations of allochthonously derived dissolved organic carbon (mean, 60 mg of C · liter⁻¹) were measured in the lake water. In Mahoney Lake, liberation of phosphorus from upwelling purple sulfur bacteria and degradation of allochthonous dissolved organic carbon as an additional carbon source render heterotrophic bacterial production largely independent of the photosynthesis of phytoplankton. A recycling of inorganic nutrients via phototrophic bacteria also appears to be relevant in other lakes with anoxic bottom waters.

The factors controlling biomass and growth of heterotrophic bacterioplankton have been investigated in numerous studies of marine and limnetic systems (5, 9, 11–13, 23, 25, 38). A compilation of these data yielded highly significant correlations between the biomasses of phytoplankton and bacteria and between the production levels of both physiological groups (5, 9). Provided that the growth of bacterioplankton is limited by the availability of organic carbon substrates (13), this correlation suggests that heterotrophic bacteria depend on phytoplankton as the main source of reduced carbon. However, recent investigations have demonstrated that heterotrophic bacteria can be phosphorus limited in freshwater (23, 25), which would indicate a significant supply of allochthonous carbon (11).

In many small lakes, the bottom water layers are temporarily or permanently anoxic and contain hydrogen sulfide. Frequently, such lakes harbor dense populations of phototrophic sulfur bacteria (18, 24, 30, 33). Although their photosynthetic carbon fixation can exceed that of the phytoplankton (2), it was proposed that the cell carbon of phototrophic sulfur bacteria does not enter the mixolimnion food web (16).

At least in some meromictic lakes, the primary production of the phytoplankton in the mixolimnion and the concomitant heterotrophic bacterial production are not balanced. Bacterial production can exceed primary production by as much as a factor of 6 (e.g., in the saline Big Soda Lake, Nev. [8, 52]). It is possible that in such cases the growth of aerobic bacteria is based largely on allochthonous dissolved organic carbon (DOC), similar to the situation in heterotrophic estuaries (15)

and humic lakes (21, 43). The significance of phototrophic sulfur bacteria for aerobic heterotrophic bacterioplankton has not been investigated in such systems, however.

An extremely dense layer of the purple sulfur bacterium *Amoebobacter purpureus* is present in meromictic Mahoney Lake, British Columbia, Canada (26, 30). The biomass concentration of *A. purpureus* exceeds all values reported so far by a factor of ≥ 10 , and anoxygenic photosynthesis accounts for 60% of the total photosynthetic production of the lake (29). In recent years, a large amount of purple aggregates has been observed floating at the surface of Mahoney Lake in late summer, concurrent with a drastic decrease of *A. purpureus* biomass in the chemocline (29). These features make Mahoney Lake ideal for the study of interactions between photosynthetic bacteria and the aerobic heterotrophic bacterioplankton in the mixolimnion. Given the present uncertainties about the significance of phototrophic sulfur bacteria for microbial growth in oxic water layers, an analysis of the population dynamics and physiological activity of aerobic heterotrophic bacteria in Mahoney Lake would aid in a general understanding of the factors controlling bacterioplankton in lakes with anoxic bottom waters.

MATERIALS AND METHODS

Sampling procedures. All water samples were obtained over the deepest site in the center of Mahoney Lake with a Van Dorn bottle for sampling of the mixolimnion and a syringe sampler for sampling of the chemocline at 2.5-cm intervals (30). Sediment traps were positioned in the lake at 5.5-, 8.0-, and 12.0-m depths (vertical position of openings) and consisted of three 55-cm-long, 10-cm-diameter polyvinyl chloride tubes at each depth. Measurements and incubations were performed on 13 dates between April 1992 and December 1993.

Physical and chemical parameters. The water temperature and conductivity were measured with a YSI no. 33 meter (Yellow Springs Instrument Co., Yellow Springs, Ohio) with calibration and correction of the conductivity values (19).

The stability of a water column is defined as the minimum amount of wind energy required to mix a stratified water column to uniformity without external

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FIG. 1. (A) Isopleths (in kilograms per cubic meter) of water density in Mahoney Lake during 1993. The shaded area indicates the presence of Bchl *a* (detection limit, 0.02 μg · liter⁻¹). (B) Stability of the mixolimnion (left ordinate) and its potential energy of stratification per unit surface area (right ordinate). Values are calculated down to the chemocline for 1993.

transfer of heat or solute. Stability coefficients were calculated for each sampling date as described previously (24, 45):

$$S = 1/A_0 \cdot \int_0^{z_m} [(z - z_{g,z}) \cdot (\rho_z - \rho) \cdot A_z] dz$$

where A_0 and A_z are the surface area and the area at depth z , z_m is the maximum depth, $z_{g,z}$ is the depth of the volumetric center of gravity of water above z , ρ_z is the density of lake water at each depth, and ρ is the mean density of the lake water. The densities were calculated from conductivity and temperature profiles by using a calibration curve (32). Stability was calculated at consecutive 1-m intervals for the mixolimnion and monimolimnion but in 10-cm steps across the chemocline.

The total soluble phosphorus in 0.2-μm-pore-size-membrane-filtered water samples was determined after digestion with H₂SO₄ plus K₂SO₄. Samples were run on a QuikChem AE autoanalyzer (Lachat, Milwaukee, Wis.).

Dissolved inorganic carbon (DIC) and DOC were measured in triplicate samples with a Shimadzu total-carbon analyzer (TOC-500) equipped with an autosampler (AS 1-502). For DIC determinations, the instrument was calibrated with solutions of Na₂CO₃. Water samples for DOC analysis were filtered through 0.2-μm-pore-size membrane filters (cellulose nitrate), acidified to pH 2.0, and purged with nitrogen gas overnight. After this treatment, the DIC levels in the samples were below the detection limit (1 mg of C · liter⁻¹). Potassium phthalate was used for the calibration of DOC measurements. Controls run with DOC-free artificial Mahoney Lake water (31) demonstrated that leaching of the membrane filters or the high salt concentrations in Mahoney Lake water did not interfere with our DOC measurements. Furthermore, we determined the recovery of DOC in Mahoney Lake water samples spiked with phthalate to be 97.8% ± 1.0%.

On three dates, the DOC was analyzed further by fractionation into molecular size classes of >10,000 Da, 10,000 to 1,000 Da, and <1,000 Da. For separation, 100 ml of 0.2-μm-pore-size-filtered lake water was run consecutively through YM 10 and YM 1 Amicon Diaflow ultrafilters in a no. 8200 cell. The system was pressurized to 276 kPa with nitrogen. Carbon recovery by this procedure was 100.3% ± 11.1%.

Concentrations of dissolved protein in lake water were measured by dye binding (4), and the concentration of carbohydrate was determined by the phenol method (20).

To estimate the amount of dissolved humic material, the natural fluorescence of filtered lake water was measured by the quinine sulfate method (50). Excita-

tion was set at 365 nm, and emission was recorded by scanning from 400 to 800 nm, which yielded a broad emission peak centered at 460 nm. For calibration, a 2.3 μM standard solution of quinine sulfate in 0.01 N H₂SO₄ was set to read 0.9 relative fluorescence unit. Since 80 mg of humic acids per liter exhibits the same fluorescence as a 0.184 μM quinine solution (50), the amount of dissolved humic substances in Mahoney Lake water could be estimated from the natural fluorescence values.

Microbial biomass and physiological activity. Chlorophyll *a* (Chl *a*) was measured fluorometrically (51) in the two size fractions of 0.2 to 3.0 μm and >3.0 μm by using cellulose nitrate membrane filters for sequential filtration. In autumn, large aggregates of *A. purpureus* were observed in the mixolimnion. To determine the biomass concentration of *A. purpureus*, 2 liters of lake water from each depth was strained through a 54-μm-mesh Nitex screen, the aggregates were concentrated on a Whatman GF/A fiberglass filter, and bacteriochlorophyll *a* (Bchl *a*) was measured in acetone extracts (41). No Bchl *a* could be detected in the size fraction of <54 μm. Cell numbers of chemotrophic bacteria were determined by epifluorescence microscopy after the water samples were stained with 4',6-diamidino-2-phenylindole (DAPI) (34).

Bacterial cell volumes were determined by electron microscopy. Water samples were fixed with glutaraldehyde (final concentration, 2%), and subsamples were filtered through 0.2-μm-pore-size Nuclepore polycarbonate filters, which were then rinsed with 5 ml of 0.1 M sodium cacodylate buffer (pH 7.4). After fixation with 1% OsO₄ in 0.1 M sodium cacodylate buffer for 1 h and dehydration in an ethanol series, the samples were critical-point dried (Balzers Union CPD 020), gold sputtered (Nanotech Semprep2 Sputter Coater), and subsequently viewed in a Cambridge 250 scanning electron microscope. The dimensions of bacterial cells were measured with a Kontron SEM image-processing system, and cell volumes were calculated. Bacterial cell volumes were converted to carbon units by assuming a factor of 1.21×10^{-13} g of C μm⁻³ (47).

Photosynthetic carbon fixation and excretion of photosynthetically fixed carbon were determined at 1-m intervals with NaH¹⁴CO₃ at a final activity of 7.4 kBq · ml⁻¹ (29, 33).

Simultaneously, the production of heterotrophic bacterioplankton was measured by [³H]thymidine (740 GBq · mmol⁻¹) incorporation (17). Blanks consisted of parallel samples fixed with formaldehyde (final concentration, 2%). At the end of the incubation, uptake of label in live samples was stopped by adding formaldehyde, and the samples were placed on ice and processed immediately on the lake shore. The fraction of label incorporated into DNA was determined on one date (13 June 1994). Additional parallel samples were incubated at 0-, 3-, and 6-m depths and afterwards hydrolyzed for 15 min at 100°C in 5% trichloroacetic acid (39) to measure the amount of ³H in hot-trichloroacetic acid-insoluble (i.e., non-DNA) macromolecules. We found that 45.0% ± 16.2% incorporated into cellular DNA. All the radioactivity was measured by liquid scintillation counting (Beckman LS 6000 TA).

We used the conversion factor from the literature of 2×10^{18} cells · (mol of thymidine)⁻¹ (25) for the calculation of bacterial growth rates from thymidine incorporation rates.

To investigate a possible nutrient limitation of heterotrophic bacterioplankton, water samples from 3 m deep were diluted 10-fold with sterile filtered lake water from the same depth and spiked with either carbon (16 μM each glucose and sodium acetate), nitrogen (20 μM NH₄Cl), or phosphorus (20 μM Na₂HPO₄) substrate or with a mixture of yeast extract and Casamino Acids (0.008% [wt/vol] each), leaving an unspiked sample as a control. To prevent the stimulation of phytoplankton growth (which could result in an excretion of organic carbon substrates for heterotrophic bacteria), the incubation bottles were wrapped completely in aluminum foil. At the beginning and after 48 h of incubation, subsamples were assayed for [³H]thymidine incorporation.

The activities of the hydrolytic enzymes β-glucosidase, lysozyme, phosphatase, and protease were assayed with methylumbelliferyl (MUF)-β-D-glucoside, MUF-β-D-N,N',N''-triacetylchitotriose, MUF-phosphate, and L-leucine-7-amino-4-methylcoumarin, respectively. Stock solutions of these substrates were prepared in advance and transported frozen to the lake. Enzyme assays were performed within 1 h of sampling and at room temperature (6). Fluorescence was measured with a fluorometer (Turner Designs, Sunnyvale, Calif.).

Stable-carbon analysis. Plant material for stable-carbon analysis was rinsed with 1 N HCl and then with distilled water and dried at 105°C. Stoneworts (members of the Characeae) are the main macrophytes found in the Mahoney Lake littoral. Before drying the specimen, it was necessary to incubate it in 1 N HCl for 24 h to remove carbonate precipitates from the plants. For the determination of δ¹³C values specifically for the purple sulfur bacterium *A. purpureus*, 500-ml samples of the bacterial layer were concentrated by filtration on fiberglass filters (Whatman GF/A) and extracted with acetone. Extracts were concentrated by rotary evaporation, and photosynthetic pigments were isolated by thin-layer chromatography (Merck silica gel 60, developed with petroleum ether).

Particulate organic carbon (POC) was filtered onto precombusted fiberglass filters (Whatman GF/C; nominal pore size, 1.2 μm). The filters were incubated in 0.1 N HCl and dried overnight. Copepods were collected over a 102-μm Nitex mesh plankton net and immediately frozen on dry ice. The DOC in 200 ml of a filtered and acidified (see the section on DOC determination, above) sample was oxidized with dichromate (1), and the liberated CO₂ was trapped in 2 N NaOH. After addition of BaCl₂, the precipitated BaCO₃ was centrifuged, dried, and analyzed by mass spectrometry on a Prism series I Triple Collector mass spec-

FIG. 2. (A) Biomass of chemotrophic bacterioplankton in 1993. Values of isopleths are given in micrograms of carbon per liter. (B) Isopleths of population growth rates for heterotrophic bacteria (in reciprocal days).

trometer (VG Isotech, Cheshire, United Kingdom) coupled to a CN analyzer (NA 1500).

All $\delta^{13}\text{C}$ values are quoted in per mille with respect to the Pee Dee belemnite standard. Analyses were reproducible to $\pm 0.1\text{‰}$.

RESULTS

Stratification and stability. After ice break, a low-salinity water layer was present at the surface of Mahoney Lake and persisted throughout the spring of 1993. Between February and May, the temperature of the upper water layers increased from below zero to 22°C , but it increased to only 6°C at depths below 5 m. These vertical differences in water temperature and salinity resulted in steep vertical gradients of water density in the mixolimnion (Fig. 1A).

The total (thermal plus chemical) stability of Mahoney Lake water increased steeply between the chemocline and 10 m deep. Stability values for the whole water column did not show any seasonal trend between April and November 1993 (mean, $236 \pm 34 \text{ kg} \cdot \text{m}^{-1}$). In contrast, the stability of the mixolimnion alone declined sharply between May and July 1993 (Fig. 1B) in parallel with the disappearance of the low-salinity surface water layer, the warming of the lower water layers, and the resulting loss in density stratification.

Microbial biomass. Until July, the biomass of heterotrophic bacteria remained almost constant around $100 \mu\text{g}$ of $\text{C} \cdot \text{liter}^{-1}$, but during late summer and autumn, it increased exponentially (Fig. 2A). This was caused by an increase in both bacterial cell numbers and bacterial cell volumes (Fig. 3B). However, at the same time, growth rates were low, indicating a low physiological activity of heterotrophic bacteria in the mixolimnion during autumn (Fig. 2B and 3B). The maximum growth rates for 1993 occurred much earlier in the year (at the end of May). Consequently, decreased loss rates rather than increased growth rates must be the reason for the autumnal rise in bacterial biomass in the mixolimnion.

With one exception (April 1992, $5.18 \mu\text{g}$ of $\text{Chl } a \cdot \text{liter}^{-1}$), chlorophyll a concentrations in the mixolimnion remained around $1 \mu\text{g} \cdot \text{liter}^{-1}$ or below. A high percentage of $\text{Chl } a$ was found in small particles in the size fraction from 0.2 to $3 \mu\text{m}$ (Fig. 3A). In September 1993, numerous aggregates of *A. purpureus* were observed floating in the mixolimnion, and the concentrations of their specific pigment, Bchl a , were significantly higher than those of $\text{Chl } a$ (Fig. 3A). At the same time, the mean concentration of DOC in the mixolimnion declined sharply by a factor of 4 (Fig. 3C) and concentrations of total soluble phosphorus remained below the detection limit of $0.1 \mu\text{M}$.

Compared with most other aquatic systems, bacterial numbers were very high relative to $\text{Chl } a$ concentrations, and these parameters were not significantly correlated ($r^2 = 0.277$; $P > 0.1$) (see Fig. 5).

Microbial physiological activity. Integrated bacterial production reached a transient peak in May 1993 (Fig. 4) and

FIG. 3. (A) Concentrations of $\text{Chl } a$ (●) and Bchl a (○), and percentage of $\text{Chl } a$ in the size fraction of $<3 \mu\text{m}$ (◇). (B) Bacterial cell numbers (▽), cell volume (▼), and specific growth rate (□). (C) Mean concentrations of DOC (▲). Error bars indicate standard deviations. All datum points are mean values for measurements in the mixolimnion (0 to 6 m) in 1993.

FIG. 4. Integrated primary production (●) and bacterial production (▽) in the mixolimnion of Mahoney Lake during 1993. Both were measured simultaneously during incubation around solar noon.

increased continuously and steeply between July and November, parallel to the increase in bacterial biomass. This time course of integrated bacterial production was distinctly different from that of the photosynthetic production of phytoplankton (Fig. 4); the parameters were not significantly correlated ($r^2 = 0.014$; $P > 0.1$). Total bacterial production calculated for 1993 was $153 \text{ g of C} \cdot \text{m}^{-2} \cdot \text{year}^{-1}$ ($105 \text{ g} \cdot \text{m}^{-2} \cdot \text{year}^{-1}$ if corrected for the actual volume of the different water strata), while primary production in the mixolimnion was much lower ($23.6 \text{ g of C} \cdot \text{m}^{-2} \cdot \text{year}^{-1}$; corrected value, $14.8 \text{ g} \cdot \text{m}^{-2} \cdot \text{year}^{-1}$).

Because of this remarkable imbalance between heterotrophic and autotrophic processes in the mixolimnion, the factors controlling the growth of bacterioplankton in Mahoney Lake were investigated. First, we calculated coefficients of correlation between the bacterial growth rate and various parameters in the mixolimnion for the year 1993. Because of the steep gradients of physicochemical parameters which persisted throughout the spring, we used values for different depths separately instead of mean values for the mixolimnion in this analysis. Growth rates were not correlated with bacterial cell numbers, primary production, water temperature, or oxygen concentrations. The only significant correlation found was between bacterial growth rates and water temperatures if restricted to the time interval from February to May 1993 ($r^2 = 0.483$; $P < 0.001$).

Thus, additional factors must be crucial to the growth of aerobic bacteria. Because of the very low concentrations of total soluble phosphorus, the role of inorganic nutrients was investigated. Thymidine incorporation rates were stimulated after incubation with inorganic nutrients or with a combination

TABLE 1. Relative increase of [^3H]thymidine uptake after incubation of 3-m-deep water samples with various substrates

Substrate ^a	Concn.	Relative increase in [^3H]thymidine uptake on ^b :				
		April 4	May 25	Aug 20	Sept 25	Nov 6
Glc + Ac	16 μM	0.61	1.42	0.90	1.41	0.56
NH_4^+	20 μM	2.84	1.46	1.23	1.17	1.49
PO_4^{3-}	20 μM	0.95	2.65	4.18	1.12	1.36
YE + CA	0.008%	0.37	1.63	3.71	29.24	0.66

^a Glc, glucose; Ac, acetate; YE, yeast extract; CA, Casamino Acids.

^b Data are from 1993.

of yeast extract plus Casamino Acids but not with the combination of glucose plus acetate (Table 1). An extremely strong stimulation by the yeast extract-Casamino Acids mixture was observed in September 1993, when none of the other substrates showed a pronounced effect. In November 1993, none of the substrates increased thymidine incorporation significantly.

The measurement of alkaline phosphatase activity in algae has been suggested as a procedure for detecting phosphorus deficiency in aquatic ecosystems. Of the six ectoenzymes assayed, alkaline phosphatase exhibited by far the highest activity, exceeding those of the other enzymes by 3 orders of magnitude (Table 2). All K_m values were on the order of several micromolar. Glucuronidase activity was not observed. We computed the specific activities for alkaline phosphatase for the mixolimnion between May and November 1993 (Table 2). Since this enzyme is associated with phytoplankton as well as with heterotrophic bacteria (49), the biomass values for both groups were included in these calculations. The specific phosphatase activity increased over the summer and dropped by a factor of 10 during the presence of *A. purpureus* aggregates in the mixolimnion between September and November 1993.

DOC. DOC concentrations were around $60 \text{ mg of C} \cdot \text{liter}^{-1}$ except during September (Table 3; Fig. 3C). Carbohydrates and protein constituted 10 and 1%, respectively, of the total DOC. The major part of the DOC was equally distributed between the $<1,000\text{-Da}$ and the $1,000\text{- to }10,000\text{-Da}$ fractions. Although Mahoney Lake water is not brown or yellow, the DOC in lake water still exhibited a marked autofluorescence. Standardization and conversion of the fluorescence values to concentrations of humic substances (see Materials and Methods) yielded values which were very similar to those of total DOC. Therefore, the DOC in Mahoney Lake seems to consist mainly of aromatic and heterocyclic compounds.

To elucidate the possible source of the high DOC concen-

TABLE 2. Enzyme activities measured as release of 4-methylumbelliferone or 7-amino-4-methylcoumarin in water samples from 3 m deep

Enzyme	Date (day/mo/yr)	K_m (μM)	$V_{\max}$ ($\text{nM} \cdot \text{h}^{-1}$)	Specific $V_{\max}^a$ ($\text{nmol} \cdot \mu\text{g of C}^{-1} \cdot \text{h}^{-1}$)
Phosphatase	24/05/93	12.5	5,700	39.5
	20/08/93	16.5	16,130	89.3
	24/09/93	ND ^b	6,000	11.6
	05/11/93	ND	10,426	8.5
Protease	06/11/93	19.6	59.5	
Lysozyme	26/05/93	2.9	2.6	
	24/09/93	— ^c	0.0	
	05/11/93	ND	2.2	
α -Glucosidase	15/07/93	3.4	8.9	
	20/08/93	6.2	8.3	
β -Glucosidase	17/08/93	5.9	8.9	
	24/09/93	ND	9.2	
	05/11/93	ND	7.3	
Glucuronidase	17/08/93	—	0.0	

^a Calculated from the sum of phytoplankton and bacterial cell carbon. Phytoplankton carbon was estimated from Chl *a* with a conversion factor of $30 \mu\text{g of C} \cdot \mu\text{g of Chl } a^{-1}$.

^b ND, not determined.

^c —, determination not possible, because no enzyme activity was present.

TABLE 3. Summary of the properties of DOC in the mixolimnion of Mahoney Lake, determined at regular intervals during 1993

Parameter	Value		n
	Mean	SD	
Total DOC (mg of C · liter ⁻¹) ^a	62.5	11.3	7
Fraction (%)			
>10,000 Da	18.5	6.6	3
10,000–1,000 Da	44.7	21.1	3
<1,000 Da	43.4	14.0	3
Carbohydrate (mg of C · liter ⁻¹) ^b	6.1	1.2	5
Protein (mg of C · liter ⁻¹) ^c	0.71	0.56	5
Natural fluorescence			
Equivalent Concn of quinine (μM)	0.276	0.015	5
Equivalent concn of humic matter (mg of C · liter ⁻¹) ^d	64.4	3.54	5

^a Mean value in the mixolimnion (0 to 6 m deep). The unusually low value recorded in September 1993 was omitted.

^b Calculated as carbon of glucose equivalents.

^c Calculated from the mean carbon content of 46.2% for intracellular amino acids (derived from the data in reference 36 and confirmed for natural bacterioplankton [39]).

^d Estimated by using the conversion factor derived from reference 50 as described in the text, and assuming a carbon content of 53.7% for humic matter (27).

trations in the lake water, stable carbon isotope ratios were determined for a variety of carbon pools in and around Mahoney Lake (Table 4). The total POC in the mixolimnion, the POC sedimenting out of the mixolimnion, or the POC recovered from the surface of the bottom sediment at 14.5 m deep all had $\delta^{13}\text{C}$ values very similar to those for DOC. The $\delta^{13}\text{C}$ values for POC at 3 m deep and in sediment traps decreased below -25.7‰ in August 1993, which could be the result of the increased CO_2 fixation by phytoplankton during summer (Fig. 4). Because of the rapid clogging of filters (probably by colloidal organic material) and the very low Chl *a* concentrations, no value for Chl *a* as an indicator of phytoplankton biomass could be obtained. The values measured for copepods (*Diaptomus connexus*), as another member of the pelagic food chain, however, were significantly lower than those for DOC or total POC. In pure cultures of *A. purpureus*, grown as described previously (32), the carbon fractionation values of okenone ($\delta^{13}\text{C} = -35.1\text{‰}$), Bchl *a* (-35.2‰), and total organic carbon (-37.4‰) were almost identical. Consequently, the low values determined for photosynthetic pigments of *A. purpureus* cells in the chemocline of Mahoney Lake excluded this compartment as the main source of the DOC. We collected plant material in the littoral (stoneworts, Characeae) of the lake as well as from ponderosa pine (*Pinus ponderosa*), salt grass (*Distichlis stricta*), and American bulrush (*Scirpus americanus*) on the lake shore. Of these, only dry needle litter of the Ponderosa pine had a $\delta^{13}\text{C}$ value similar to those of the DOC and total POC (Table 4).

DISCUSSION

The yearly bacterial production in Mahoney Lake exceeded primary production by a factor of 7. In a closed ecosystem, bacterial production can exceed primary production by a factor of 1.3 (37), which is a result of carbon cycling. Even if the mean error of our production estimates (7% for bacterial, 14% for primary production) is considered, the pronounced imbalance of primary and bacterial production in Mahoney Lake cannot be explained by carbon cycling alone. Furthermore, bacterial

production appears much too high for such an oligotrophic lake (compare the values in reference 9).

Theoretically, both allochthonous organic carbon and the upwelling purple sulfur bacterial biomass could be invoked to explain the high heterotrophic bacterial production in the mixolimnion of Mahoney Lake. The absence of a correlation between bacterial numbers and Chl *a* (Fig. 5) could be explained by a phosphorus limitation of the bacterioplankton (12, 23, 25). Therefore, the role of inorganic nutrients in the limitation of heterotrophic bacterial growth in Mahoney Lake must be considered.

Limitation of bacterial growth. Until September 1993, bacterial thymidine incorporation was stimulated by the addition of ammonium or phosphate salts. A significant stimulation by carbon substrates was not observed. The activities of alkaline phosphatase determined in mixolimnion water samples from Mahoney Lake were 24 to 53 times higher than values reported for other nonacidic lakes (11, 40), indicating extreme phosphorus shortage of microorganisms (bacteria plus phytoplankton) in the mixolimnion. As far as we know, phosphatase activities of the same order of magnitude as in Mahoney Lake have been measured only in acidic lakes (3, 28) and in one other Canadian saline lake (44).

In general, the production of alkaline phosphatase is derepressed only after depletion of extracellularly available P_i and intracellularly stored phosphorus, and it is induced by phosphoester substrates (7). During autumn, the specific activity of phosphatase decreased 10-fold and bacterial biomass and mean bacterial cell volume increased simultaneously, indicating an improvement of the substrate supply (cf. reference 35). In September, growth was exclusively and strongly stimulated by yeast extract plus Casamino Acids, and in November, no significant stimulation was observed with any of the substrates tested. In summary, all these results indicate an increase in the supply of phosphorus with the appearance of *A. purpureus* aggregates in the mixolimnion.

Source and significance of DOC. The growth of heterotrophic bacterioplankton was never limited by carbon substrates.

TABLE 4. Carbon isotope composition^a of various carbon pools in Mahoney Lake^b

Pool	$\delta^{13}\text{C}$	n
DOC mixolimnion (0–6 m)	–23.1	1
POC 3 m deep	–23.8 ± 1.3	4
POC chemocline	–23.2 ± 1.0	3
POC 12 m deep	–22.5 ± 0.2	3
POC sedimenting (5.5 m)	–23.1 ± 2.1	4
POC sediment (14.5 m)	–23.3	1
<i>A. purpureus</i>		
Bchl <i>a</i>	–27.8 ± 0.7	3
Okenone	–27.2 ± 1.9	3
<i>Diaptomus connexus</i>	–27.4 ± 1.9	4
<i>Pinus ponderosa</i>		
Needles	–27.2	1
Needle litter	–24.5	2
<i>Distichlis stricta</i>	–27.3	1
<i>Scirpus americanus</i>	–28.2	2
Characeae	–16.4	2

^a ‰ relative to the Pee Dee belemnite standard, given as mean values ± standard deviation.

^b Samples were obtained between September 1992 and November 1993 except for September 1993, during the upwelling of *A. purpureus*.

FIG. 5. Concentrations of Chl *a* (in micrograms per liter) and corresponding bacterial cell numbers (in reciprocal milliliters) in Mahoney Lake during 1992 and 1993 (●) compared with values from the literature. ---, regression computed by using 142 datum points from various seawater and freshwater locations; —, observed threshold of bacterial cell numbers (5); ◇, two datum points obtained for the Wedell Sea (10); □, data from various humic lakes (21, 43). The arrow indicates the transition from values measured during a spring algal bloom (▲) to those obtained upon decay of the bloom (△) in the eutrophic Plüsee (6).

Therefore, the carbon supply in the mixolimnion was sufficient throughout the whole year, even before the upwelling of *A. purpureus*. Consequently, an additional source of organic carbon must exist in Mahoney Lake. The DOC concentrations measured in Mahoney Lake water are extremely high compared with most other nonhumic lakes (14, 25). We conducted several control experiments (see Materials and Methods) to ensure that our measurements are not biased by artifacts. Values for DOC comparable to those in Mahoney Lake have been reported mainly for lakes containing high concentrations of dissolved humic matter (43). However, the water of Mahoney Lake does not show the strong yellow or brown color and high light attenuation associated with high concentrations of humic substances. Interestingly, values between 20 and 72 mg of C · liter⁻¹ were also reported for alkaline Big Soda Lake (52) and for nonhumic saline lakes in the Canadian prairies (44). The fluorescence of humic compounds depends strongly on their molecular mass and is 2 orders of magnitude higher for the 3- to 50-kDa fraction than for the 100- to 300-kDa fraction (22). Because most of the DOC in Mahoney Lake has a molecular mass similar to that of fulvic acids (<10 kDa), we conclude that the marked fluorescence of the lake water is caused by fulvic acid-like compounds.

Allochthonous and littoral carbon sources are known to contribute a large portion of the total carbon input of small lakes (about 70% in a hard-water lake [48]). In fact, the $\delta^{13}\text{C}$ values we obtained indicate that a terrestrial origin of the DOC in Mahoney Lake is most likely. The difference between the values for bulk POC and *A. purpureus* shows that degradation of the latter does not contribute significantly to the persisting DOC. Independent evidence for a high rate of input of allochthonous organic carbon comes from our investigation of the sulfur cycle in Mahoney Lake (31). As calculated from the rate of diffusion of sulfide from the monimolimnion into the chemocline, bacterial sulfate reduction in the monimolimnion would require a mean rate of carbon supply of 244 mg of C · m⁻² · day⁻¹. A corresponding flux of sedimenting POC of 233 mg of C · m⁻² · day⁻¹ was measured with sediment traps positioned at 12 m deep. In contrast, the mean primary productivity in the mixolimnion was much lower (66.1 mg of

C · m⁻² · day⁻¹), leaving the majority to be explained by allochthonous input of organic carbon.

Although some heterotrophic bacteria are able to grow at the expense of dissolved humic matter (43), a major portion of the DOC in lakes is refractory (42). The increase in the heterotrophic bacterial biomass between August and September 1993 (ca. 300 μg of C · liter⁻¹) explains only 0.5% of the concomitant drop in DOC (61.4 mg of C · liter⁻¹ [Fig. 3C]). Thus, it is unlikely that the whole decrease in DOC is due to degradation by heterotrophic bacteria. Rather, other mechanisms such as coagulation to colloids or absorption of DOC by *A. purpureus* aggregates dominate.

Significance of purple sulfur bacteria. Between August and September 1993, 86% of the *A. purpureus* biomass in the bacterial plate (1,092 mg of Bchl *a* · m⁻²) entered the mixolimnion of Mahoney Lake (29). This is equal to an increase of 6.29 mg of C · liter⁻¹ from the *A. purpureus* carbon component (employing the specific Bchl *a* content of 19.9 mg of Bchl *a* · g of C⁻¹ [29] and correcting for the dilution in the greater volumes of upper water layers). At a C/P ratio of 53:1, a complete mineralization of this biomass would increase the phosphate concentration of the lake water by 314 μg of P · liter⁻¹. Only 16% of this (50 μg of P · liter⁻¹) is required to explain the autumnal increase in the biomass of heterotrophic bacteria following the upwelling event. No erosion of the chemocline was observed during the upwelling event, which excludes a significant contribution of phosphate-rich bottom water to the fertilization of the mixolimnion.

Rather, our results indicate that the enormous *A. purpureus* population is the major source of phosphorus for the growth of heterotrophic bacteria in the mixolimnion of Mahoney Lake. The large amount of organically bound phosphorus present as *A. purpureus* aggregates in the mixolimnion, inorganic phosphorus concentrations below the detection limit, and high phosphatase activities all indicate a rapid turnover of the soluble phosphorus pool and a rapid incorporation of liberated phosphorus into heterotrophic bacterial biomass. The upwelling of purple bacterial biomass, which has already been monitored for five consecutive years, represents a recycling mechanism for inorganic nutrients and minimizes nutrient loss due to sedimentation of *A. purpureus* cells to the lake bottom.

One question remains: is the autumnal appearance of *A. purpureus* aggregates in the mixolimnion caused by physical mixing of the water column down to the chemocline or caused by a decrease in the buoyant density of *A. purpureus* cells?

The impact of wind-driven vertical mixing on the upwelling of *A. purpureus* can be assessed by comparing the time course of stability values in Fig. 1B with the wind energy available and the time of appearance of aggregates in the mixolimnion.

Over the summer of 1993, the stability of stratification of the mixolimnion decreased steeply (Fig. 1B). An average wind speed of 2.17 m · s⁻¹ was measured at Mahoney Lake in previous years (46). The corresponding energy imparted by wind between May and July 1993 (32.2 J · m⁻²) does not explain the drop in stability observed (186 J · m⁻² [Fig. 1B]). Temperature isopleths indicate that similar to solar lakes, the more saline layers below 1 m deep in Mahoney Lake warmed more rapidly between April and July than did the low-salinity layer on top. The resulting penetrative convection, rather than wind-driven mixing, caused the observed loss in stability between May and July 1993.

Upwelling of *A. purpureus* did not occur parallel to this drop in stability of the mixolimnion but took place much later. Because the stratification of the chemocline (at 7 m deep) remained stable after July (Fig. 1A), *A. purpureus* must have risen out of the steep vertical density gradient and entered the

mixing currents in the mixolimnion. Our results indicate that convective mixing aids in the sudden vertical distribution of *A. purpureus* throughout the mixolimnion, but this physical factor is not the trigger that initiates the massive flotation of cells into the upper water layers.

In pure cultures, *A. purpureus* cells reach minimum buoyant densities of $1,002 \text{ kg} \cdot \text{m}^{-3}$ as a result of the formation of gas vesicles (32). During summer, the supply of electron-donating substrates for anoxygenic photosynthesis was limited (29), and levels of intracellular ballast compounds of *A. purpureus* decreased sharply. After the upwelling, *A. purpureus* aggregates remained neutrally buoyant throughout the mixolimnion (29). These observations are consistent with the view that the buoyant density of *A. purpureus* changes in response to the physiological state of the cells and that this process results in a concerted flotation of cells up to the lake surface.

In conclusion, allochthonously derived organic matter seems to provide a significant part of the carbon substrates for chemoheterotrophic bacteria in the mixolimnion, but bacterial production reaches maximum values only after the input of other nutrients (notably P) present in upwelling cells of purple sulfur bacteria. There is evidence from other lakes with anoxic bottom waters (Lake Cisó [24] and Lake Buchensee [28a]) that phototrophic bacterial biomass frequently enters the mixolimnion. We propose that chemocline populations of anaerobic bacteria might be of general significance for nutrient supply to aerobic bacterioplankton in meromictic lakes.

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