Solar Lake (Sinai). 3. Bacterial distribution and production[1]

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Abstract

The relations between photosynthetic and nonphotosynthetic (chemoorganotrophic and chemolithotrophic) microorganisms in Solar Lake were studied during the annual limnological cycle. Six different bacterial plates were observed during stratification by direct and viable bacterial counts, light and dark CO_2 incorporation, chlorophyll *a*, protein, ATP, and ETS determinations. A maximal dark CO_2 incorporation of 1,014 mg C m^{-3} d^{-1} may represent as much as 16,900 mg C m^{-3} d^{-1} of chemoorganotrophic bacterial production, on the assumption that these bacteria assimilate an average of 6% CO_2 of their total carbon uptake. This calculated production is very high in comparison to the recorded photosynthetic maximum of 4,960 mg C m^{-3} d^{-1}. The organic carbon needed for such a high chemoorganotrophic production may be supplied by the benthic cyanobacterial mats. Extremely high specific activities of ATP and ETS for the layer immediately above the thermocline indicate a very active bacterial plate at this layer.

Early in the study of the productivity of aquatic environments emphasis was placed on the role of photosynthetic organisms as "primary producers." The contribution of heterotrophic organisms was often neglected or considered to be limited to the stages of degradation and mineralization. However, it has become evident that there are many special cases of natural communities having high dark CO_2 incorporation, some times even higher than the light incorporation. Only a small portion of this dark CO_2 uptake can be attributed to algal CO_2 incorporation in the dark (Goldman et al. 1972; Galloway et al. 1974). Since considerable dark CO_2 uptake takes place along with phytoplankton CO_2 photoassimilation, several investigators have tried to evaluate the role of bacteria in the production of total biomass in lakes (e.g. Sorokin 1965; Kusnetsov and Romanenko 1966). They have developed methods of correlating direct bacterial counts and calculated bacterial biomass with dark CO_2 incorporation values obtained simultaneously and have found that dark CO_2 incorporation can account generally for 3.5–6% of total bacterial production and that 94–96.5% must be derived from various organic sources. Using this approach, Overbeck (1972*a*,*b*, 1974), Tilzer (1972), Takahashi and Ichimura (1970), and Seki et al. (1972) studied several lakes of different productivity and arrived at about the same average values (about 6%) for dark CO_2 incorporation. Overbeck (1972*a*), Tilzer (1972), and Sorokin (1969, 1970) stated that the amount of CO_2 incorporation by the bacterial flora in various strata of a lake may fluctuate as widely as 3.5–60%.

Biological assimilation of CO_2 in aquatic environments can occur either photosynthetically or in the dark. In addition to dark CO_2 incorporation by photosynthetic organisms, three other groups fix CO_2 in the dark (Sorokin 1969): chemolithotrophic bacteria in which 80–90% of the organic carbon can be attributed to CO_2 incorporation (Rittenberg 1972), mixotrophic organisms in which 30–90% of total cell carbon is derived from CO_2 incorpora-

[1] This work was made possible partially by grants of the Deutsche Forschungsgemeinschaft (Kr 333/6–11) and a grant to the Department of Microbiological Chemistry of the Hebrew University.

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tion, and heterotrophic organisms incorporating small amounts of CO_2 mainly through the Wood-Werkman reaction (Wood and Stjernholm 1962). In cases where the bulk of the photosynthetic algae and bacteria is spatially separated from the heterotrophic and chemolithotrophic bacteria, crude calculations of bacterial production can be made on the basis of dark fixation and bacterial biomass (Sorokin 1970; Tilzer 1972; Seki et al. 1972).

Highly productive Solar Lake, with its relatively low biomass, marked stratification, and spatial separation of bacterial populations (Cohen et al. 1977*b*), seemed to be appropriate for a study of bacterial productivity. In this work we have tried to estimate bacterial productivity in the different bacterial plates that develop during stratification. The estimates were based on comparison of direct and viable bacterial counts with light and dark CO_2 fixation, chlorophyll *a*, and protein determinations, as well as ATP and ETS measurements.

We are indebted to the staff of the H. Steinitz Marine Biological Laboratory for help in carrying out the fieldwork and to B. Golek and G. Koch for their aid in preparing this manuscript. A critical review of the first draft by R. Daley contributed substantially to the present manuscript.

Material and methods

Viable counts—Water samples were taken aseptically at station A (*see* Cohen et al. 1977*a*) with a Cobet water sampler and a ZoBell (J-Z) water sampler at 50-cm vertical intervals. The water samples were transferred to the laboratory within a half hour and inoculated within 2 h.

Serial dilutions in 75% aged holomictic Solar Lake water were inoculated into the following selective media.

1. Medium 2216 E, after ZoBell (1946), was used to isolate aerobic heterotrophic proteolytic bacteria as pour plates with 1.5% Difco agar, in triplicate. This medium was prepared with 75% Solar Lake water (120‰ salinity), 75% seawater (30‰ salinity), and distilled water (4‰, due to inoculate salinity). Parallel inocula for all the different salinities were incubated at 20°, 35°, and 48°C.

2. The 2216 E medium of ZoBell with additional 0.5% glucose and 0.1% ascorbic acid was used to isolate facultative anaerobic proteolytic bacteria. Triplicate 1.5% Difco agar shake tubes were prepared in 75% Solar Lake water, sealed with paraffin, and incubated at 35°C.

3. Medium for *Desulfovibrio* consisted of (in g liter^{-1}): NH_4Cl (1), $MgSO_4$ (1), $CaCl_2$ (0.1), K_2HPO_4 (0.5), Na_2SO_4 (0.2), Na lactate (3.5), and agar (1.5) at pH 6.8. The medium was prepared in 75% Solar Lake water. Before inoculation one drop of a freshly prepared 10% $FeSO_4$ solution was added to culture tubes containing 15 ml of medium. A second medium for sulfate-reducing bacteria consisted of medium 3 with additional 0.05% asparagine, 0.1% ascorbic acid, and 0.1% sodium thioglycolate. Inoculation and incubation were as described for medium 2.

4. Medium for bacteria-degrading organic sulfur compounds consisted of (in g liter^{-1}): yeast extract (1), Difco neopeptone (0.1), cysteine (0.5), NH_4Cl (0.5), K_2HPO_4 (0.5), and agar (1.5) at pH 7.6. The medium was prepared in sulfate-free artificial seawater (120‰). Inoculation and incubation were as for medium 2.

Bacterial colonies were counted after 7 and 14 days incubation.

Direct microscopical counts of microorganisms—Water samples (500 ml) taken with a modified 1-liter Niskin bottle were centrifuged at 10,000 rpm for 30 min. The pellets were resuspended in a known volume of sterile Solar Lake water. Microorganisms were classified morphologically and each type was counted under phase contrast in a Petroff-Hauser cell in statistically significant numbers.

Light and dark CO_2 uptake—Vertical profiles of light and dark CO_2 uptake were determined according to the Steemann Nielsen method (1952) modified as follows: 130-ml BOD bottles were completely filled with samples taken at 50-cm depth intervals at station A. To each bottle

[^{14}C]$NaHCO_3$ (Amersham, England) (60.3 mCi mmole^{-1} in 0.1 M tris buffer pH 9.2) was added to a final activity of 65 μCi per bottle. Pairs of light and dark bottles were placed at the original depth. After 3 h incubation (always 1000 to 1300 hours) bottles were transferred in a dark, cool box (4°C) to the laboratory and filtered within 30 min through 0.45-μm-mean-pore-size Millipore filters. Different amounts (30–130 ml) were filtered, according to cell density, from different depths. Filters were rinsed with 100 ml of 0.01 N HCl prepared with filtered Solar Lake water. Radioactivity was then measured by a gas flow counter (Nuclear Chicago, model 1412B) after filters were dried at room temperature overnight.

Samples were taken simultaneously to measure alkalinity according to Strickland and Parsons (1968) after removal of sulfide by addition of a 2% $CdCl_2$ solution.

Chlorophyll—Water samples (100–500 ml) were filtered through Whatman GFC 0.45-μm filters. Filters were extracted twice with 5 ml of 90% acetone in the dark. Extracts were centrifuged 10 min. The extinction of the acetone extract was measured spectroscopically at 750, 665, 630, and 480 nm, and the chlorophyll content was calculated according to Strickland and Parsons (1968).

Biomass was initially calculated from direct microscopical counts (Am. Public Health Assoc. 1971). Calculations were based on volume determination of the various morphological types of microorganisms counted. Simultaneous protein determinations were carried out according to Lowry et al. (1951).

Protein and TOC data were compared. Protein content was 50% of the TOC of the bacteria examined. The exception was cyanobacteria with a protein content of 40% of the TOC determined. From these findings, we calculated a suitable conversion factor for each sample regarding the cyanobacteria and other bacteria volume ratios found by direct microscopic counts. Biomass was then generally calculated from protein determinations and expressed in organic carbon.

ATP content was measured according to Holm-Hansen and Booth (1966). Water samples (500 ml) were filtered through a glass fiber filter (GF/C, 0.45-μm pore size) under reduced vacuum. Immediately after filtration, the filters were boiled 5 min in 0.02 M tris buffer at pH 7.75. Samples were cooled and frozen until analysis. Before analysis 0.5 ml of EDTA (0.5 M) was introduced to remove divalent ions and the samples were filtered again. Commercial firefly extract (Sigma) was prepared in $MgSO_4$ and arsenate buffer. The suspended enzyme solution was incubated at 4°C overnight. Sterile tris buffer (1.7 ml) 0.02 M, pH 7.75 was put in a scintillation vial, and 0.1 ml of enzyme was added. Background was counted at room temperature in a scintillation counter (gain 52%, window 50–1,000) for 10 s. Aliquots of ATP extract (0.2–1.0 ml) were added and light emission counted for 10 s, exactly 10 s after addition of the ATP extract. A standardization curve was prepared with dilution series of ATP in tris buffer (0.02 M, pH 7.75).

Electron transport system (ETS) potential activity (potential respiration rate) was measured according to Packard (1971). Water samples (500 ml) were filtered through a glass fiber filter (Whatman GF/C, 0.45-μm pore size). Filters were transferred to 5 ml of suspension mixture consisting of 0.01 M phosphate buffer (pH 7.7), 75 μM $MgSO_4$, 0.5 mM glutathione, and 15 mg ml^{-1} of polyvinylpyrolidone. Filters were homogenized in a Sonifier at 4°C for 1 min. To 1 ml of homogenate, 3 ml of 0.5 mM NADH, 0.15 mM NADPH, and 80 mM Na succinate in 60 mM phosphate buffer (pH 7.7) were added. Then 1 ml of 0.8 mM 3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) was added. This reaction mixture was incubated for 30 min at 30°C in the dark. The reaction was stopped by adding 1 ml of formaldehyde (37%). Then 1 ml of $FeCl_3$ (0.15 M) and 4 ml of acetone and tetrachloroethylene (1.5:1.0 vol:vol) were added and the extinction of the furmazan which formed was measured spectroscopically in the acetone-tetrachlorocthylene

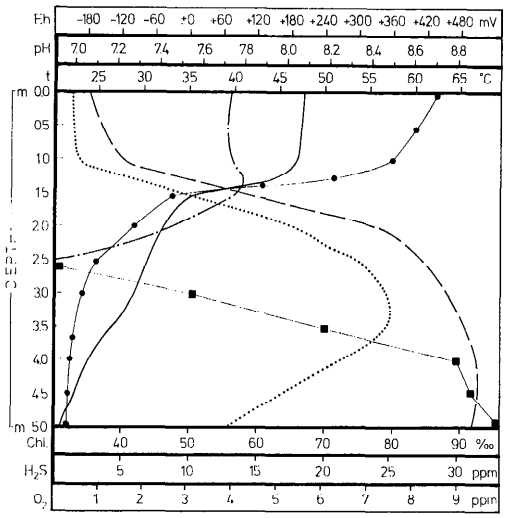

Fig. 1. Physical and chemical limnology during maximal stratification (March): (.....) temperature; (——) pII; (—●—●—●—) redox potential; (— — —) chlorosity; (■—■—■—) H_2S; (—·—·—·) oxygen.

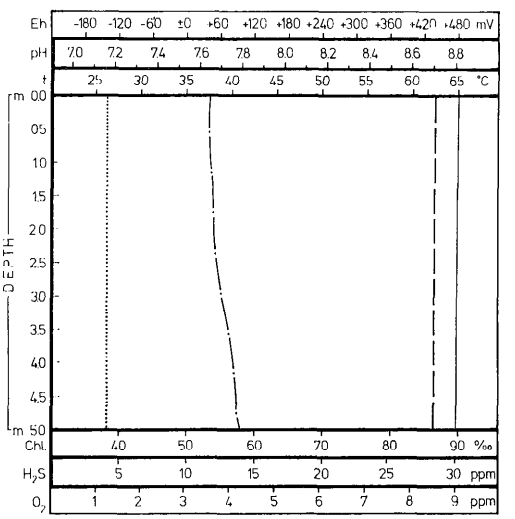

Fig. 2. Physical and chemical limnology during holomixis (August). All parameters are evenly distributed. Symbols as in Fig. 1.

phase at 490 nm. Results were calculated in M O_2 h^{-1} mg protein^{-1}, using the absorbancy of formazan calculated to be 1 O.D. = 1.42×10^4 mol^{-1} cm^{-1}.

Results

Solar Lake is a small (140 × 70 m), shallow (max depth 5 m), hypersaline coastal pond 18 km south of Elat, Israel. Because of seawater seepage onto the surface of this wind-shielded pond, a pycnocline builds up in September. Solar heating produces a mesothermal temperature curve. During stratification from September–July, anoxic conditions develop with up to 39 mg of H_2S per liter at the bottom together with pH decrease (from 8–6.9), redox potential gradient (+390 to −185 mV), and pronounced light absorption (Cohen et al. 1977*a*). Overturn occurs in summer and the holomictic period lasts for 4–13 weeks. The major limnological parameters during stratification and holomixis are shown in Figs. 1 and 2.

Direct counts of microorganisms—The seasonal and vertical distributions of the major morphological types of microorganisms are summarized in Figs. 3 and 4. During holomixis (Fig. 3) most of the morphological types were evenly distributed throughout the water column. All the heterotrophic bacteria show an increase toward the bottom, where they reach a maximum of 6.7×10^5 cells ml^{-1}. The dominant types of heterotrophic bacteria were vibrios, rods, and cocci. Budding bacteria and spirochaetes appear in lower numbers. During holomixis photosynthetic communities are limited throughout the water column and reach maximal concentrations close to the bottom (1.2×10^4 cells or trichomes ml^{-1}).

The vertical distribution of the major morphological types of microorganisms during stratification (Fig. 4) is in extreme contrast to the relatively even distribution during holomixis. The epilimnion (0–1.0 m) contains low concentrations of microorganisms, mainly budding bacteria, some of which were identified as *Caulobacter*, which occur only in the epilimnion, and various rods. The total number of bacteria in the epilimnion is 2×10^5 cells ml^{-1}. A limited population of diatoms also appears, together with a few (10^3 cells ml^{-1}) unicellular cyanobacteria.

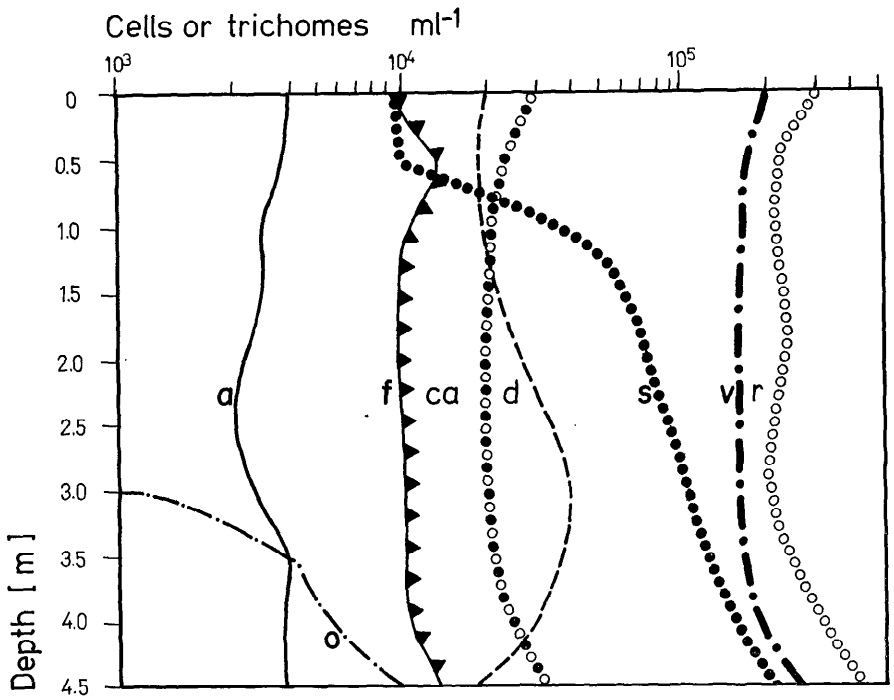

Fig. 3. Vertical distribution of major morphological groups of microorganisms during holomixis. Direct counts of cells or trichomes at each depth from samples taken on 27 July 1970: d—diatoms (*Nitzschia sp., Amphora sp., Naviçula sp.*); o—filamentous cyanobacteria (*Oscillatoria limnetica, Oscillatoria salina, Microcoleus* sp.); ca—*Caulobacter* sp. and other budding bacteria; f—filamentous bacteria; a—unicellular cyanobacteria (*Aphanothece halophytica, Aphanocapsa littoralis*); s—spirochaetes; r—rods; v—vibrios.

In the metalimnion (1–2.5 m) high numbers of spirochaetes, spore-bearing bacteria, filamentous bacteria, and various rods form a dense heterotrophic community (9.5×10^6 cells ml^{-1}). Spirochaetes and filamentous bacteria are restricted to the metalimnion. The major photosynthetic microorganism appearing in the metalimnion is *Chromatium violescens*, reaching maximal densities of 10^6 cells ml^{-1}.

In the hypolimnion the numbers of rods and cocci decrease to about 1.8×10^6 cells ml^{-1}. Extremely high numbers (up to 2×10^6 cells ml^{-1}) of the green sulfur bacterium *Prosthecochloris* sp. form a second photosynthetic bacterial plate in the upper hypolimnion. Filamentous cyanobacteria (*Oscillatoria limnetica, Oscillatoria salina*) dominate the lower hypolimnion and reach densities of 3×10^4 trichomes ml^{-1}.

Thus, in winter, defined strata of mainly photosynthetic microorganisms alternate with strata where chemoorganotrophic or chemolithotrophic bacteria, or both, predominate.

Viable counts of heterotrophic bacteria —The vertical distributions of aerobic chemoorganotrophic bacteria, sampled during stratification and holomixis and capable of growth at different salinities and temperatures, appear in Fig. 5. In summer holomixis, viable counts in 30‰ salinity (75% seawater) and in 120‰ salinity (75% Solar Lake water) are relatively uniform throughout the water column. The highest viable counts (4×10^4 cells ml^{-1}) were obtained in 120‰ at 20°C, conditions most closely resembling the in situ conditions during holomixis. At increased incubation temperatures, viable counts drop drastically. This effect is most pronounced in the 120‰ incubation series: an increase

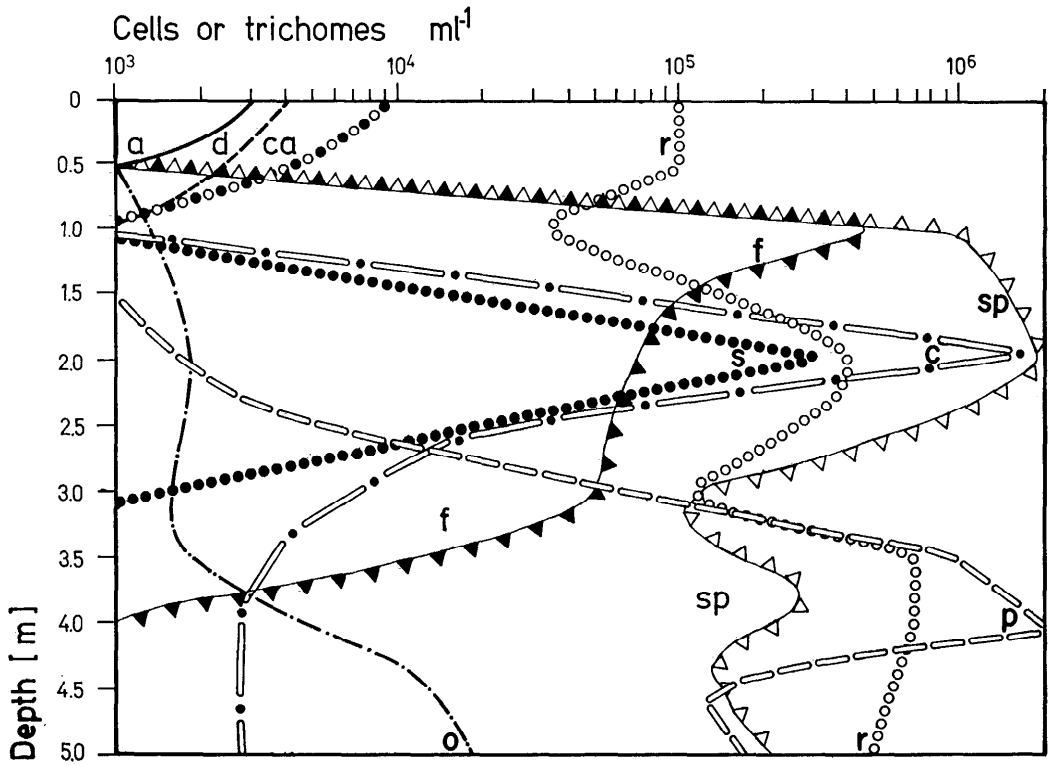

Fig. 4. Vertical distribution of major morphological groups during stratification. Direct counts made on 27 March 1971: d—diatoms (*Nitzschia* sp., *Amphora* sp., *Navicula* sp.); o—filamentous cyanobacteria (*Oscillatoria limnetica, Oscillatoria salina*); f—filamentous bacteria; ca—*Caulobacter* spp. and other budding bacteria; a—unicellular cyanobacteria (*Aphanothece halophytica, Aphanocapsa littoralis*); r—rods; sp—bacterial spores; c—*Chromatium violescens*; p—*Prosthecochloris* sp.; s—spirochaetes.

in incubation temperature from 20 to 35°C decreases the viable count by an order of magnitude; a further increase to 48°C brings an additional drop by two orders of magnitude, so that numbers at 48°C are <0.1% of the counts at 20°C. This effect is less pronounced in 30‰ and 4‰ (distilled water) media. At 20°C viable counts tend to decrease with decreasing salinities. The relatively high counts in 4‰ media with surface water samples may reflect contamination by terrestrial bacteria.

In winter during stratification viable counts of aerobic heterotrophs decrease drastically at 2.5-m depth in comparison with the counts from the epilimnion. For example, in 30‰ at 35°C a difference of three orders of magnitude is observed. The high counts from the epilimnion (0–1 m) isolates grown at 35°C in seawater media are noteworthy. The decreased numbers of aerobic organisms at all salinities and temperatures at the greater depths are correlated with anaerobic conditions in the hypolimnion (Cohen et al. 1977*b*).

Viable counts for anaerobic proteolytic bacteria grown in medium 2 in Solar Lake water at 35°C for the period of holomixis (August) and stratification (March) are shown in Fig. 6. During holomixis when the whole lake is oxygenated, numbers of anaerobes are extremely low (about 500 cells ml^{-1}) throughout the water column. During stratification, the numbers of anaerobic heterotrophs increase considerably with depth, from 40 cells ml^{-1} at the surface to 9×10^4 cells ml^{-1} at the bottom.

Tests for sulfate-reducing bacteria were

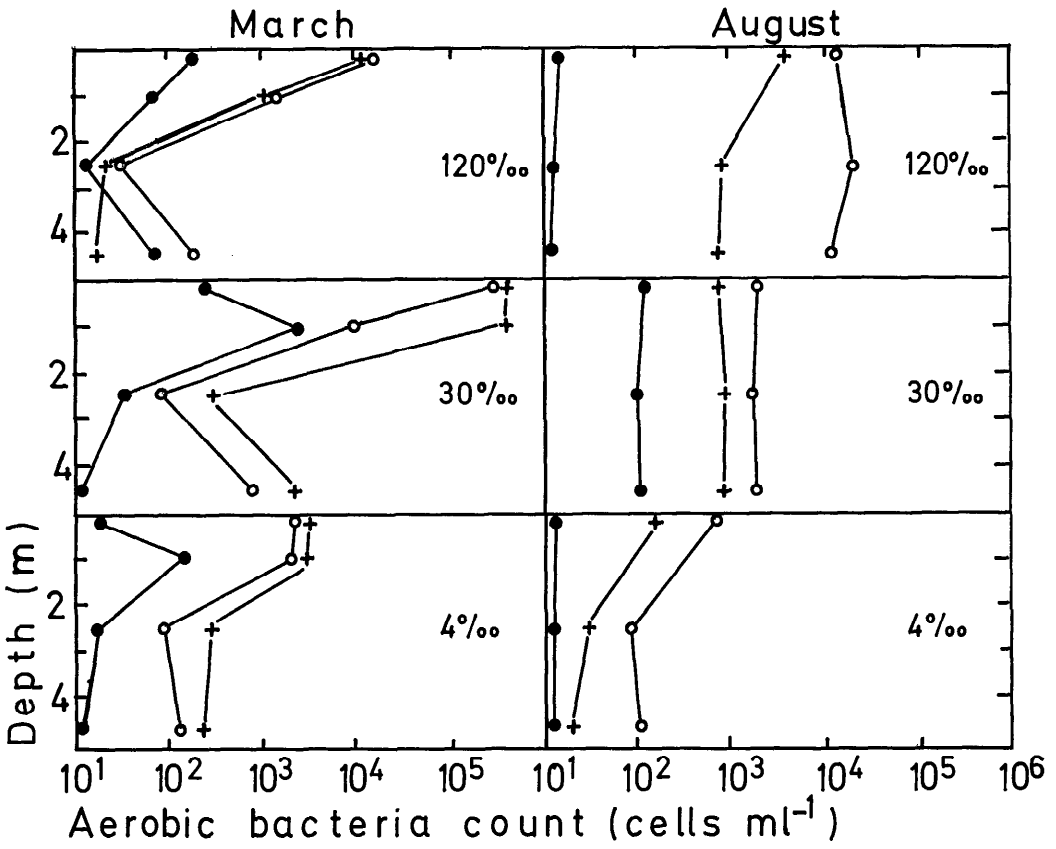

Fig. 5. Viable counts at different salinities and temperatures of aerobic heterotrophic bacteria from various depths of stratified (March) and holomictic (August) Solar Lake. Sampling and viable counts described in methods; incubation temperatures (°C): 20—○; 35—+; 48—●.

negative for all depths in both summer and winter. H_2S producers (180 cells ml^{-1}) from cysteine (putrefaction processes) were found only in the bottom water samples during winter.

Viable counts for thiobacilli showed up to 10^3 cells ml^{-1} above the thermocline (2-m water depth). In the epilimnion and the hypolimnion, thiobacilli were not recovered by selective media.

Dark CO_2 uptake—A comparison of the development of the daily vertical distribution of CO_2 photoassimilation during stratification (Fig. 7) with simultaneous dark CO_2 uptake (Fig. 8) accords well with the distribution of the microorganisms shown in Fig. 4. Primary production shows three defined peaks in the water column. The epilimnetic maximum (80 mg C m^{-3} h^{-1}) corresponds to the community of diatoms and unicellular cyanobacteria at 1 m. The second maximum (227.8) appears in the metalimnion at 3 m, the location of the O_2-H_2S chemocline, where a dense *C. violescens* plate develops. A still higher peak (356.0) occurs at 4 m, owing to the *Prosthecochloris* sp. plate together with *Oscillatoria* spp. The *Oscillatoria* (*see Fig. 4*) increases in numbers toward the bottom and is responsible for the high bottom production measured (238.5).

In contrast, the dark CO_2 uptake (Fig. 8) shows two smaller peaks, corresponding to the diatom layer in the epilimnion (14.2 mg C m^{-3} h^{-1}) and a *Prosthecochloris-Oscillatoria* layer in the lower hypolimnion

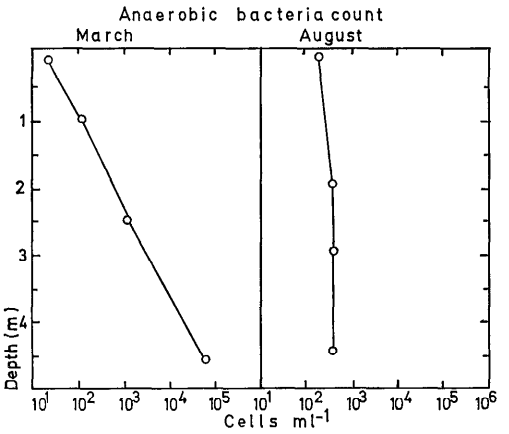

Fig. 6. Viable counts of anaerobic heterotrophic bacteria grown in Solar Lake water (120‰ salinity) at 35°C from various depths of stratified (March) and holomictic (August) Solar Lake.

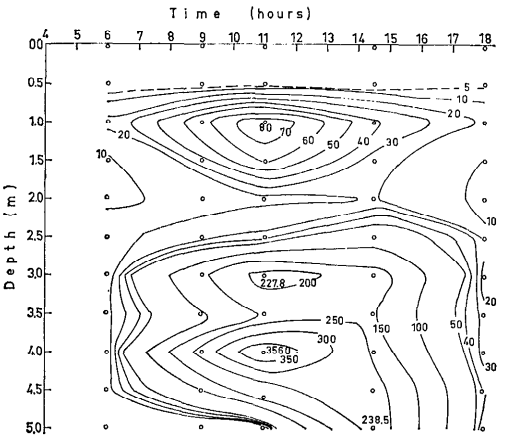

Fig. 7. Diurnal vertical distribution of ^{14}C photoassimilation during stratification, 24 March 1974 (expressed in mg C m^{-3} h^{-1}).

(20.7). The third and most pronounced peak of dark CO_2 incorporation (26.4) appears at 2.5 m, where a minimum of CO_2 photoassimilation was recorded (18.1 mg C m^{-3} h^{-1}).

In addition to the spatial separation, a temporal difference in CO_2 photoassimilation and dark CO_2 uptake was observed during the course of a day. The highest dark CO_2 uptakes were recorded in the afternoon. A time lag of 3.5 h between the maximal CO_2 photoassimilation and maximal dark CO_2 uptake may be a response to excretion of photosynthetic products.

In contrast to the stratified period, a comparison of holomictic CO_2 photoassimilation and dark CO_2 uptake (Fig. 9) shows an even distribution of the two parameters throughout the water column. The dark CO_2 uptake is higher than photoassimilation and increases toward the bottom. Three peaks of dark CO_2 uptake occur during stratification, two of which overlap the CO_2 photoassimilation peaks.

The seasonal and vertical distributions of dark CO_2 fixation are presented in Fig. 10. In August, during holomixis, dark CO_2 fixation was low (11 mg C m^{-3} d^{-1}) at the surface and increased close to the bottom (135). In early November, with the beginning of stratification, the dark CO_2 incorporation values started to form two vertically separated peaks: at the thermocline (496 mg C m^{-3} d^{-1}) and at the bottom (902). These two peaks were maintained and increased during the following 4 months, reaching the recorded maximum of 996 m^{-3} d^{-1} at 1 m and 1,014 at the bottom in March 1971. A third minor peak of 428 first appeared in March at a depth of 3.5 m. CO_2 photoassimilation was low in the epilimnion at this time (about 50 mg C

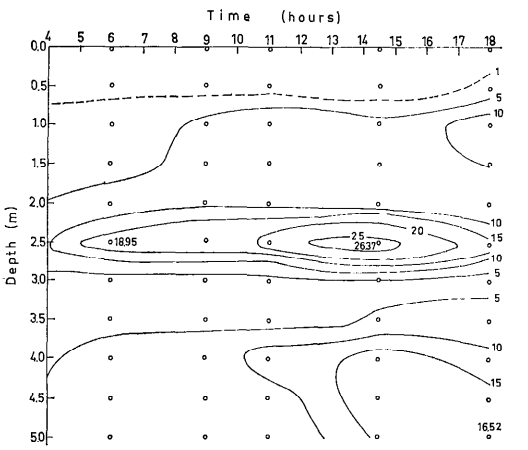

Fig. 8. Diurnal vertical distribution of dark CO_2 uptake during stratification, 24 March 1974 (expressed in mg C m^{-3} h^{-1}).

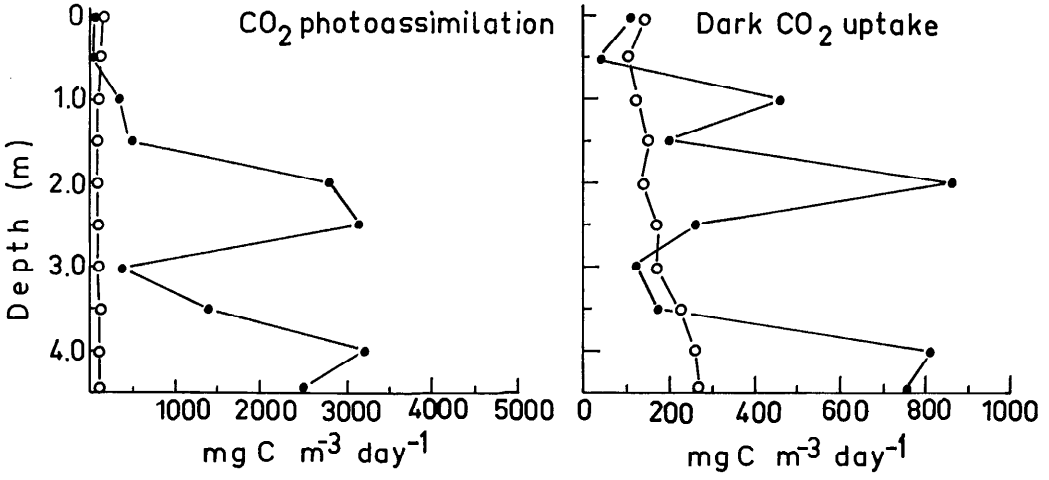

Fig. 9. Vertical distribution of CO_2 photoassimilation and dark CO_2 uptake during holomixis (○) and stratification (●). (For Figs. 9, 11–15, date of measurement is 15 August 1973 for holomixis and 24 April 1974 for stratification.)

m^{-3} d^{-1}). In general the annual course of dark CO_2 peaks follows that of CO_2 photoassimilation (Cohen et al. 1977*b*). However, the dark CO_2 uptake peak in the epilimnion is 0.5 m above the CO_2 photoassimilation peak. The former peak can be related to the introduction of organic matter by flash floods, which accumulates at the pycnocline and provides a suitable energy source for the dense heterotrophic bacterial community. These floods cause partial mixing and may also effect a transfer of organic material and nutrients from the metalimnion and upper hypolimnion to the upper layers of the lake, enhancing dark CO_2 uptake above the pycnocline (0–1.0 m) in March.

During June and July, between the stages of stratification and total mixing of the lake, dark CO_2 incorporation is relatively high in the entire water column (about 180 mg C m^{-3} d^{-1}). With the establishment of holomixis, the dark CO_2 fixation values decrease to the minimum.

To better understand the microbial activities in Solar Lake, we also studied chlorophyll *a*, biomass, ATP, and ETS potential activity during holomixis (15 August 1973) and during maximal stratification (24 April 1974) (Figs. 11, 12). All parameters were low and evenly distributed throughout the water column during holomixis, except for a generally slight increase toward the bottom.

Biomass as calculated from protein (Fig. 11) shows three defined peaks during stratification (1.5, 2.5, and 4 m). The 1.5-m and 4.0-m peaks coincide with maxima of chlorophyll *a* (Fig. 11). The small biomass peak at 2.5 m coincides with a plate of photosynthetic sulfur bacteria (Fig. 4) and therefore does not appear in the

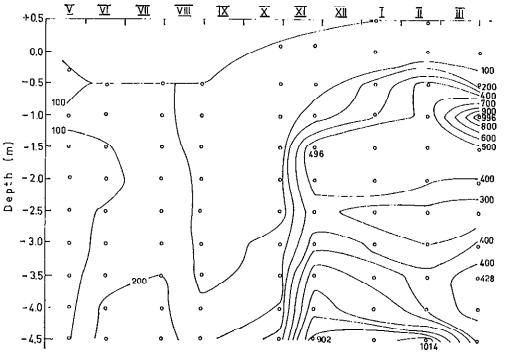

Fig. 10. Seasonal and vertical distribution of dark CO_2 uptake, May 1970–April 1971 (expressed in mg C m^{-3} d^{-1}).

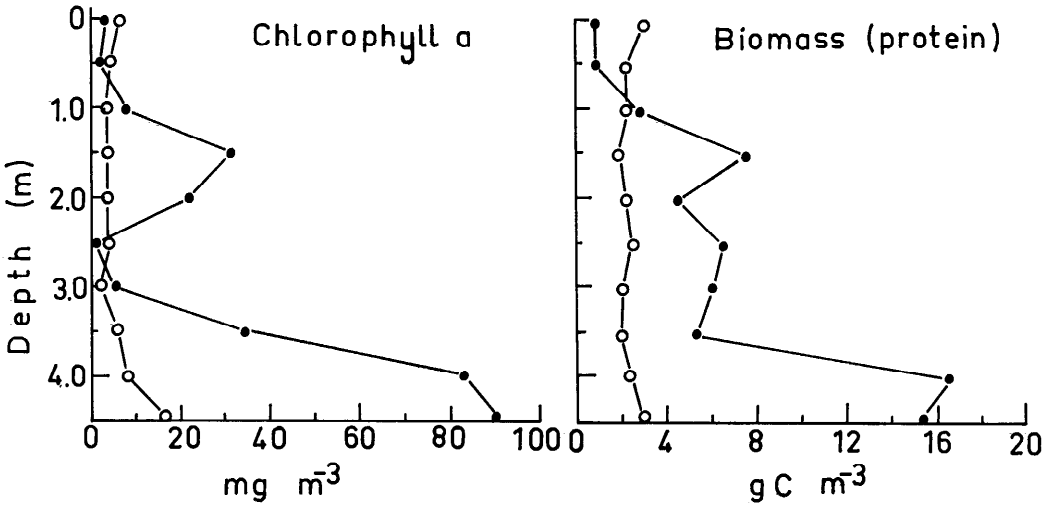

Fig. 11. Vertical distribution of chlorophyll *a* and biomass calculated from protein during holomixis (○) and stratification (●).

chlorophyll *a* graph. The dominance of the cyanobacterial communities at 1–1.5 and at 4.0 m is reflected by the coincidence of high numbers of cyanobacteria with chlorophyll *a* and protein (Figs. 4, 11).

A high activity coefficient of dark CO_2 uptake (up to 0.85), as shown in Fig. 13, suggests the presence of a community of chemolithotrophic bacteria. During maximal stratification, a defined layer of budding bacteria occurs at the surface and a *Thiobacillus* layer at 2-m depth.

When Figs. 9, 10, 11, and 13 are compared, an alternation with depth of dark CO_2 uptake and CO_2 photoassimilation is apparent. This, according to Figs. 3 and 4 and our results on viable counts of bacteria, can be described as follows: A chemo-

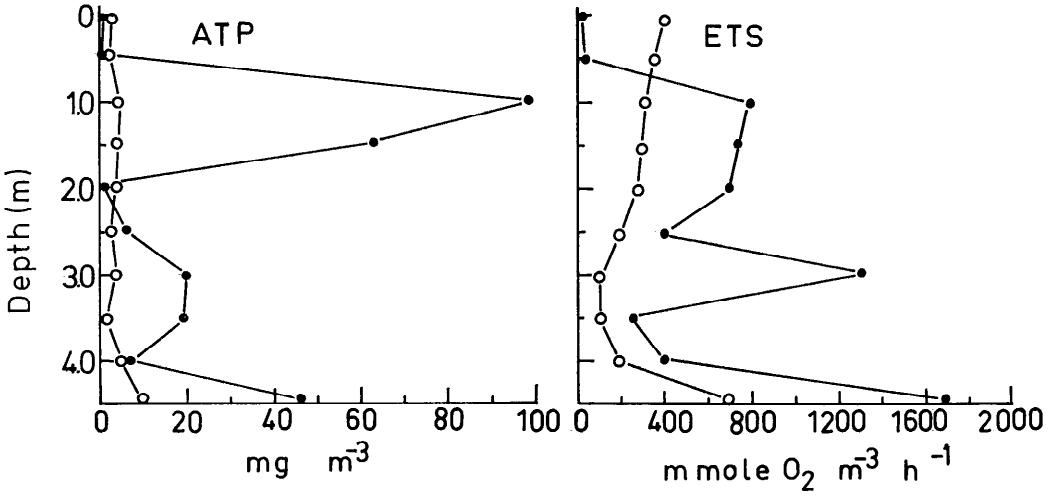

Fig. 12. Vertical distribution of adenosine triphosphate (ATP) and potential dehydrogenase activity (ETS) determined by tetrazolium reduction during holomixis (○) and stratification (●).

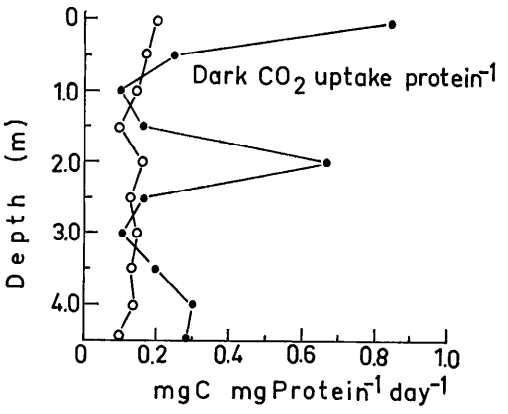

Fig. 13. Vertical distribution of dark CO_2 uptake : protein ratios during holomixis (○) and stratification (●).

lithotrophic community at the surface is situated above a mainly chemoorganotrophic bacterial layer at 1 m. Below that a mixed population of chemolithotrophic and phototrophic organisms appears at 2 m. At 2.5 m a mainly phototrophic community occurs, and the chemolithotrophic populations disappear. Peculiar minima of CO_2 uptake activity and low total biomass occur at 3.0–3.5 m. Close to the bottom, at 4.0 m, a major zone of both chemoorganotrophic and phototrophic communities appears.

ATP concentrations and ETS potential activities (Fig. 12) during maximal stratification show three distinct highly active layers at the thermocline (1.0 m), at 3 m, and at the bottom (4.5 m).

The specific activities of ATP and ETS per unit protein (Fig. 14) exhibit remarkably high peaks at 1.0 m and 3 m, respectively, with lesser peaks close to the bottom for both.

Discussion

The microorganisms in Solar Lake during holomixis are few, evenly distributed, and show both low and uniform specific activities for the biomass parameters measured. The holomictic period is very brief in contrast to other monomictic lakes of the northern hemisphere and occurs in summer.

However, during winter stratification, defined layers of mainly photosynthetic microorganisms alternate with layers where chemoorganotrophic or chemolithotrophic bacterial communities predominate. This spatial separation makes it easier to analyze the role of chemoorganotrophic and chemolithotrophic communi-

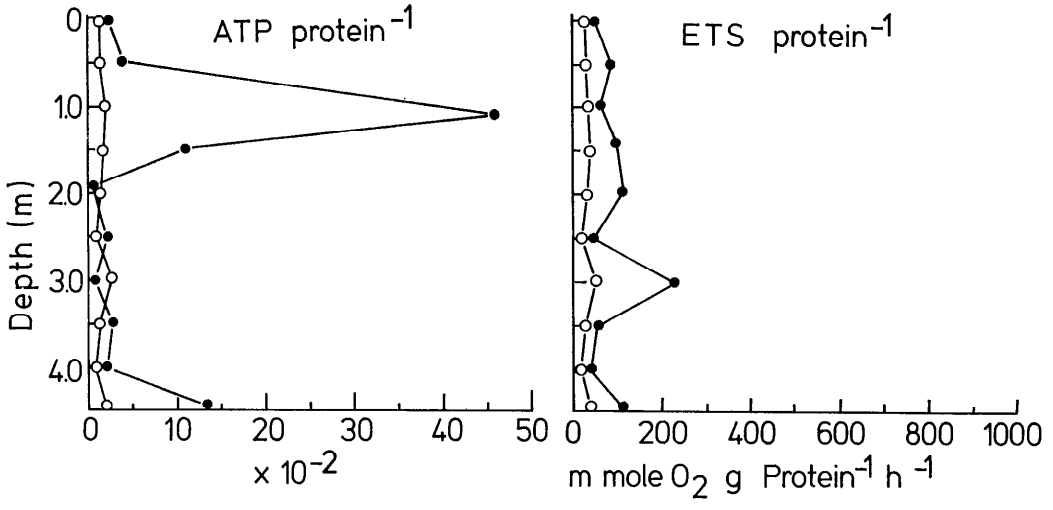

Fig. 14. Vertical distribution of adenosine triphosphate (ATP) : protein and potential dehydrogenase activity (ETS) : protein during holomixis (○) and stratification (●).

ties in the overall productivity of the lake. From our data on the vertical distributions of different types of microorganisms we are able to assign light and dark CO_2 incorporation maxima to specific physiological groups of microorganisms. The alternation of six separate bacterial plates, each with different specific activities, through a water column only 4.5 m deep, together with CO_2 incorporation rates as high as 4,960 mg C m^{-3} d^{-1} for light CO_2 incorporation (Cohen et al. 1977*b*) and 1,014 for dark CO_2 incorporation, makes Solar Lake an interesting model for studying the interrelations among phototrophic, chemoorganotrophic, and chemolithotrophic productivity.

According to Sorokin and Kadota (1972) an estimate of bacterial production could be made by assuming that 6% of the organic C produced by bacteria comes from CO_2 fixation. Sorokin (1969), Overbeck (1972*a*,*b*), and Tilzer (1972) have discussed this original assumption of Romanenko (1964) and Kusnetsov and Romanenko (1966). It has been stated however that the presence of chemolithotrophic bacteria (Sorokin 1969) and the effects of stratification in lakes may necessitate modification of this value, and Overbeck (1972*b*, 1974), from laboratory experiments, has pointed out that the generalization of Kusnetsov and Romanenko (1966) should be examined separately for each lake. Since there is no method yet available to estimate both CO_2 *and* dissolved organic carbon uptake at the same time, we are not able to judge the validity of the 6% assumption for Solar Lake during the stratified period. This point has often been missed in earlier studies.

In applying the 6% rule to a cold, high mountain lake, Tilzer (1972) suggested that the high bacterial production values he calculated were the result of winter degradation of organic material that was accumulated by photosynthetic communities in summer. Primary production in Solar Lake during stratification is extremely high, but it is not sufficient to sustain the enormous potential rates of bacterial production that are calculated from dark CO_2 uptake using the 6% ratio. The phototrophic production would be exceeded by a factor of 3 (4,960 vs. 16,900 mg C m^{-3} d^{-1}). Dark CO_2 incorporation by photosynthetic organisms is not responsible for the high dark CO_2 uptake values. For Solar Lake this can be seen from Figs. 9 and 11 where dark CO_2 uptake does not correspond spatially to biomass peaks of photosynthetic organisms. Galloway et al. (1974) and Goldman et al. (1972) have also indicated that algal dark CO_2 incorporation cannot explain the rates occurring in nature.

If the cyanobacteria are not contributing to the dark CO_2 uptake totals, it then follows either that the Romanenko rule does not apply to Solar Lake or that enormous amounts of dissolved organic carbon must be supplied to the water column from the benthic communities. Since Solar Lake's benthic communities are extremely productive photosynthetically (5–12 g C m^{-2} d^{-1}) (Krumbein et al. 1977) we cannot exclude the latter possibility. In fact, since the highest dark CO_2 uptakes were measured close to the bottom, and since all parameters measured during holomixis increase toward the bottom, it seems likely that heterotrophic bacterial productivity is higher than photosynthetic productivity in certain layers of Solar Lake during holomixis and stratification. Benthic production together with the photosynthetic productivity in the water column could furnish the organic matter necessary for the high potential chemoorganotrophic bacterial values calculated from the 6% rule.

Yet the validity of the 6% rule for certain specific strata of Solar Lake during stratification seems highly improbable. From Fig. 13 we can derive that the specific dark CO_2 incorporation is around 6% throughout the water column during holomixis. The same is true for stratification, except for the surface layer and a layer at 2 m where we calculated ratios of 40% and 35%. Chemolithotrophic or mixotrophic bacteria are present here, with budding bacteria predominating in the surface layer and thiobacilli at 2 m.

Another indication for high nonphotosynthetic bacterial production can be derived from ATP and ETS determinations and especially from the specific activities of the two parameters. During stratification ATP and ETS show extremely high peaks at 1.0-m depth, 0.5 m above the zone of major photosynthetic activity (Fig. 12). The ATP:protein ratio at this water depth is 4,500, far above ratios usually recorded from aquatic environments. Ratios between 4,000 and 100 have been recorded from Lake Kinneret (B. Cavari pers. com.). The high figure from Solar Lake may be explained by a combination of high nutrient concentrations immediately above the chemocline and a constant grazing stress exerted by a dense population of *Artemia salina* thriving at this layer. In all other layers of the stratified lake, and throughout the water column, during holomixis ATP:protein ratios are around 250, agreeing well with the average ratios found by Hamilton and Holm-Hansen (1967).

In contrast to ATP measurements, the ETS data do not show a high specific activity for the layer immediately above the chemocline. For all layers of the stratified lake and the whole holomictic lake ETS specific activities are quite low.

The heterogenity of the different bacterial communities in Solar Lake during stratification makes the estimation of productivity a difficult task. Present methods for determining bacterial productivity, and therefore also photosynthetic productivity, are not satisfactory. Accurate methods for comparing CO_2 uptake with dissolved organic carbon uptake are also needed. No common DOC source can be used for every lake. We would need to know the physiology of the major bacterial groups in a specific lake to be able to estimate uptake rates of specific organic compounds suitable for each known community. Our data, though an estimate, may help to clarify the role of chemoorganotrophic and chemolithotrophic productivity in comparison to photosynthetic productivity.

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Submitted: 16 April 1975
Accepted: 26 October 1976