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High chemoautotrophic primary production in Lake Kinneret, Israel: A neglected link in the carbon cycle of the lake

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Abstract

Intensive chemosynthetic microbial activity fueled by H_2S oxidation with dissolved O_2 was measured by ^{14}C fixation in the dark and in presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea in Lake Kinneret waters. This process occurred in water collected below the photic zone (20 m) at the chemocline in the late autumn (November–January) and close to the sediment water interface in May when the chemocline starts to form. Averaged depth-integrated chemoautotrophic primary production at the chemocline was 16% and 24% of the photosynthetic primary production during May and autumn, respectively. The maximal rates were measured in December 1992, reaching values of >90% of the photosynthetic rate. The $\delta^{13}C$ of particulate organic matter at the chemocline ranged between −27‰ and −39‰, the latter being associated with intensive chemosynthesis. These $\delta^{13}C$ values support our earlier hypothesis that chemoautotrophic bacteria constitute, directly or indirectly (through the microbial loop), a ^{13}C-depleted food source for the zooplankton in the lake during autumn and early winter. Mass and isotopic balance of carbon and H_2S suggest that chemosynthetic productivity may constitute 20%–25% of the primary production in Lake Kinneret annually.

Lake Kinneret, Israel, is a freshwater, warm monomictic lake, stratified for 7–8 months of the year. During stratification, aerobic warm (18°C–28°C) epilimnion and anoxic relatively cold (14°C–16°C) hypolimnion are formed. Primary production in the epilimnetic trophogenic layer reaches annual average values of ~1.8 g C m^{-2} d^{-1} (Berman et al. 1995). About 1% of this production is due to anoxygenic bacterial photosynthesis in the metalimnion that occurs mostly from June to September, when stable thermocline and a matching oxycline are observed between 14 and 20 m depth (Butow and Bergstein-Ben Dan 1992). In October, the thermocline and the oxycline deepened below the euphotic zone, excluding the photosynthetic metalimnetic primary production. However, the potential for chemosynthetic primary production, which can proceed in the dark at oxic-anoxic interfaces, still exists.

Oxic-anoxic interfaces are favorable for growth of chemosynthetic bacteria that oxidize H_2S and other reducing substances (ammonium, thiosulfate, and tetrathionate) to obtain energy for dark fixation of CO_2. Dark CO_2 fixation was reported in several marine locations, e.g., Saanich Inlet, British Columbia, Canada; Gotland Deep, Central Baltic; the Black Sea; Cariaco Trench; and the Solar Lake in Sinai (Jorgensen et al. 1979; Tuttle and Jannasch 1979; Juniper and Brinkhurst 1986; Brettar and Rheinheimer 1991) and in marsh sediments (Peterson et al. 1980), cold seeps, and deep-sea hydrothermal vents (Jannasch and Wirsen 1979). This system is often characterized by a variety of bacterial symbioses within marine invertebrates (Spiro et al. 1986). It was suggested that chemosynthesis, rather than photosynthesis, is the major source of reduced carbon in both hydrothermal vent communities and symbiotic vent invertebrates (Cavanaugh et al. 1981; Southward et al. 1981). In the last decade, chemoautotrophic activity was recorded in freshwater systems: Lake Cadagno (Switzerland), Lake Ciso (Spain), and Lake Mekkojarvi (Finland) (Pedros-Alio and Guererro 1991; Kuuopo-Leinikki and Salonen 1992; Camacho et al. 2001).

Carbon flow between different trophic levels in marine and freshwater ecosystems can be traced by stable carbon isotope ratios of various components in the food web (e.g., Fry and Sherr 1984; Rau et al. 1990; Kelley et al. 1998). Analysis of the Lake Kinneret food chain with repeated observations at different years showed that during the late fall to early winter, the zooplankton is characterized by very low $\delta^{13}C$ values (around −34‰) (Zohary et al. 1994). These negative $\delta^{13}C$ values for the zooplankton did not correspond to their classical potential food organisms (nanoplanktonic algae) which were 6‰–10‰ more positive. Sulfur oxidizing chemoautotrophic bacteria show highly negative $\delta^{13}C$ values (Ruby et al. 1987), similar to those displayed by Lake Kinneret zooplankton during the fall and after overturn (Zohary et al. 1994), suggesting that chemoautotrophs may serve as carbon source for zooplankton during this period. However, the role of chemoautotrophic bacteria in the food web of Lake Kinneret has never been documented directly, and their contribution to primary production (carbon fixation) in the

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lake has been considered to be of low importance (e.g., Serruya et al. 1980). In general, the contribution of chemoautotrophic bacteria to aquatic primary production has been considered unimportant (Rheinheimer 1992), and their role in the food web have been largely overlooked in freshwater ecosystems. The objectives of the present study were to examine the chemosynthetic processes at the oxic-anoxic interface in Lake Kinneret and to evaluate their significance in organic matter production and the carbon cycle in the lake.

Materials and methods

Sampling and field measurements—The study was conducted during the early (May–June) and late (October–December) stages of thermal stratification in Lake Kinneret between December 1992 and 1996, at Sta. A, the deepest part in the lake (42 m). Water samples were collected with a 2-liter horizontal PVC sampler or by use of a pump into dark sterile bottles, at 0.25 or 0.50 m intervals in the chemocline/thermocline zone and transferred to the laboratory. One liter was used for ^{14}C incubations, chlorophyll, bacterial counts, and chemical analysis, and the other liter was used for $\delta^{13}C$ measurements. Temperature was measured on board with an Applied Microsystems STD or with a YSI thermistor probe.

Chemosynthesis (^{14}C fixation)—Water from each depth was sampled with a narrow tube (avoiding air and gas exchange) into 60-ml glass bottles with glass stoppers (Wheaton). Each set consisted of light and dark treatments, with and without the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; Sigma) (5×10^{-6} M) to separate bacterial photosynthesis from eukaryotic one. The potential for ammonium oxidation was examined by addition of 15 μM of the inhibitor 2-chloro-6-trichloromethylpyridine (N-Serve; Sigma). Carbon fixation was measured by the addition of 200 μl of 25 μCi ml^{-1} $NaH^{14}CO_3$ (Amersham) to the experimental bottles, which were incubated for 3 h at 18°C–20°C. Two parallel controls for each depth were run: a time 0, filtered immediately after addition of the radiotracer, and a killed sample, to which 1 ml of 40% formaline + $HgCl_2$ was added and incubated as above. The fixation was terminated by filtering the samples on 0.45 μm nitrate cellulose membrane filters (Poretics) and washed five times with 10 ml of distilled water. The filters were put in scintillation vials and exposed overnight to concentrated fuming HCl, to eliminate unfixed ^{14}C. A volume of 10 ml scintillation liquid (Ultima Gold, Packard) were added to each vial and the vials were counted in a Kontron liquid scintillation counter. The importance of H_2S as an electron donor was studied by the addition of 10–200 μM of S^{-2} to water samples taken immediately after overturn in winter and at the formation of the chemocline above the sediments in May.

H_2S, O_2, NH_4^+, and NO_3^- measurements—Samples for H_2S and dissolved O_2 were collected in 300-ml BOD bottles. H_2S samples were treated with 2 ml of zinc acetate (2N) and 2 ml of sodium hydroxide (6N) on board. Concentrations of S^{-2} were measured by use of the iodometric method (American Public Health Association 1992). Dissolved O_2 was determined by the Winkler method (American Public Health Association 1992), and NH_4^+ and NO_3^- were measured spectrophotometrically according to standard methods (American Public Health Association 1992).

Chlorophyll *a* was measured by fluorometry after acetone extraction, according to the method of Holm-Hansen et al. (1965). Bacteria were counted under an epifluorescence microscope after staining with 4′,6-diamidino-2-phenylindole (DAPI; Sigma; Porter and Feig 1980). Protozoa counts were carried out after staining with DAPI, as described by Hadas and Berman (1998).

$\delta^{13}C$ of total particulate organic matter—Each bottle that contained suspended particles was separately filtered through a 47 diameter, precombusted GF/F filter. Isotopic measurements were conducted by use of an automated carbon nitrogen analyzer (ANCA-SL, Europa Scientific) connected to a VG-602 ratio mass spectrometer. Samples of ~0.5 mg of organic matter were placed within tin-foil capsules that were tightly squeezed and then automatically dropped into a combustion tube of 1,000°C under a stream of He. Combustion is commenced with addition of highly purified O_2 gas, followed by a Cu reduction furnace at 600°C. The CO_2 and N_2 gasses produced are separated by a GC column and are injected via a capillary tube directly to the source of the mass spectrometer. The masses 44 and 45 are integrated for each sample, and standards of known isotopic composition (our working standard is glycine, BDH, with $\delta^{13}C$ of −35.85‰) are used to calibrate the system. The masses 28 and 29 were measured and used only for the C/N ratios. All samples were analyzed in duplicates, and standards were analyzed in pairs after every eight samples. The average precision of $\delta^{13}C$ duplicates run consecutively was ~0.1‰, whereas the standards treated as samples at random give an average precision of ~0.2‰.

Results

In situ measurements—Oxygen was evenly distributed within the water column during winter, reaching values of ~10 mg L^{-1}, and oversaturation occurred in the epilimnion during the annual Peridinium bloom in spring (Fig. 1A). Since April, the oxic–anoxic (H_2S–O_2) interface was formed near the sediments and rose to a 14-m depth in June, where it remained stable until late September (Fig. 1B). The depths profiles of temperature, H_2S, and oxygen in the water column in autumn 1993 (Fig. 2) showed great similarities to those obtained at previous years and are considered here as representative for the yearly process of thermocline/chemocline deepening below the euphotic zone during autumn. The epilimnetic temperature was high (26.7°C) in mid-October but decreased to 16.9°C by January, just before overturn. During that period, the thermocline deepened by 15 m, and epilimnetic oxygen concentrations were in the range of 7–9 mg L^{-1}. Hypolimnetic sulfide concentration increased with time from 0.2 mg L^{-1} to a maximum of 13 mg L^{-1} as the result of intensive sulfate reduction processes. The chemocline was characterized by steep gradients of temperature, sulfide, and oxygen and a narrow (0.5–5 m) layer where the two coexisted (up to 0.4 mg L^{-1} of oxygen and 0.2 mg L^{-1} of H_2S).

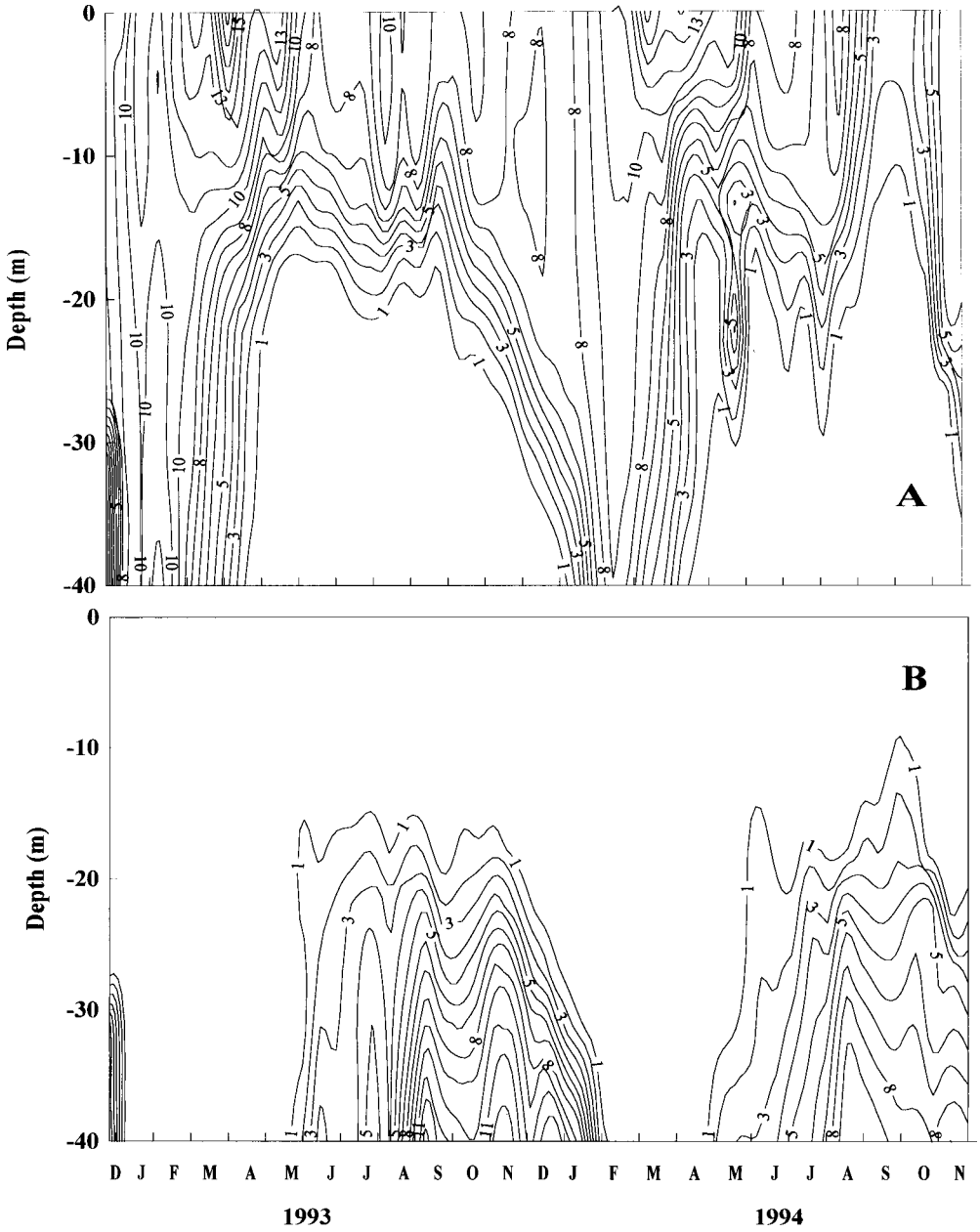

Fig. 1. (A) Oxygen (mg L^{-1}) and (B) sulfide (mg L^{-1}) distribution in the water column at Sta. A in Lake Kinneret during the years 1993–1994.

As the thermocline deepened, the temperature gradient was smaller, but the steep gradients of O_2 and H_2S at the interface (the chemocline) were maintained or even increased (Fig. 2). From the onset of stratification, NH_4^+ accumulated in the hypolimnion, reaching a maximum of 1.89 mg N L^{-1} at 40 m early in January 1994, before overturn (Fig. 3). Generally, NH_4^+ values in the hypolimnion in autumn were in the range of 0.44–1.42 mg N L^{-1}, and NO_3^- was depleted then from the hypolimnion. In the pelagic water column of Lake Kinneret during autumn, the chemocline depth corresponds to the thermocline, whereas during spring, when the thermocline and chemocline begin to develop, they are at different depths. The thermocline develops by warming of surface water in the epilimnion, whereas the chemocline is developed at the sediment-water interface as a result of the anaerobic mineralization processes. Organic matter supplied by the sinking remains of the *Peridinium* bloom and intensive sulfate reduction in the upper layer of the sediments resulted in an H_2S–O_2 interface and the formation of the water column chemocline. At the shallower areas of the lake, the H_2S–O_2 interface was favorable for benthic Beggiatoa growth that progressed toward pelagic regions.

$^{14}CO_2$ fixation through chemosynthetic activity—To exclude anoxygenic photosynthesis, $^{14}CO_2$ fixation at Sta. A was studied when the interface was below the euphotic zone.

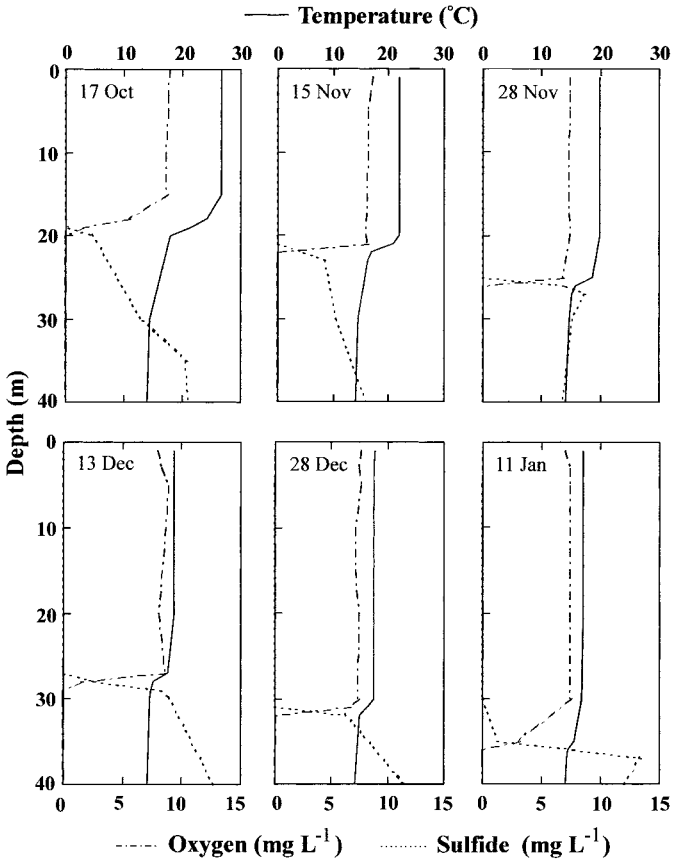

Fig. 2. Deepening of the thermocline/chemocline during autumn 1993 in Lake Kinneret.

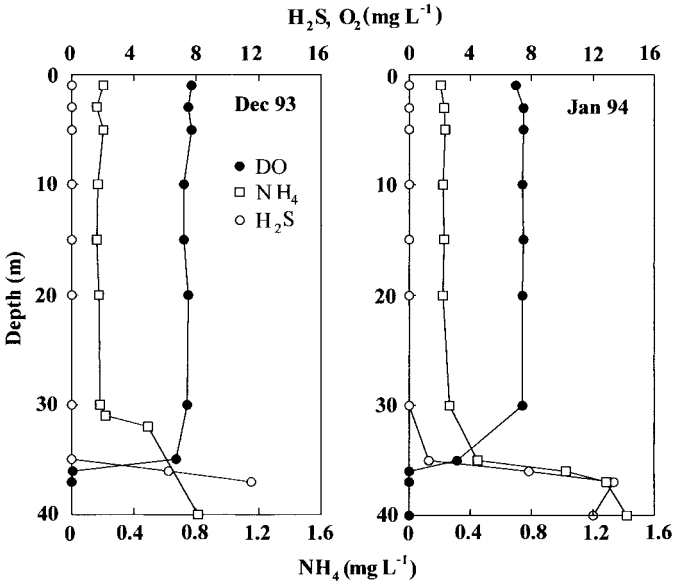

Fig. 3. Depth profiles of dissolved oxygen (DO), NH_4, and H_2S concentrations (mg L^{-1}), in Lake Kinneret in December 1993 and January 1994, representative of the period before the overturn.

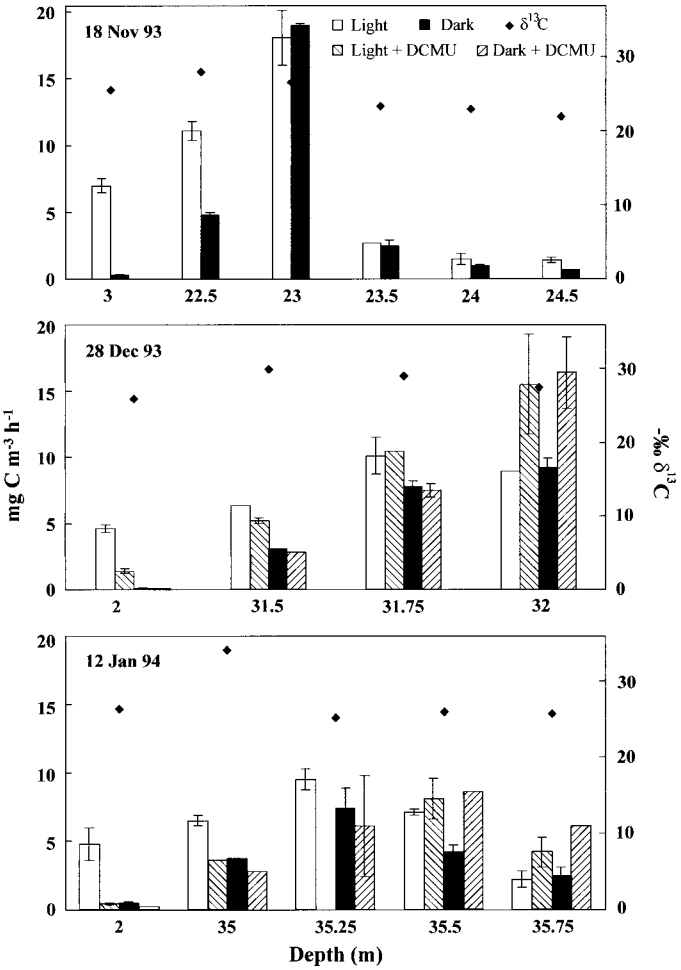

Fig. 4. Rates of photosynthetic and chemosynthetic carbon fixation with and without the addition of DCMU and the isotopic composition of the total particulate organic matter at 2–3 m and at the chemocline depths at Sta. A during autumn 1993.

Measured rates of dark $^{14}CO_2$ fixation were in the range of 0.4–40 mg C m^{-3} h^{-1}, with highest values at 32.5 m depth at Sta. A in December 1996 (Figs. 4–6). In December 1994, a chemocline of ~2 m was located at 32.5–34 m depth (Fig. 6). The fixed carbon values were much higher at 32.5–33 m in the chemocline (23.4 ± 2.2 mg C m^{-3} h^{-1}) compared with the photosynthetic activity in the euphotic zone at 2m depth (5 ± 0.9 mg C m^{-3} h^{-1}). Furthermore, in water from 2 m depth, carbon fixation was totally inhibited in the dark and in the presence of DCMU, because nanoplanktonic algae were dominant in photosynthetic carbon fixation at this depth. At the same time, dark carbon fixation at the chemocline was entirely microbial and DCMU resistant. Similarly, the 4 m chemocline region, which expanded from 38 to 42 m during May 1995 (Fig. 6), showed chemosynthetic activity in the range of 9.83 ± 0.15 to 12 ± 0.15 mg C m^{-3} h^{-1}, which was not affected by the addition of the photosynthetic inhibitor DCMU.

NH_4^+ oxidation—NH_4^+ accumulated in the hypolimnion (see above), and carbon fixation due to chemosynthetic ac-

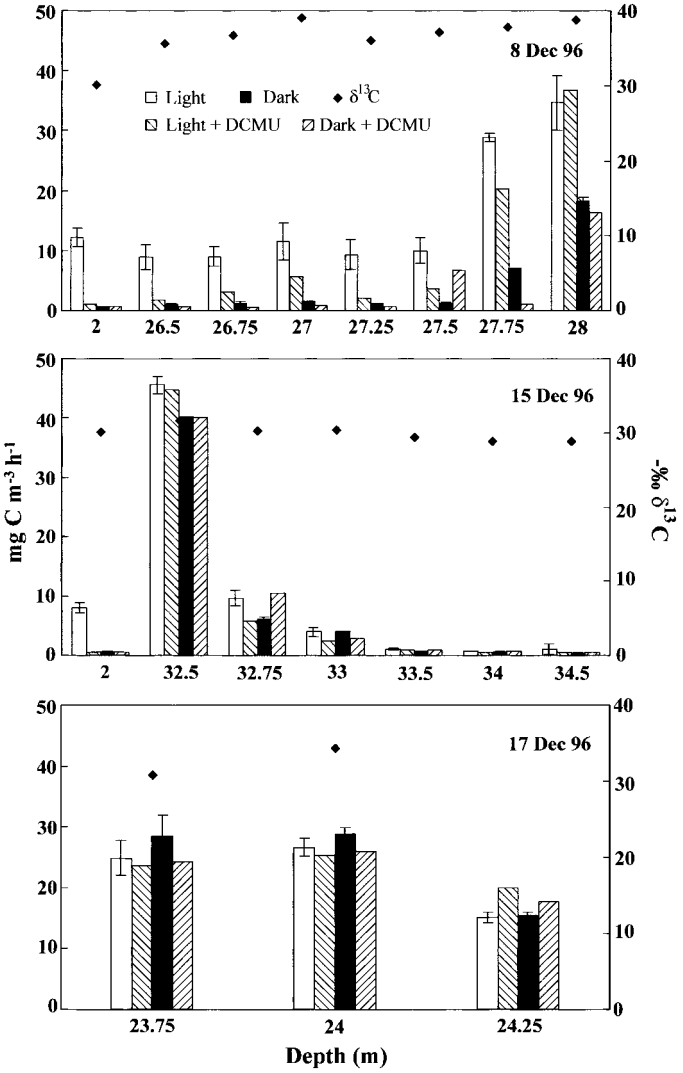

Fig. 5. Rates of photosynthetic and chemosynthetic carbon fixation and the shifting of the chemocline in December 1996.

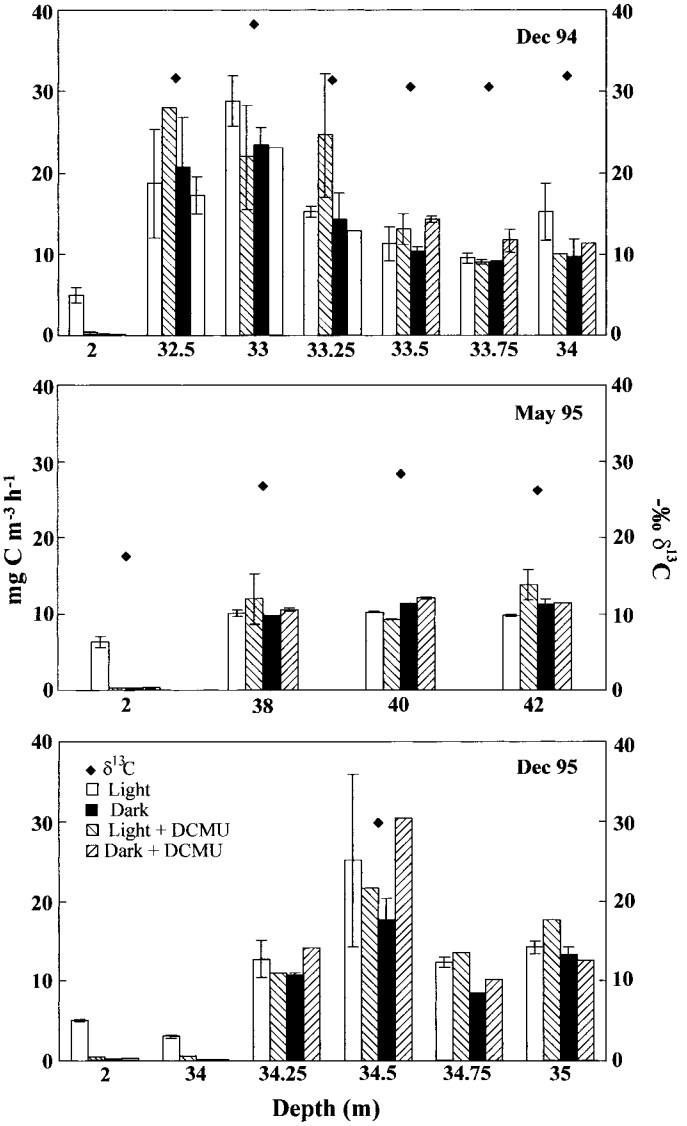

Fig. 6. Rates of photosynthetic and chemosynthetic carbon fixation with and without the addition of DCMU and the isotopic composition of the total particulate organic matter at 2–3 m and at the chemocline depths at Sta. A in December 1994 and May and December 1995.

tivity by ammonia oxidizers could not be excluded. Because inhibition of ammonia oxidizers by addition of N-Serve resulted in mainly unchanged carbon synthesis rates, the role of ammonia oxidizers proved to be negligible (Table 1). The same trend was observed in three experiments done in different years. Furthermore, concentrated bacteria samples from the respective depths were sent for 16 S rRNA hybridization with specific probes to identify ammonia oxidizers (Nitrosomonas, Nitrospira, unpubl. data). The abundance of these bacteria was low, which confirmed the results obtained in our experiments and implied that the chemoautotrophic population was mostly composed of H_2S oxidizers at the depths studied. This was confirmed in studies with sediment

Table 1. The effect of N-serve on chemosynthetic activity (mg C m^{-3} h^{-1}) in the chemocline at station A in Lake Kinneret.

Depth (m)	Light	Dark	Light + N-serve	Dark + N-serve
2	8 ± 0.9	0.6 ± 0.04	8.58 ± 0.42	0.7 ± 0.06
32.5	45.56 ± 1.48	40.17 ± 2.74	44.97 ± 4.96	43.45
32.75	9.6 ± 0.02	6.14 ± 0.48	5.48 ± 0.65	13.13 ± 0.47
33	3.94 ± 0.8	3.98 ± 1.74	3.3 ± 0.15	3.4 ± 0.86
33.5	1.06 ± 0.12	0.76 ± 0.05	1.15 ± 0.01	1.08 ± 0.13
34	0.7 ± 0.02	0.57 ± 0.03	0.71 ± 0.01	0.72 ± 0.05

Table 2. Chemosynthetic activity (carbon fixation, mg C m^{-3} h^{-1}) at the formation of the chemocline (May) at Station A in Lake Kinneret, and the effect of the addition of S^{-2}.

				S^{-2} added (μm)				
Date	Depth (m)	Light	Dark	10	20	40	100	200
03 May 93	2	133.9 ± 7.2	0.69 ± 0.3					
03 May 93	42	4.2 ± 0.2	0.97 ± 0.04				26.2 ± 1.5	5.5 ± 2.2
03 May 93	Overlying water	23.7 ± 1.4	16 ± 3.5				34.1	11.2
17 May 93	42	25.36	30.3 ± 2.3	32.3 ± 6.1	33.8 ± 2.3	38.4 ± 2.4	26 ± 0.1	

overlying water sampled 2 d after overturn (the whole water column was oxygenated), when dark carbon fixation was only 7% of the photosynthetic carbon fixation (3.1 and 45.7 mg C m^{-3} h^{-1}, respectively). The addition of 150 μl of S^{-2} to these samples resulted in an increase in dark carbon fixation to values of 50.3 mg C m^{-3} h^{-1}, which suggests depletion in electron donor source (S^{-2}) but the presence and potential activity of the chemoautotrophic population. The same trend was observed in May, during the formation of the chemocline (Table 2).

$\delta^{13}C$ of total particular carbon (TPC)—The $\delta^{13}C$ values in the chemocline were negative in the range of −27‰ to −39‰ with the most negative values in autumn, when the thermocline/chemocline was below 25 m (Figs. 4–6). In May, the $\delta^{13}C$ at 2 m depth was −17‰ representing the euphotic zone at the end of the *Peridinium gatunense* bloom, when it is most enriched in ^{13}C (Erez et al. 1998). At this period, the degradation of the *P. gatunense* bloom occurred, and part of the cells sank toward the hypolimnion and the sediments, contributing to the heavier isotopic composition of TPC in the chemocline compared with the chemocline in autumn (Fig. 6). The most negative $\delta^{13}C$ values of the TPC were always associated with highest chemosynthetic rates and hence can be attributed mostly to H_2S oxidizing bacteria. There are two main possibilities to explain the low $\delta^{13}C$ values displayed by the chemosynthetic bacteria: (1) the $\delta^{13}C$ of the hypolimnis low in the range of −6‰ to −8‰ (Stiller and Nissenbaum 1999), and (2) the pH in these waters is 7.8 and hence the $CO_{2(aq)}$ concentrations are high, well above 100 μM, so that the bacteria can fractionate carbon isotopes at their maximum capacity. This subject, however, was not investigated directly during this research and requires further study.

Protozoa composition—With the deepening of the thermocline and oxycline below the euphotic zone in October, there was an increase in both numbers and biovolumes of ciliates in this region. In December 1996 at 32.5 m depth, almost a pure culture of *Coleps hirtus* was observed, (180 *Coleps* ml^{-1}), corresponding to high dark carbon fixation (~40 mg C m^{-3} h^{-1}) and a low $\delta^{13}C$ value of −31.59‰, which suggests feeding of *Coleps* on chemoautotrophic bacteria (Fig. 5). Other protozoa present were the ciliates *Cyclidium, Vorticella mayerii,* and small flagellates. Ciliate population, obtained by differential filtration, showed $\delta^{13}C$ of −27‰, which suggests that ciliates were feeding not only on bacteria but also on nano- and pico-sized algae, as well as flagellates and small ciliates with higher $\delta^{13}C$ composition. This feeding behavior was also confirmed by microscopic observations. During the formation of the chemocline (April–May), high numbers of ciliates (64 ml^{-1}), were observed near the bottom sediments. They consisted mainly of anaerobic or facultative ciliates (*Saprodinium dentatum, Plagiopyla*), which were feeding on sulfur cycle bacteria (i.e., *Beggiatoa*).

Chlorophyll and bacteria numbers—In autumn, phytoplankton populations in the euphotic zone consisted mostly of chlorophytes and cyanophytes with small biomass and low chlorophyll values of 6.7–15.8 μg L^{-1}. In the chemocline below the euphotic zone, chlorophyll values were lower (3.3 ± 0.86 μg L^{-1}), consisting of small numbers of decomposing algae such as *Melosira, Cyclotella, Synedra, Microcystis, Chroococcus, Scenedesmus,* and *Cosmarium.* In April–May, during the formation of the chemocline and before thermal stabilization, chlorophyll values near the bottom ranged from 11.2 to 67 μg L^{-1} because of sinking algae. In the euphotic zone chlorophyll values were high (~150 μg L^{-1}), because it was the end of the *Peridinium* bloom. Bacteria numbers were in the range of 2–7 × 10^6 ml^{-1}, but no specific distribution trend was observed. On 24 April 1994, thymidine uptake at the chemocline depth (40 m) was 20—50 times higher than in the rest of the water column, implying high bacterial activity (data not shown). Microscopic observation of samples from the chemocline depth revealed dense populations of chemoautotrophic bacteria, such as *Macromonas bipunctata, Thiovulum,* and *Beggiatoa,* all of which contained sulfur granules.

Discussion

Dark CO_2 fixation in Lake Kinneret—Intensive dark inorganic carbon fixation was observed during the course of the present study in Lake Kinneret at the oxic-anoxic interface where sulfide and oxygen coexist, and this was attributed mainly to H_2S-based bacterial chemosynthesis. Earlier qualitative single observation on dark CO_2 fixation in Lake Kinneret was reported by Serruya (1972), who noted increased ^{14}C fixation at the chemocline, relating it to chemosynthetic processes. More recently, Herzig and Butow (1993) described high levels of dark $^{14}CO_2$ fixation especially at chemocline depth in Lake Kinneret, which suggests that algal photosynthetic primary producers were responsible for these dark carbon fixations. In view of the present findings, it is most probable that their observations might be explained by H_2S-based chemosynthetic primary production.

Photosynthetic primary production in the euphotic zone

Table 3. Chemosynthetic production in comparison to photosynthetic production at Sta. A. Data represent depth integrated values for three characteristic seasons in Lake Kinneret.

Period	Primary production (mg C m^{-2} d^{-1})	Chemosynthetic production (mg C m^{-2} d^{-1})	% of chemosynthetic out of primary production
Beginning of summer (May)	3276 ± 972 (n = 4)	533 ± 453 (n = 4)	16% (range 9–28)
Autumn (Oct–Dec)	1300 ± 686 (n = 12)	314 ± 172 (n = 12)	24% (range 8–92)
Summer (June 1997)	1883 (n = 4)	305.7 (n = 2)	16% (range (11–16)

Integrated depths for photosynthetic primary production 1–15 m, chemosynthetic production at the chemocline ranging from 20 to 35.5 m.

(0–15 m) from October to December averaged 1,300 ± 686 mg C m^{-2} d^{-1} during the years 1992–1996. Chemoautotrophic primary production at the chemocline (below the euphotic zone) at the same period averaged 314 ± 172 mg C m^{-2} d^{-1}—i.e., 24% of the photosynthetic primary production. Maximal monthly value for chemosynthesis was observed in December 1992, when it reached 92% of the photosynthetic primary production. In May, photosynthetic and chemosynthetic primary production values averaged 3276 ± 972 and 533 ± 453 mg C m^{-2} d^{-1}, respectively—i.e., chemosynthetic production was 16% of photosynthetic production (Table 3). The difference in primary production between the two periods was due to the characteristic yearly bloom of *Peridinium gatunense* in winter/spring, with 7–10 times higher biomass than that of nano-phytoplankton in summer/autumn (Berman et al. 1995).

These results suggest that bacterial chemolithotrophic production in Lake Kinneret is significant, and it must be considered in the carbon cycling and budget of the lake. It should be noted, however, that this production was energetically derived from organic carbon, which at an earlier stage was fixed by photosynthesis. The use of reduced compounds (sulfide, ammonium, and methane) that originate from energetically inefficient anaerobic metabolism can be partly compensated by use of the energy transferred to these compounds. In this way, at least a part from the photosynthetic energy potentially convertible to biomass under aerobic conditions can be gained back by chemosynthesis, which otherwise would be completely lost for the system. In the Black Sea and the Cariaco Trench, maximum dark CO_2 fixations were in the range of 3–6 mg C m^{-2} d^{-1}, much lower than those in Lake Kinneret. However, the rates of photosynthetic primary production in these locations (140–480 mg C m^{-2} d^{-1}) were also much lower than those measured in Lake Kinneret. In Sannich Inlet, British Columbia, Canada, photosynthetic productivity rates (1,100 mg C m^{-2} d^{-1}) were closer to the rates observed in Lake Kinneret, whereas the chemosynthetic dark fixation was about 20 times lower, in the range of 24 mg C m^{-2} d^{-1} (Tuttle and Jannasch 1979; Juniper and Brinkhurst 1986). In Lake Ciso (Spain), dark carbon fixation was between 1% and 20% of oxygenic carbon fixation during holomixis and stratification, respectively (Pedros-Alio and Guerrero 1991).

The thickness and depth of the chemocline layer in the pelagic waters in the lake changed because of internal seiches, induced by daily westerly winds during the stratified period. The amplitude of the thermocline tilt during the seiches can reach 10 m within a period of 24 h, with decreasing oscillations after the wind stops. This generates shear flow and turbulence contributing to mixing and displacement of water layers within the metalimnion (Serruya 1975; Ostrovsky et al. 1996). These daily mixing events may potentially enhance the chemosynthetic process because it promotes the mixing between O_2 and H_2S within the chemocline. In our study, during 2 d between 15 and 17 December 1996, the location of the chemocline shifted upward by 8 m, from 32 to 24 m, resulting in chemoautotrophic dark CO_2 fixation of 40 and 29 mg C m^{-3} h^{-1}, respectively (Fig. 5).

Chemosynthesis in Lake Kinneret is probably not restricted to the chemocline of the pelagic waters only but may also exist in the benthic environments and particularly in the littoral zone (Hadas et al. 2000). This region of the lake is characterized by the highest primary productivity during the period of Peridinium bloom. The appearance of white filamentous bacterial mat communities (i.e., *Beggiatoa*), on sulfide-rich sediments, indicates high organic load and oxygen depletion (Sweerts et al. 1990), which suggests that high chemosynthetic activity may occur at this area. Anyhow, because photosynthesis is usually also higher at the littoral areas, the proportion or photosynthetic to chemosynthetic production in the littoral may possibly be unchanged.

Other anoxygenic autotrophic bacteria in the lake are the photosynthetic bacteria appearing in the metalimnion from June to September, with a bloom of the green sulfur bacterium *Chlorobium phaeobacteroides*, which may persist from 6 to 13 weeks. However, the contribution of sulfur photosynthetic bacteria to the total primary productivity of the lake was negligible, ~1% (Butow and Bergstein-Ben Dan 1992). The bacterial chemoautotrophic activity measured in this study was not inhibited in the dark or DCMU, meaning that photosynthetic activity by bacteria, algae, or cyanobacteria below the euphotic zone in the pelagic waters was insignificant.

The role of chemoautotrophs in the Kinneret food web as determined from stable carbon isotope ratios—In a previous study in Lake Kinneret, we found that $\delta^{13}C$ declined throughout autumn reaching minimal values in zooplankton (−33.8‰) in January, shortly after the overturn (Zohary et al. 1994). It was then suggested that this large isotopic shift might be explained by chemosynthetic production with $\delta^{13}C$ of −35‰. In the present study, we found that in the chem-

ocline the $\delta^{13}C$ of the organisms (and their protistan predators) was very negative, ranging between −27‰ and −39‰, and epilimnetic TPC was 2‰–9‰ more positive (ranging −18‰ to −30‰). Zooplankton, which seem to feed mainly on the chemosynthetic community (probably also on their predator ciliates), displayed very low $\delta^{13}C$ values (−30‰ to −34‰). It is possible that the zooplankton prefer the chemosynthetic production, because it is concentrated in a rather narrow and very dense layer (of roughly 1–2 m). During the early stratified period (May), when chemosynthesis occurs near the sediment water interface, a difference approximately −9% is observed between the chemosynthetic and the photosynthetic producers. During the fall and early winter, the chemosynthetic primary production provides enough carbon to isotopically label the entire zooplankton with its negative $\delta^{13}C$ within ~2 months. This implies that very high rates of chemosynthesis occurred during this period, as indeed were observed by our ^{14}C incubations.

A high degree of fractionation is characteristic of sulfur oxidizing chemoautotrophs in general (Ruby et al. 1987). The $\delta^{13}C$ of dissolved inorganic carbon in the hypolimnion of Lake Kinneret is about −6‰ to −8‰ (Stiller and Nissenbaum 1999), and $CO_{2(aq)}$ must be lower by 8‰–9‰ (Deuser et al. 1968). If the average isotopic composition of the chemosynthetic bacteria is −35‰, they seem to fractionate by ~20‰ relative to the $CO_{2(aq)}$ source in agreement with the study of Ruby et al. 1987.

Measurements from 28 temperate lakes showed that zooplankton were depleted in ^{13}C relative to smaller planktonic fractions, and metalimnic zooplankton were lighter than any other fraction in the food web (Del Giorgio and France 1996), similar to the situation found in Lake Kinneret.

Protozoa distribution profiles showed that ciliates were abundant in the lake at the chemocline-thermocline region during autumn and at the sediment-water interface in spring. Their species composition was dominated by ciliates feeding on bacteria of the sulfur cycle (Hadas and Berman 1998; Madoni 1990). The fact that we did not find large changes in bacterial numbers in the water column in autumn supports the idea that at the chemocline as well as in other water layers grazing controlled bacteria numbers. Similar ciliate distribution and grazing pressure at the oxic-anoxic interface was described for the Danish Fjords (Fenchel et al. 1990). In the Black Sea, in the O_2–H_2S boundary layer, protist community was considered to be the main factor in consumption of chemoautotrophic bacterial production, and the microplankton community can be, to a certain extent, a factor in the biological control of H_2S in the water column (Zubkov et al. 1992).

References

AMERICAN PUBLIC HEALTH ASSOCIATION. 1992. Standard methods for examination of water and waste water, 18th ed. APHA.

BERMAN, T., L. STONE, Y. Z. YACOBI, B. KAPLAN, M. SCHLICHTER, A. NISHRI, AND U. POLLINGHER. 1995. Primary production and phytoplankton in Lake Kinneret: A long-term record (1972–1993). Limnol. Oceanogr. **40:** 1064–1076.

BRETTAR, I., AND G. RHEINHEIMER. 1991. Denitrification in the Central Baltic: Evidence for H_2S oxidation as motor for denitrification at the oxic-anoxic interface. Mar. Ecol. Prog. Ser. **77:** 157–169.

BUTOW, B., AND T. BERGSTEIN-BEN DAN. 1992. Occurrence of *Rhodopseudomonas palustris* and *Chlorobium phaeobacteroides* blooms in Lake Kinneret. Hydrobiologia **232:** 193–200.

CAMACHO, A., J. EREZ, A. CHICOTE, M. FLORIN, M. M. SQUIRES, C. LEHMANN, AND R. BACHOFEN. 2001. Microbial microstratification, inorganic carbon photoassimilation and dark carbon fixation at the chemocline of the meromictic Lake Cadagno (Switzerland) and its relevance to the foodweb. Aquat. Sci. **63:** 91–106.

CAVANAUGH, C. M., S. L. GARDINER, M. L. JONES, H. W. JANNASCH, AND J. B. WATERBURY. 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila Jones:* A possible chemosynthetic symbiont. Science **213:** 340–342.

DEL GIORGIO, P. A., AND R. L. FRANCE. 1996. Ecosystem specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. Limnol. Oceanogr. **41:** 359–365.

DEUSER, W. G., E. T. DEGANS, AND R. R. L. GUILLARD. 1968. Carbon isotope relationship between plankton and sea water. Geochim. Cosmochim. Acta **32:** 657–660.

EREZ, J., A. BOUEVITCH, AND A. KAPLAN. 1998. Carbon isotope fractionation by photosynthetic aquatic microorganisms: Experiments with Synechococcus

PCC 7942 and simple carbon flux model. Can. J. Bot. **76:** 1109–1118.

FENCHEL, T., L. D. KRISTENSEN, AND L. RASMUSSEN. 1990. Water column anoxia: Vertical zonation of planktonic protozoa. Mar. Ecol. Prog. Ser. **62:** 1–10.

FRY, B., AND E. SHERR. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and fresh waters ecosystems. Contrib. Mar. Sci. **27:** 13–47.

HADAS, O., AND T. BERMAN. 1998. Seasonal abundance and vertical distribution of protozoa (flagellates, ciliates), and bacteria in Lake Kinneret, Israel. Aquat. Microb. Ecol. **14:** 161–170.

———, N. RUSHANSKY-MALINSKY, R. PINKAS, E. HALICZ, AND J. EREZ. 2000. High chemoautotrophic primary production across a transect in Lake Kinneret, Israel. Arch. Hydrobiol. Spec. Iss. Adv. Limnol. **55:** 413–420.

HERZIG, R., AND B. BUTOW. 1993. The underestimated importance of dark carbon fixation to primary productivity in Lake Kinneret. Wat. Sci. Tech. **27:** 373–380.

HOLM-HANSEN, O., C. J. LORENZEN, R. W. HOLMES, AND J. D. H. STRICKLAND. 1965. Fluorometric determination of chlorophyll. J. Cons. Perm. Int. Explor. Mer. **30:** 3–15.

JANNASCH, H. W., AND C. O. WIRSEN. 1979. Chemosynthetic primary production at East Pacific sea floor spreading centers. BioScience **29:** 592–598.

JORGENSEN, B. B., J. G. KUENEN, AND Y. COHEN. 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol. Oceanogr. **24:** 799–822.

JUNIPER, S. K., AND R. O. BRINKHURST. 1986. Water-column dark CO_2 fixation and bacterial mat growth in intermittently anoxic Saanich Inlet, British Columbia. Mar. Ecol. Prog. Ser. **33:** 41–50.

KELLEY, C. A., R. B. COFFIN, AND L. A. CIFUENTES. 1998. Stable isotope evidence for alternative bacterial carbon sources in the Gulf of Mexico. Limnol. Oceanogr. **43:** 1962–1969.

KUUPPO-LEINIKKI, P., AND K. SALONEN. 1992. Bacterioplankton in a small polyhumic lake with an anoxic hypolimnion. Hydrobiologia. **222:** 159–168.

MADONI, P. 1990. The ciliated protozoa of the monomictic Lake Kinneret (Israel): Species composition and distribution during stratification. Hydrobiologia. **190:** 111–120.

OSTROVSKY, I., Y. Z. YACOBI, P. WALLINE, AND I. KALIKHMAN.

1996. Seiche induced mixing: Its impact on lake productivity. Limnol. Oceanogr. **41:** 323–332

PEDROS-ALIO, C., AND R. GUERRERO. 1991. Abundance and activity of bacterioplankton in warm lakes. Verh. Int. Verein Limnol. **24:** 1212–1219.

PETERSON, B. J., F. HOWARTH, F. LIPSCHULTZ, AND D. ASHENDORF. 1980. Salt marsh detritus: An alternative interpretation of stable carbon isotope ratios and the fate of *Spartina alterniflora.* Oikos **34:** 173–177.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943–948.

RAU, G. H., J.-L. TEYSSIE, F. RASSOULZADEGAN, AND S. W. FOWLER. 1990. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size-fractionated marine particles: Implications for their origin and trophic relationships. Mar. Ecol. Prog. Ser. **59:** 33–38.

RHEINHEIMER, G. 1992. Production and breakdown of organic substances, p. 183–210. *In:* G. Rheinheimer [ed.], Aquatic microbiology. John Wiley & Sons.

RUBY, E. G., H. W. JANNASCH, AND W. G. DEUSER. 1987. Fractionation of stable carbon isotopes during chemoautotrophic growth of sulfur-oxidizing bacteria. Appl. Environ. Microbiol. **53:** 1940–1943.

SERRUYA, C. 1972. Metalimnic layer in Lake Kinneret, Israel. Hydrobiologia **40:** 355–359.

———. 1975. Wind, water temperature and motion in Lake Kinneret: General pattern. Int. Verh. Theor. Angew. Limnol. Verh. **19:** 73–87.

———, M. GOPHEN, AND U. POLLINGHER. 1980. Lake Kinneret: Carbon flow patterns and ecosystem management. Arch. Hydrobiol. **88:** 265–302.

SPIRO B., P. B. GREENWOOD, A. J. SOUTHWORD, AND P. R. DANDO. 1986. $^{13}C/^{12}C$ ratios in marine invertebrates from reducing sediments: Confirmation of the nutritional importance of chemoautotrophic endosymbiotic bacteria. Mar. Ecol. Prog. Ser. **28:** 233–240.

SOUTHWARD, A. J., E. C. SOUTHWARD, P. R. DANDO, G. H. RAU, H. FELBECK, AND H. FLUGEL. 1981. Bacterial symbionts and low $^{13}C/^{12}C$ ratios in tissues of *Pogonophora* indicate unusual nutrition and metabolism. Nature **293:** 616–620.

STILLER, M., AND A. NISSENBAUM. 1999. A stable carbon isotope study of dissolved in organic carbon in hardwater Lake Kinneret (Sea of Galilee). S. Afr. J. Sci. **95:** 166–170.

SWEERTS, J.-P., AND OTHERS. 1990. Denitrification of sulfur oxidizing Beggiatoa spp. mats on freshwater sediments. Nature **344:** 762–763.

TUTTLE, J. H., AND H. W. JANNASCH. 1979. Microbial dark assimilation of CO_2 in the Cariaco Trench. Limnol. Oceanogr. **24:** 746–753.

ZOHARY, T., J. EREZ, M. GOPHEN, I. BERMAN-FRANK, AND M. STILLER. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnol. Oceanogr. **39:** 1030–1043.

ZUBKOV, M. V., A. F. SAZHIN, AND M. V. FLINT. 1992. The microplankton organisms at the oxic-anoxic interface in the pelagical of the Black Sea. FEMS. Microbiol. Ecol. **101:** 245–250.

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