Aquat.sci.63 (2001) 91–106
1015-1621/01/010091-16 $ 1.50+0.20/0

Aquatic Sciences

Microbial microstratification, inorganic carbon photoassimilation and dark carbon fixation at the chemocline of the meromictic Lake Cadagno (Switzerland) and its relevance to the food web

Antonio Camacho[1,*], Jonathan Erez[2], Alvaro Chicote[3], Máximo Florín[3], Margaret M. Squires[4], Christine Lehmann[5] and Reinhard Bachofen[5]

[1] Department of Microbiology and Ecology, and Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, E-46100 Burjassot, Spain
[2] Institute of Earth Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
[3] Department of Ecology, Universidad Autonoma de Madrid, E-28049 Madrid, Spain
[4] Department of Geography, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6
[5] Institute of Plant Biology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich, Switzerland

Key words: Chemocline, deep chlorophyll maximum, photo- and chemoautotrophic bacteria, dark carbon fixation, oxic-anoxic food web.

ABSTRACT

The microstratification of the microbial community at the chemocline of Lake Cadagno and the associated inorganic carbon fixation activity was studied by fine layer sampling. A deep chlorophyll maximum caused by diatoms overlying *Cryptomonas* was found at the upper edge of the chemocline. A high population density of phototrophic sulphur bacteria, mainly *Amoebobacter* cf. *purpureus*, occurred closely below the oxic-anoxic boundary. Despite the small fraction of total lake volume represented by the chemocline, half of the total carbon photoassimilation of the lake occurred within the chemocline with approximately equal contributions by oxygenic and anoxygenic phototrophs. Rates of dark carbon fixation in the chemocline were even higher than rates of photoassimilation, especially at the depths where oxygen and sulphide coexisted during part of the day. These results indicate a substantial contribution by chemolithotrophic organisms to the carbon cycle in Lake Cadagno. Analysis of stable carbon isotopes suggests that zooplankton may obtain as much as half of its carbon at the chemocline, indicating a strong link between production in anoxic waters and the food web in the oxic part of the lake.

Introduction

Spatial heterogeneity in microbial ecosystems implies that physical and chemical conditions differ between two points in space, thus giving rise to defined gra-

* Corresponding author, e-mail: antonio.camacho@uv.es

dients (Wimpenny, 1993). Such gradients can be found at the chemocline of stratified lakes. As a consequence, specific autotrophic microorganisms form dense populations over small depth intervals according to their ecophysiological characteristics (see review by Van Gemerden and Mas, 1995). Different microbial activities may be coupled to changes in the concentrations of oxygen, sulphide, and nitrogen and phosphorus compounds, as well as to changes in light intensity and light quality (Børsheim et al., 1985; Fischer et al., 1996).

Oxygenic and anoxygenic photoassimilation by finely layered phototrophic microbial populations in deep waters may contribute substantially to overall inorganic carbon photoassimilation in stagnant lakes (Camacho and Vicente, 1998). Moreover, dark carbon fixation by chemolithotrophic microorganisms may also be important where chemical gradients are steep (Jørgensen, 1982; Shively et al., 1998). Aerobic zooplankton feeding on these deep microbial populations may represent an important link between oxic and anoxic lake food chains (Salonen and Lehtovaara, 1992; Massana et al., 1994). Here we studied the microstratification of phototrophic microorganisms and the photosynthetic and chemolithotrophic microbial activity at the chemocline of the meromictic Lake Cadagno to evaluate the relative contribution of these microbial populations to lake carbon metabolism.

Material and methods

Study site

Lake Cadagno is an alpine lake situated on the South face of the Gotthard massif in Switzerland, 1923 m above sea level. The lake is about 800 × 400 m in size with an average surface area of 24 ha and a maximum depth of 21 m. Lake Cadagno is permanently stratified due to higher salt content in bottom waters relative to surface waters. Orographic and limnological details are summarized in Peduzzi et al. (1998).

Sampling

Techniques suitable for sampling on the order of cm's were required to determine the chemical gradients and vertical distribution of microorganisms occupying the chemocline of the lake (e.g. Børsheim et al., 1985). Sampling was performed from a fixed platform positioned above the deepest point of the lake (20.7 m). The chemocline was sampled at 10–20 cm intervals with an improved version of a fine-layer pump sampler described by Jørgensen et al. (1979). Zooplankton samples were collected from the platform in a vertical tow from 14 m to surface, using a 50 μm mesh net. We sampled the lake seven times over a three day period in September 1999. A similar pattern of physical and chemical features, microorganism distribution, inorganic carbon assimilation and isotopic carbon ratios was found among the different samplings.

Results exposed here correspond to sampling and assays performed around noon on September 13, which are totally representative of the above mentioned pattern.

Physical and chemical parameters

In situ profiles of dissolved oxygen, temperature, conductivity, redox potential, pH and turbidity were obtained with a YSI model 6920 multisonde (Yellow Springs Instruments). Ammonia, nitrate plus nitrite and soluble orthophosphate were determined from samples filtered immediately after collection with a GF/F glass fiber filtre to avoid nutrient release from cells during the subsequent chemical analyses. Equipment used for *in situ* filtration and chemical analyses was acid-washed to avoid chemical contamination. The indophenol-blue method was used for ammonia determinations (APHA-AWWA-WEF, 1992). Nitrate plus nitrite was measured after reduction of nitrate to nitrite and its reaction with sulphanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride. Soluble orthophosphate was determined by the phosphomolybdic acid – ascorbic method (Golterman et al., 1978). Total phosphorus was analyzed in non-filtered water as orthophosphate after persulphatic-acid hydrolysis at 135 °C for 2 hours. Total inorganic carbon was calculated from pH, temperature, ionic strength and alkalinity after neutralization with HCl and titration with NaOH (Golterman et al., 1978).

Counting procedures

Phototrophic sulphur bacteria were collected on 0.2-µm pore diameter cellulose acetate filters, treated with erythrosine (Jones 1979), and the dried filters were counted at 1250× with a Zeiss phase-contrast microscope until 3000 cells per species were found. Identification was done according to Staley et al. (1989), Guyoneaud et al. (1998) and Imhoff (1999). Algal numbers were determined from Lugol-fixed samples with an Olympus IX50 inverted microscope (200× and 400×) by using the Utermöhl (1958) sedimentation method, until a minimum of 500 individuals of each of the main species (see Fig. 3) were found. Algae were identified according to Anton and Duthie (1981), Komárek and Fott (1983), and Krammer and Lange-Bertalot (1991). Algal biovolume was determined with an Image Processing and Analysis System (LEICA Qwin Standard v.2.2) on a minimum of 100 individuals of each species. Autotrophic picoplankton (APP) was counted on 0.2 µm black membrane filters (Isopore GTBP, Millipore) in a Zeiss III fluorescence microscope at 1250×, using the filter combination recommended by MacIsaac and Stockner (1993). Total bacterial counts were made by epifluorescence microscopy (G 365 exciting filter, LP 420 suppression filter) on the same type of black filters after filtering 2 ml of sample and staining with DAPI (4′,6-diamidino-2-phenylindole) at a final concentration of 0.0013 µg ml^{-1} for 3 min. A minimum of 5000 cells were counted per sample for APP and total bacteria. Zooplankton samples were fixed with formalin

and counted after sedimentation in a Nikon TMS inverted microscope at 100×. Selected water samples were further analysed for particle number and size using the Microcyte flow cytometer (Optoflow, Oslo), instrument spans from 0.4 to 15 µm. Counts for 20 1 µl samples were averaged.

Light measurements and photosynthetic pigments

Photosynthetically active radiation (PAR, 400–700 nm) was measured at discrete depths several times with a 2-π cosine corrected irradiance sensor and once with a 4-π scalar irradiance sensor (Li-Cor LI-192SA and LI-193SA, respectively). During the photoassimilation experiments surface irradiance was measured continuously and stored in a LI-1000 data-logger (Li-Cor Inc.). Chlorophyll *a* and bacteriochlorophyll *a* determinations were performed by HPLC after extraction in 100% acetone, as described by Borrego and Garcia-Gil (1994). In vivo pigment determinations were performed according to Trüper and Yentsch (1967).

In situ *photoassimilation experiments*

Water samples were incubated in 60 ml polycarbonate bottles (®Nunclon, InterMed, >95% transmitance from 390 to 850 nm, 50%T at 350 nm, 0%T at 295 nm) after flushing three times when filling to avoid oxygen contamination. To differentiate between oxygenic and anoxygenic photoassimilation two transparent bottles and two dark bottles were coupled with two transparent bottles supplemented with 10 µM (final concentration) DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) to inhibit oxygenic photosynthesis, plus a control bottle treated with buffered formalin (2% final concentration) at each depth (Pedrós-Alió et al., 1993). $NaH^{14}CO_3$ was added to a specific activity of 0.08 µCi ml^{-1}. Bottles were kept in the dark during manipulation and checked for the absence of bubbles before being suspended on a floating hanger at 10 to 20 cm depth intervals (depths indicated in Figures). Samples were incubated for two and a half hours. After retrieval samples were protected from light and fixed with buffered formaldehyde to 2% final concentration (Camacho and Vicente, 1998). Duplicate subsamples from each bottle (10 to 30 ml depending on cell concentrations) were filtered through 25 mm diameter 0.2 µm polycarbonate filters (Nuclepore), washed twice with 2% HCl (v/v) to remove ^{14}C-carbonate precipitates, then washed with distilled water. After drying for 24 h filters were placed in 20 ml vials containing 10 ml of scintillation cocktail (Ultima Gold, Canberra). Radioactivity was determined by a Beckman LS7800 liquid scintillation counter. After correcting for counts in the formalin-treated control bottles (<80 dpm) carbon assimilation was calculated by considering the total amount of inorganic carbon in the water. Maximal variation in carbon fixation rates among duplicate subsamples was 8.3%, and a mean value is presented. Total carbon fixation during the day-time in Lake Cadagno on September 13

was calculated with the appropriate weighting according to the lake hypsography (Peduzzi et al., 1998).

Stable carbon isotopes

Samples for analysis of stable carbon isotopes were filtered onto pre-combusted GF/F glass fibre filters and analysed with an automated combustion GC system ANCA-SL (Europa Scientific) connected to VG 602 dual-collector isotope-ratio mass spectrometer (Zohary et al., 1994). The results are reported as $\delta^{13}C$ values (‰ deviations relative to the PDB standard), with a precision determined on standards of ± 0.25‰.

Results

Water stratification and physical and chemical variables

In September 1999 the lake was thermally and chemically stratified. Temperature declined between 4 and 10 meters from 15 to 7 °C (Fig. 1A) and conductivity increased between 5 and 11 meters from 100 to 320 µS cm^{-1}, indicating a density gradient driven by both temperature and salinity. Dissolved oxygen concentrations were approximately 0.3 mM and rose slightly to a maximum near the upper edge of the thermocline (Fig. 2A) before declining with increasing depth. Anoxic conditions prevailed below 11.2–11.4 meters and sulphide concentration increased with increases in depth (Fig. 2A). As a consequence a strong Eh gradient occurred between 10 and 12 meters (Fig. 1A). Epilimnetic pH was slightly alkaline (pH 8.4) but pH decreased at the thermocline and fell to 6.8 in the monimolimnion. Concentrations of inorganic nitrogen and phosphorus were low in the epilimnion (Fig. 1B), ammonia < 1 µM, nitrate plus nitrite between 0.1 and 1.2 µM, and soluble phosphorus (SRP) < 0.03 µM. The concentrations of these nutrients increased within the chemocline and were maximal in the monimolinion (ammonia between 10 and 70 µM and SRP between 0.7 and 4.1 µM). In contrast, the highest concentrations of total phosphorus (mostly particulate) occurred at the chemocline, due to the accumulation of planktonic microorganisms.

Vertical distribution of planktonic microorganisms

Epilimnetic phytoplankton was dominated by the chlorophyte *Echinocoleum elegans*, a green alga with a carbohydrate cover that improves buoyancy. Other chlorophyta, diatoms, and autotrophic picocyanobacteria were also present in the epilimnion (Fig. 3A). Dense layers of algae and phototrophic bacteria were found at the chemocline (Fig. 4). A dense population of diatoms, mainly *Fragilaria capucina* and *Cyclotella comensis*, was present in the oxic water at the upper edge of the chemocline (10.8–11.2 m). At the oxic-anoxic boundary the

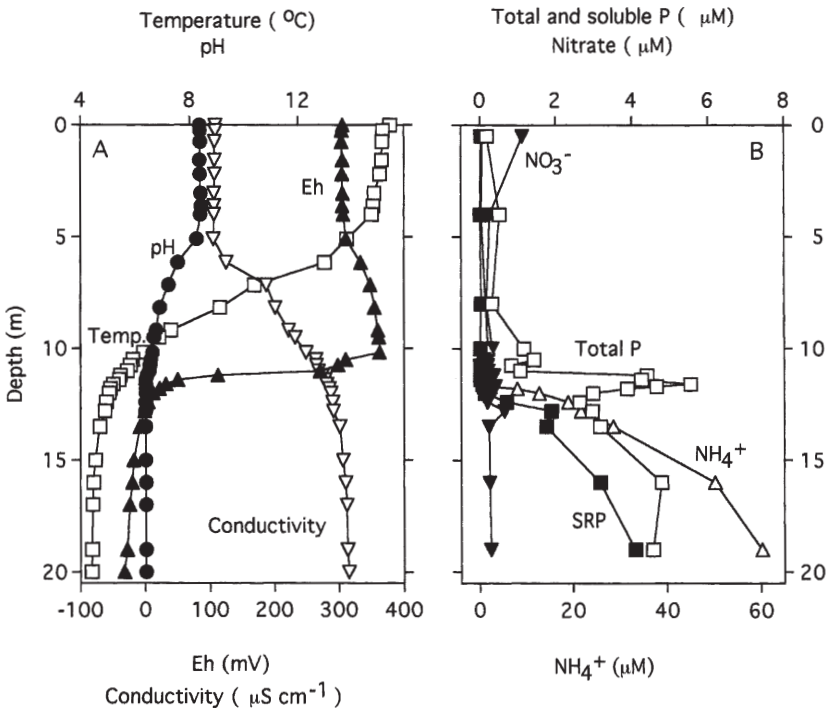

Figure 1. Vertical profiles of (A) temperature (°C), conductivity (μS cm^{-1}), Eh (mV), and pH, and (B) concentrations (μM) of soluble reactive phosphorus (SRP), total phosphorus (TP), nitrate and ammonium in Lake Cadagno. September 13, 1999, 12:00 h

cryptomonads *Cryptomonas erosa* and *Cryptomonas phaseolus* were abundant. Below the cryptophyte maximum, high densities of mostly-vacuolated phototrophic sulphur bacteria resembling *Amoebobacter* cf. *purpureus* were found. The larger sulphur bacteria *Chromatium okenii* occurred throughout the hypolimnion but at much lower densities than *Amoebobacter*. Despite smaller size *Amoebobacter* cf. *purpureus* greatly dominated phototrophic bacterial biomass. The abundance of DAPI-stained non-photosynthetic bacteria also peaked at the chemocline. Maximal *in situ* turbidity corresponded exactly with the distribution of planktonic microorganisms (Fig. 2B).

Cladocera and rotifers dominated the zooplankton (Fig. 3B). Although *Daphnia* gr. *longispina* and *Bosmina longirostris* were the only cladoceran species found in the lake, they comprised more than 40% of zooplanktonic organisms. Among rotifers, *Conochilus* sp. accounted for 35% of total zooplankton but *Asplanchna priodonta* was also abundant (10%). The calanoid copepod *Acanthodiaptomus denticornis* comprised approximately 9.5% and the cyclopoid *Cyclops abyssorum* represented 2% of total zooplankton.

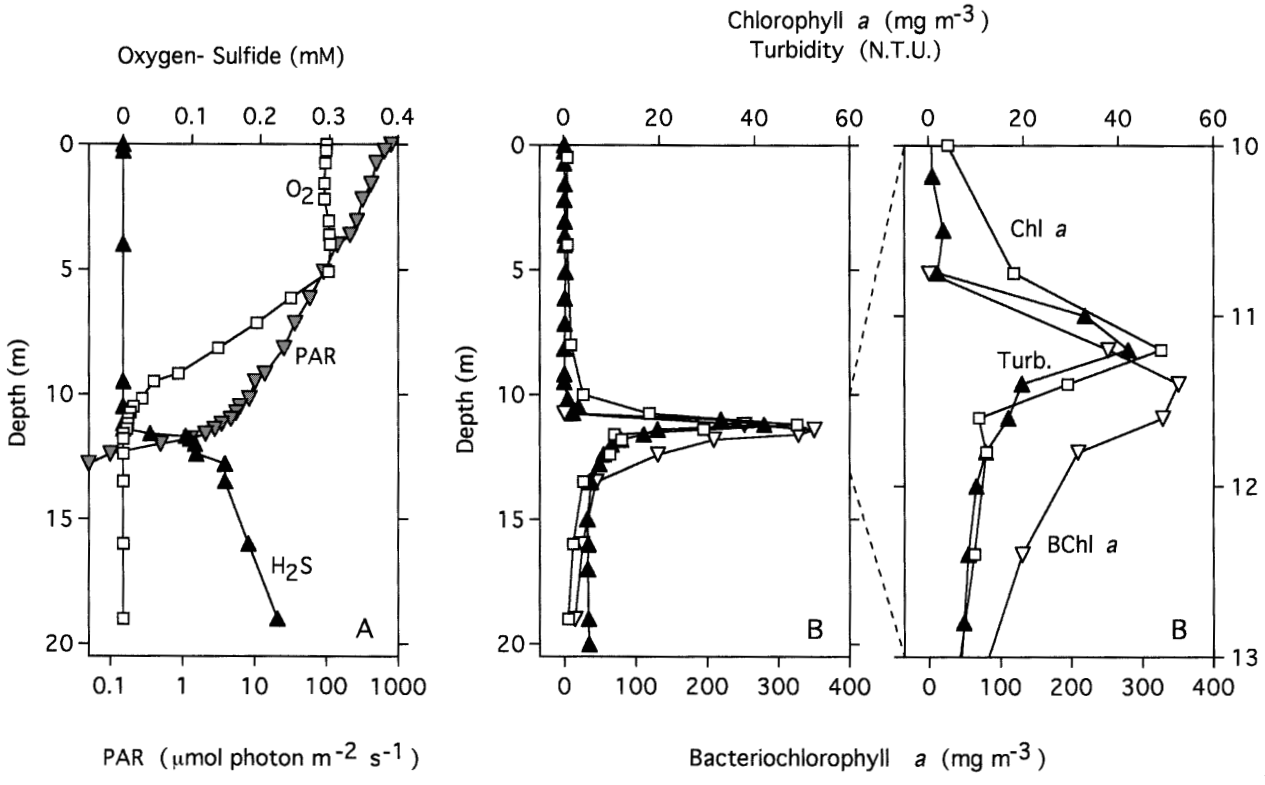

Figure 2. Vertical profiles of (A) oxygen and sulfide (mM) and light (PAR 2π, µmol photon m^{-2} s^{-1}), and; (B) total profile and zoom at the chemocline of chlorophyll *a* and bacteriochlorophyll *a* (mg m^{-3}) and turbidity (Turb., N.T.U) in Lake Cadagno. September 13, 1999, 12:00 h

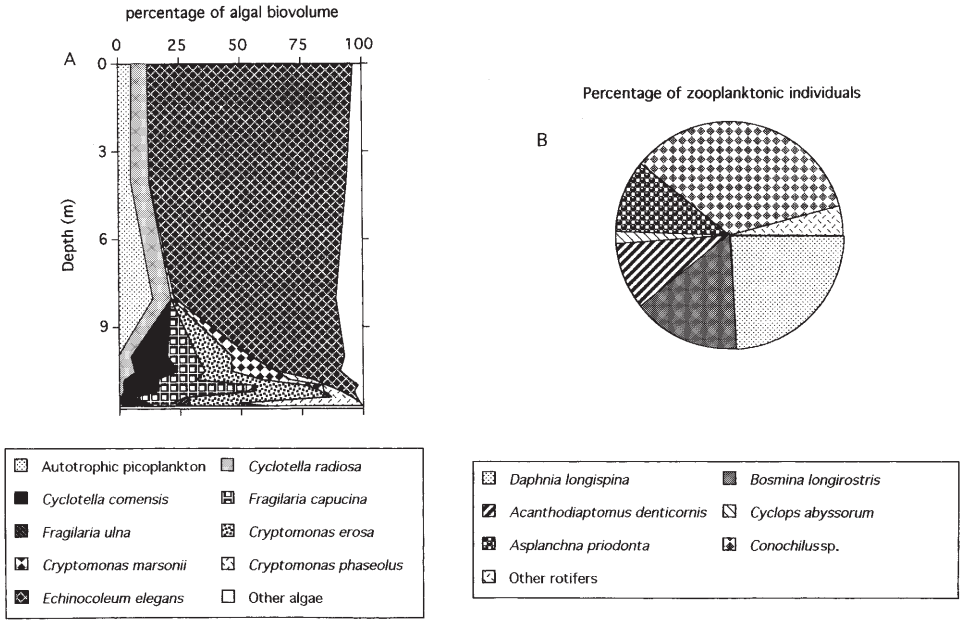

Figure 3. (A) percentage of algal biovolume in the oxic waters and; (B) percentage of zooplanktonic individuals in a vertical tow collected at the sampling station in Lake Cadagno. September 13, 1999

Light and photosynthetic pigments

Light availability at the top of the chemocline was approximately 1.5 % of surface irradiance. Attenuation increased through the chemocline due to absorption and scattering of light by the dense populations of phototrophic microorganisms resulting in negligible light (measured with a 2π sensor) at the bottom of the chemocline (Fig. 2 A).

Vertical distribution of chlorophyllic pigments (Fig. 2 B) corresponded to the distribution of photosynthetic microorganisms. In the epilimnion, chlorophyll *a* (Chl *a*) was found at concentrations < 1 mg m^{-3}, whereas concentrations as high as 50 mg m^{-3} were found at the chemocline. Bacteriochlorophyll *a* (BChl *a*) maxima (350 mg m^{-3}) occurred below the Chl *a* peak coincident with the dense layer of *Amoebobacter* cf. *purpureus*. *In vivo* pigment analysis detected phycoerythrin when *Cryptomonas* spp. were present (not shown).

Inorganic Carbon fixation

The highest rates of inorganic carbon fixation in Lake Cadagno were found at the chemocline (Fig. 5). Maximum rates occurred at 11.4 meters coincident with the oxic-anoxic boundary. In the epilimnion, rates of oxygenic carbon photoassimilation ranged between 4.9 and 7.8 mg C m^{-3} h^{-1}. Rates increased substan-

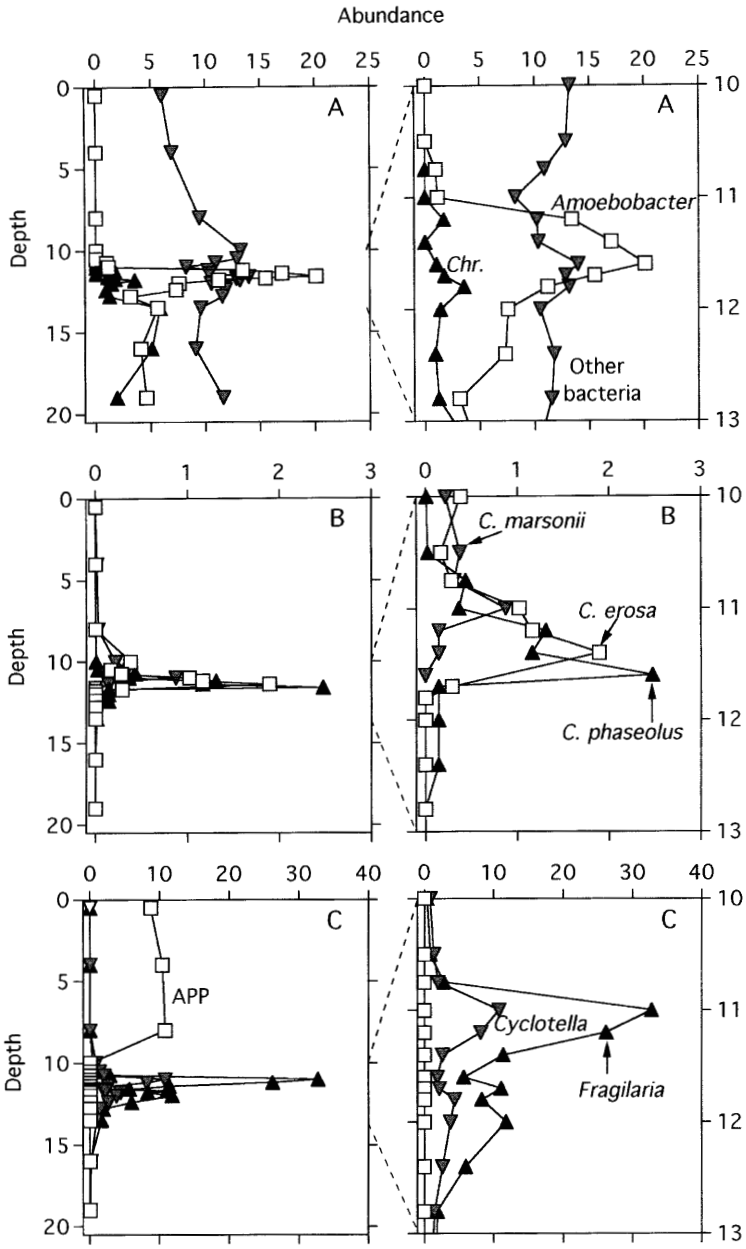

Figure 4. Vertical distribution of the main phototrophic microorganisms and DAPI stained bacteria in Lake Cadagno. September 13, 1999, 12:00 h. (A) *Amoebobacter* cf. *purpureus* (cells $ml^{-1} \times 10^5$), *Chromatium okenii* (*Chr.*, cells $ml^{-1} \times 10^3$), DAPI-stained non-photosynthetic bacteria (other bacteria, cells $ml^{-1} \times 10^6$). (B) *Cryptomonas* spp. (cells $ml^{-1} \times 10^3$). (C) Diatoms (*Cyclotella comensis* and *Fragilaria capucina*, cells $ml^{-1} \times 10^3$) and autotrophic picoplankton (APP, cells $ml^{-1} \times 10^5$). Distribution through the total vertical profile is shown on left graphs, whereas a zoom at the chemocline is shown on the right

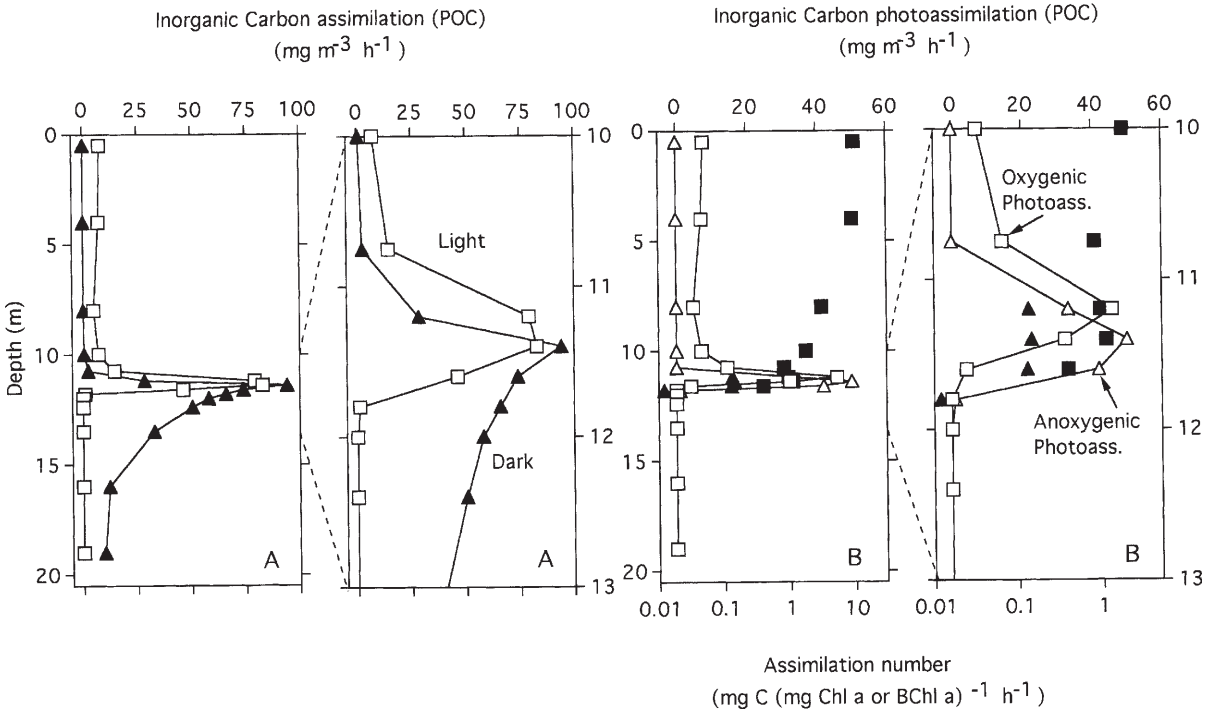

Figure 5. (A) Light and dark inorganic carbon assimilation (POC, mg m^{-3} h^{-1}). (B) Oxygenic and anoxygenic photoassimilation (POC, mg m^{-3} h^{-1}) and assimilation numbers (mg C (mg Chl *a* or Bchl *a*) m^{-3} h^{-1}) for oxygenic (■) and anoxygenic phototrophs (▲). Lake Cadagno, September 13, incubations at noon. Zooms at the chemocline zone are shown on the right part of each graph

tially below 10.5 m to a maximum of 45.9 mg C m^{-3} h^{-1} near the oxic-anoxic boundary. Rates of anoxygenic photoassimilation were as high as 50.1 mg C m^{-3} h^{-1} immediately below the oxic-anoxic boundary. Integrated carbon photoassimilation was as high as 130 mg C m^{-2} h^{-1} from which 60.8 mg C m^{-2} h^{-1} was due to carbon photoassimilation at the chemocline (31.2 and 29.6 mg C m^{-2} h^{-1} for oxygenic and anoxygenic photoassimilation respectively). Assimilation numbers (assimilation normalised to the chlorophyll or bacteriochlorophyll content) for oxygenic microorganisms decreased steadily with depth but increased slightly at the chemocline (Fig. 5). Assimilation numbers for anoxygenic photosynthesis were 3–7 times lower than for oxygenic phototrophs (Fig. 5).

Interestingly, the highest rates of inorganic carbon fixation in Lake Cadagno were not due to photoassimilation but to dark assimilation. Maximal dark assimilation rate was 94.1 mg C m^{-3} h^{-1} and coincided with the oxic-anoxic boundary where both oxygen and sulphide were present at very low concentrations. High rates (58.0–74.0 mg C m^{-3} h^{-1}) were also found throughout the middle and the bottom part of the chemocline corresponding with maximal bacterial densities, and rates >10 mg C m^{-3} h^{-1} occurred in the hypolimnion. By contrast, dark assimilation was almost negligible in the epilimnion and in the thermocline. Integration of fixation rates through the water column and considering the relative contribution of each depth layer to the total lake volume indicated that half of the total inorganic carbon fixation in Lake Cadagno during the day-time was due to dark assimilation.

Stable isotopes

$\delta^{13}C$ values were most negative (–36‰ to –39‰) for seston at the chemocline (Fig. 6), whereas the epilimnetic seston was the least negative (–27‰). $\delta^{13}C$ for zooplankton was –32‰, intermediate between epilimnetic seston and seston at the chemocline.

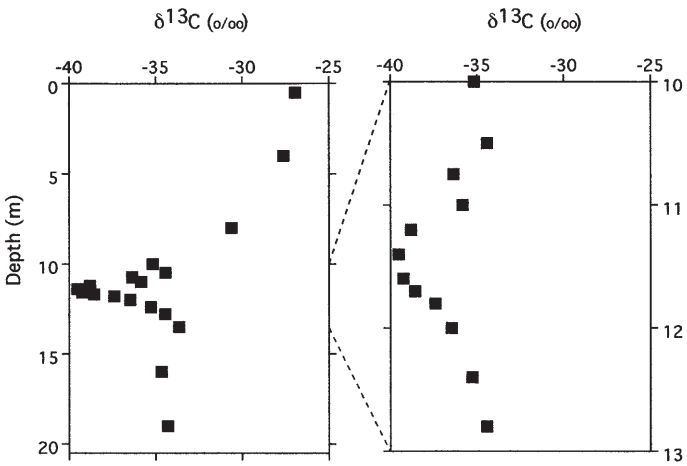

Figure 6. $\delta^{13}C$ (‰) of seston samples obtained in Lake Cadagno on September 13, 1999, 12:00 h

Discussion

In Lake Cadagno, density gradients due to differences in salinity and temperature induce permanent stratification. One consequence of permanent stratification is the formation of ecologically significant gradients of oxygen, sulphide and nutrients. Chemical and physical gradients lead to stratification of the microbial populations, which become concentrated in a narrow depth range within the chemocline. Development of anoxia coincident with increases in sulphide permits the development of anoxygenic phototrophic sulphur bacteria, which benefit from increases in inorganic nitrogen and phosphorus availability in the chemocline and hypolimnion.

Epilimnetic phytoplanktonic populations in Lake Cadagno are dominated by chlorophycean algae which suggests that the oxic part of the lake tends to slightly eutrophic conditions. *Fragilaria capucina*, the dominant diatom in the deep chlorophyll maximum, is also an indicator of slightly eutrophic conditions. Autotrophic picocyanobacteria (APP) are present throughout the epilimnion and the thermocline, but are scarce at the chemocline. The presence of APP in deep layers of stagnant lakes is not unusual, since these populations often have a higher photosynthetic efficiency compared to epilimnetic phytoplankton (Vila et al., 1996; Malinsky-Rushansky et al., 1997), although in Lake Cadagno the contribution of APP to the deep chlorophyll maximum was negligible. In contrast, a dense population of diatoms, which were not present in the epilimnion, was found in the upper chemocline. Schanz and Stalder (1998) have demonstrated that these diatoms develop in the epilimnion before sinking to a depth which corresponds to neutral buoyancy.

Cryptomonas phaseolus and *Cryptomonas erosa* also formed dense populations close to the oxic-anoxic boundary. Although these algae were absent in the epilimnion, cryptophytes are known to grow at the chemocline of stratified lakes (Gasol and Pedrós-Alió, 1991) where grazing pressure is low (Jones, 1993) and nutrient availability improved (Salonen et al., 1984). Diel vertical migrations throughout the oxic-anoxic boundary allows flagellated algae for separation of processes such as nutrient acquisition and photosynthesis, the later being inhibited by sulphide.

The most prevalent microorganisms in the highly dense bacterial layers were large purple bacteria and aggregates of smaller bacteria. Direct microscopical counts suggest that the small spherical purple bacteria were *Amoebobacter* cf. *purpureus*, although 16S rRNA sequence analysis indicated that they might actually represent four different bacterial populations slightly different to the type strain of *A. purpureus* (Tonolla et al., 1999; Bosshard et al., 2000a). These *Amoebobacter* cells were found below the oxic-anoxic boundary where sulphide was available as electron donor. In September 1999 *Chromatium okenii* was clearly less abundant than *A.* cf. *purpureus*. The abundant *C. okenii* in early summer (Tonolla et al., 1999) is followed by a dominance of *A.* cf. *purpureus* in late summer and early fall (Bosshard et al., 2000b).

Light availability was strongly reduced at the chemocline of Lake Cadagno due to the absorption and scatter by dense layers of phototrophic microorganisms. In the diatom maximum light was 1.5%, at the *Cryptomonas* peak 0.5%,

and at the depth of the phototrophic bacterial plate 0.35 % of surface irradiance, respectively. Accessory pigments such as okenone allow phototrophic bacteria to efficiently collect the light spectrum available at these depths. These bacteria may adapt rapidly to changes in light availability (Fischer et al., 1996). Despite low light availability, the highest rates of carbon photoassimilation in Lake Cadagno occurred at the chemocline, which accounts for about 10 % of the lake volume, reaching up to 41 % of the total carbon photoassimilation. Half of this carbon photoassimilation at the chemocline was due to anoxygenic phototrophs and half by oxygenic algae, mainly by Cryptophyta. The activity of carbon photoassimilation corresponds with the distribution of sulphide turnover rates at different depths (Lüthy et al., 2000). The average assimilation numbers reported here for the anoxygenic phototrophic bacterial population of 0.134 mg C mg $Bchla^{-1}$ h^{-1} are well within the range given by Schanz et al. (1998) (0.039 and 0.235 mg C mg $Bchla^{-1}$ h^{-1}) for Lake Cadagno. Similar rates have been reported for other stratified lakes (Overmann, 1997).

Most remarkable in Lake Cadagno were the high rates of dark carbon assimilation, which accounted for half of the total inorganic carbon assimilation in the entire lake during the hours in which light was available. High values for the activity of chemolithotrophs have also been found in Lakes Cisó (Pedrós-Alió and Guerrero, 1991), Mekkojärvi (Kuuppo-Leinikki and Salonen, 1992), Big Soda (Cloern et al., 1983), and Kinneret (Hadas, 2000), although rates as high as those found in Lake Cadagno have not commonly been reported. Although extrapolation must be made with caution, our study suggests that chemoautotrophy can be as important as photoautotrophy in some lakes. Aerobic chemoautotrophic bacteria include a variety of physiological groups. In sulphide-rich lakes, autotrophic sulphur-oxidising bacteria find optimal growth conditions at the chemocline due to co-occurrence of sulphide and oxygen (Jørgensen et al., 1979, Børsheim et al., 1985). Thiobacilli were responsible for chemolithotrophic activity at the redox transition zone in the Black Sea (Sorokin et al., 1995). Interestingly Demarta et al. (1998) found in the monimolimnion of Lake Cadagno sequences phylogenetically related to the autotrophic sulphur-oxidising bacteria *Thiovulum*. Furthermore, some phototrophic sulphur bacteria, including *Amoebobacter* sp., have the capacity for chemolithoautotrophic growth (Van Gemerden and Mas, 1995). High rates of dark fixation also occurred throughout the anoxic hypolimnion. Methanogens, acetogenic bacteria and some sulphate-reducers, which are able to grow chemolithoautotrophically in anoxic conditions (Shively and Barton, 1991), may there be responsible for dark fixation.

The distribution of stable carbon isotopes gives information on trophic interactions among planktonic organisms (Peterson and Fry, 1987; Michener and Schell, 1994). In Lake Cadagno the lowest values of $\delta^{13}C$ of –39 ‰ were found in the chemocline compared to –27 ‰ for epilimnetic seston. Similar negative $\delta^{13}C$ (lower than –35 ‰) are found at oxic-anoxic interfaces in the ocean where chemolithotrophic activity is also high (Ruby et al., 1987). Interestingly the $\delta^{13}C$ of zooplankton collected in the epilimnion was close to –32 ‰. From these data it can be calculated that the microbial community at the chemocline contributed at least 50 % to the carbon nutrition of zooplankton, providing evidence that deep production was an important food source for zooplankton, at least prior to

the study period. *Daphnia longispina*, which was abundant in Lake Cadagno, is known to efficiently feed on bacteria (Kuuppo-Leinikki and Salonen, 1992) and can tolerate low-oxygen conditions (Salonen and Lehtovaara, 1992). Stable isotope data indicated a strong link between zooplankton and algal and bacterial carbon produced at the chemocline of the lake. Vertical migration of zooplankton could potentially distribute nutrients and represent a critical link between carbon fixation at the chemocline and the rest of the lake food web. This is important, as most carbon fixation in Lake Cadagno occurred at the chemocline. Transfer of energy and nutrients from the chemocline to the epilimnion by zooplankton is undoubtedly important to the whole lake food web and may partially explain the unusually high trophic status of Lake Cadagno.

ACKNOWLEDGEMENTS

This study was carried out during the 7th International GAP Workshop, held on 9–17 Sept. 1999 in Zürich, Switzerland, and supported by the Swiss National Science Foundation (SNF), the Swiss Academies of Natural and Technical Sciences (SANW and SATW), the Swiss Society of Hydrology and Limnology (SGHL), by EAWAG, Zürich Water Supply and the University of Zürich, as well as by Hoffmann-La Roche, Lonza, Novartis, Canberra Packard S.A, Millipore AG and Faust Laborbedarf AG. Authors are also indebted to the Cadagno staff during the GAP-99 workshop, especially to Lukas Lüthy and Markus Fritz, and to the Organizing Committee for providing logistic and technical support. We are also grateful to L. Boronat for helping with the zooplankton counts.

REFERENCES

Anton, A. and H.C. Duthie, 1981. Use of cluster analysis in the systematics of the algal genus *Cryptomonas*. Can. J. Bot. 59: 992–1002.

APHA – AWWA – WEF, 1992. Standard methods for the examination of water and wastewater. 18th edition. American Public Health Association. Washington D.C., 1100 pp.

Borrego, C.M. and L.J. García Gil, 1994. Separation of bacteriochlorophyll homologues from green photosynthetic sulfur bacteria by reversed-phase HPLC. Photosynth. Res. 41: 157–163.

Børsheim, K.Y., J.G. Kuenen, K.J. Gottschal and I. Dundas, 1985. Microbial activities and chemical gradients in the chemocline of a meromictic lake in relation to the precision of the sampling procedure. FEMS Microbiol. Ecol. 31: 337–341.

Bosshard, P.P., Y. Santini, D. Grüter, R. Stettler and R. Bachofen, 2000a. Bacterial diversity and community composition in the chemocline of the meromictic alpine Lake Cadagno as revealed by 16S rDNA analysis. FEMS Microbiol. Ecol. 31: 173–182.

Bosshard, P.P., R. Stettler and R. Bachofen, 2000b. Seasonal and spatial community dynamics in the meromictic Lake Cadagno. Arch. Microbiol. 174: 168–174.

Camacho, A. and E. Vicente, 1998. Carbon photoassimilation by sharply stratified phototrophic communities at the chemocline of Lake Arcas (Spain). FEMS Microbiol. Ecol. 25: 11–22.

Cloern, J.E., B.E. Cole and R.S. Oremland, 1983. Autotrophic processes in meromictic Big Soda Lake, Nevada. Limnol. Oceanogr. 28: 1049–1061.

Demarta, A., M. Tonolla, A.-P. Caminada, N. Ruggeri and R. Peduzzi, 1998. Phylogenetic diversity of the bacterial community from the anoxic layer of the meromictic Lake Cadagno Doc. Ist. Ital. Idrobiol. 63: 19–30.

Fischer, C., M. Wiggli, F. Schanz, K.W. Hanselmann and R. Bachofen, 1996. Light environment and synthesis of bacteriochlorophyll by populations of *Chromatium okenii* under natural environmental conditions. FEMS Microbiol. Ecol. 21: 1–9.

Gasol, J.M. and C. Pedrós-Alió, 1991. On the origin of deep algal maxima: the case of lake Cisó. Verh. Internat. Verein Limnol. 24: 1024–1028.

Golterman, H.L., R.S. Clymo and M.A.M. Ohnstad, 1978. Methods for physical and chemical analysis of freshwaters. IBP Handbook no. 8, Blackwell Sci. Pub., Oxford, UK, 213 pp.

Guyoneaud, R., J. Süling, R. Petri, R. Matheron, P. Caumette, N. Pfennig and J. Imhoff, 1998. Taxonomic rearrangements of the genera *Thiocapsa* and *Amoebobacter* on the basis of 16S rDNA sequence analyses, and description of *Thiolamprovum* gen. nov. Int. J. Syst. Bacteriol. 48: 957–964.

Hadas, O., N. Malinski-Rushanski, R. Pinkas, E. Halicz and J. Erez, 2000. High chemoautotrophic primary production across a transect in Lake Kinneret, Israel. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. 55: 413–420.

Imhoff, J.F., 1999. A phylogenetically oriented taxonomy of anoxygenic phototrophic bacteria. In: Pescheck, G.A., W. Löffelhardt and G. Schmetterer (eds.), The Phototrophic Prokaryotes, Kluwer Academic/Plenum Publishers. New York, pp. 763–774.

Jones, J.G., 1979. Direct counts on membrane filters. In: A Guide to Methods for Estimating Microbial Numbers and Biomass in Freshwaters. FBA Sci. Publ. 39, Ambleside, UK pp. 19–39.

Jones, R.I., 1993. Phytoplankton migrations: Patterns, processes and profits. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. 39: 67–77.

Jørgensen, B.B., 1982. Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. Phil. Trans. Roy. Soc. London B. 298: 543–561.

Jørgensen, 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.

Komárek, J. and B. Fott, 1983. Das Phytoplankton des Süsswassers. Systematik und Biologie. *Chlorophyceae* (Grünalgen). Ordung: *Chlorococcales*. Teil 7. In: Elster, H.J. and W. Ohle (eds.) Die Binnengewässer. Einzeldarstellungen aus der Limnologie und ihren Nachbargebieten. E. Schweizerbart'sche Verlag. Stuttgart. 1044 pp.

Krammer, K. and H. Lange-Bertalot, 1991. *Bacillariophyceae*. Teil 3: *Centrales, Fragilariaceae, Eunotiaceae.* In: Ettl, H., J. Gerloff, H. Heynig and D. Mollenhauer (eds.). Süsswasserflora von Mitteleuropa, Gustav Fischer Verlag. Jena. 576 pp.

Kuuppo-Leinikki, P. and K. Salonen, 1992. Bacterioplankton in a small polyhumic lake with an anoxic hypolimnion. Hydrobiologia. 229: 159–168.

Lüthy L., M. Fritz, and R. Bachofen, 2000. *In situ* determination of sulfide turnover rates in a meromictic alpine lake. Appl. Environ. Microbiol. 66: 712–717.

MacIsaac, E.A. and J.G. Stockner, 1993. Enumeration of phototrophic picoplankton by autofluorescence microscopy. In: Kemp, K.F., B.F. Sherr, E.B. Sherr and J.J. Cole (eds.), Handbook of Methods in Aquatic Microbial Ecology, Lewis Publishers, Boca Raton, pp. 187–198.

Malinsky-Rushansky, N.Z., T. Berman and Z. Dubinsky, 1997. Seasonal photosynthetic activity of autotrophic picoplankton in Lake Kinneret, Israel. J. Plankton Res. 19: 979–993.

Massana, R., J.M. Gasol, K. Jürgens and C. Pedrós-Alió, 1994. Impact of *Daphnia pulex* on a metalimnetic microbial community. J. Plankton Res. 16: 1379–1399.

Michener, R.H. and D.M. Schell, 1994. Stable isotopes as tracers in marine aquatic food webs. In: Lajtha, K. and R. H. Michener (eds), Stable isotopes in ecology and environmental sciences, Methods in Ecology, Blackwell Scientific Publications, Oxford, pp. 138–157.

Overmann, J., 1997. Mahoney Lake: A case study of the ecological significance of phototrophic sulfur bacteria. In: Jones, J.G. (ed.), Advances in Microbial Ecology, Vol. 15, Plenum Press, New York, pp. 251–288.

Pedrós-Alió, C. and R. Guerrero, 1991. Abundance and activity of bacterioplankton in warm lakes. Verh. Internat. Verein Limnol. 24: 1212–1219.

Pedrós-Alió, C., J. Garcia-Cantizano and J.I. Calderón, 1993. Bacterial production in anaerobic water columns. In: Kemp, K.F., B.F. Sherr, E.B. Sherr and J.J. Cole (eds.), Handbook of Methods in Aquatic Microbial Ecology, Lewis Publishers, Boca Raton, pp. 519–530.

Peduzzi, R., R. Bachofen and M. Tonolla (eds.), 1998. Lake Cadagno: a meromictic alpine lake. Doc. Ist. Ital. Idrobiol. Vol 63, Verbania, It., 152 pp

Peterson, B.J. and B. Fry, 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18: 293–320.

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.

Salonen, K. and A. Lehtovaara, 1992. Migrations of haemoglobin-rich *Daphnia longispina* in a small, steeply stratified, humic lake with an anoxic hypolimnion. Hydrobiologia 229: 271–288.

Salonen, K., R.I. Jones and L. Arvola, 1984. Hypolimnetic phosphorus retrieval by diel vertical migrations of lake phytoplankton. Freshwater Biol. 14: 431–438.
Schanz, F. and S. Stalder, 1998. Phytoplankton summer dynamics and sedimentation in the thermally stratified Lake Cadagno. Doc. Ist. Ital. Idrobiol. 63: 71–76.
Schanz, F., C. Fischer-Romero and R. Bachofen, 1998. Photosynthetic production and photoadaptation of phototrophic sulfur bacteria in Lake Cadagno (Switzerland). Limnol. Oceanogr. 43: 1262–1269.
Shively, J.M. and L.L. Barton, 1991. Variations in autotrophic life. Academic Press. London. 346 pp.
Shively, J.M., G. van Keulen and W.G. Meijer, 1998. Something from almost nothing: Carbon dioxide fixation in chemoautotrophs. Ann. Rev. Microbiol. 52: 191–230.
Sorokin, Y.I., P.Y. Sorokin, V.A. Avdeen, D.Y. Sorokin and S.V. Ilchenko, 1995. Biomass, production and activity of bacteria in the Black Sea, with special reference to chemosynthesis and the sulfur cycle. Hydrobiologia 308: 61–76.
Staley, J.T., M.P. Bryant, N. Pfennig and J.G. Holt (eds), (1989). Bergey's manual of systematic bacteriology, Vol. 3, Williams and Wilkins, Baltimore.
Tonolla, M., A. Demarta, R. Peduzzi and D. Hahn, 1999. *In situ* analysis of the phototrophic sulfur bacteria in the chemocline of meromictic Lake Cadagno (Switzerland). Appl. Environ. Microbiol. 65: 1325–1330.
Trüper, H.G. and C.S. Yentsch, 1967. Use of glass filters for the rapid preparation of *in vivo* absorption spectra of photosynthetic bacteria. J. Bacteriol. 94: 1255–1256.
Utermöhl, H., 1958. Zur Vervollkommung der quantitativen Phytoplankton-Methodik. Mitt. Int. Verein. Theor. Angew. Limnol. 9: 1–38.
Van Gemerden, H. and J. Mas, 1995. Ecology of phototrophic sulfur bacteria. In: R.E. Blankenship, M.T. Madigan and C.E. Bauer (eds.) Anoxygenic photosynthetic bacteria, Kluwer Acad. Publ., Dordrecht, pp 49–85.
Vila, X., M. Dokulil, L.J. García-Gil, C.A. Abellà, C.M. Borrego and L. Bañeras, 1996. Composition and distribution of phototrophic bacterioplankton in the deep communities of several central European lakes: the role of light quality. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. 48: 183–196.
Wimpenny, J., 1993. Spatial gradients in microbial ecosystems. In: R. Guerrero and C. Pedrós-Alió (eds.), Trends in Microbial Ecology, Spanish Society for Microbiology, Barcelona, pp. 135–140.
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.

Received 1 March 2000;
revised manuscript accepted 18 July 2000.