J. Limnol., 68(1): 16-24, 2009

Fine scale analysis of shifts in bacterial community structure in the chemocline of meromictic Lake Cadagno, Switzerland

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ABSTRACT

Phototrophic sulfur bacteria in the chemocline of Lake Cadagno, Switzerland, were dominated by purple sulfur bacteria before 1998, but their composition shifted after a presumed disturbance to consist of mainly green sulfur bacteria. This study focused on comparative analyses of the distribution of the green sulfur compared to the purple sulfur bacteria by analyzing specific populations along fine scale depth profiles of the chemocline of Lake Cadagno. Water samples were collected from the chemocline on October 14th, 1998, and on September 28th, 2004. A detailed analysis of chemocline depth profiles revealed that total biomass of phototrophic sulfur bacteria was three times higher in 2004 than in 1998. The three-fold increase of biomass was entirely due to increments in abundance of one population of green sulfur bacteria, identified as Chlorobium clathratiforme*. Abundance of purple sulfur bacteria remained unchanged with respect to overall numbers in the chemocline, but also with respect to distribution of different populations in depth in the chemocline. Aggregates of small-celled purple sulfur bacteria decreased in size about four-fold, but remained associated with sulfate-reducing bacteria of the genus* Desulfocapsa*. Compared to 1998, these had increased in numbers about three times in 2004, resulting in about ten times higher numbers per aggregate. These results demonstrate long-term effects of a presumed disturbance in autumn 1999 and 2000, on environmental conditions and on green sulfur and sulfate-reducing bacteria in the chemocline, however, without changes in the abundance and in the distribution of purple sulfur bacteria.*

Key words: phototrophic sulfur bacteria, Chlorobium clathratiforme, meromictic lake, biomass distribution

1. INTRODUCTION

Phototrophic sulfur bacteria are present in freshwater environments where all physico-chemical conditions needed for their metabolism are satisfied (e.g., anoxic environment with sulfide in a sub-toxic concentration and a sufficient penetration of light) (Overmann 1997). The phototrophic sulfur bacteria comprise essentially two groups distantly phylogenetically related: the purple sulfur bacteria and the green sulfur bacteria (Pfennig & Trüper 1989). Purple and green sulfur bacteria (PSB and GSB) typically stratify at depths where light intensity and sulfide concentration favour their growth (Guerrero *et al*. 1985). They have indeed the ability to oxidize hydrogen sulfide to sulfate under anaerobic conditions in the course of their anoxygenic photosynthesis. Anoxygenic photosynthesis accounts for a large proportion of total primary production in lakes where phototrophic sulfur bactiera are present (Pfennig 1975; Overmann 1997). The function of hydrogen sulfide in these bacteria is to provide electrons for the assimilation of CO_2 *via* the reductive pentose phosphate cycle (in purple bacteria) or the reductive carboxylic acid cycle (in green bacteria) (Biebl & Pfennig 1979; Evans *et al*. 1966). Sulfate and hydrogen sulfide are the two opposite compounds of the sulfur cycle and sulfate reduction is carried out under anaerobic conditions by sulfate-reducing bacteria (SRB) which accumulate hydrogen sulfide in their environment (Pfennig 1975). The products of the degradation of settling biomass act as electron donors for the sulfate reduction (Lüthy *et al*. 2000).

In the chemocline of meromictic Lake Cadagno the population analyses demonstrated that the bacterial community included a large proportion of phototrophic sulfur bacteria representing up to 35% of these bacteria. Within the phototrophic sulfur bacteria, small-celled PSB typically accounted for up to 95% of the cells with large-celled PSB and GSB representing the remaining 5% (Tonolla *et al*. 2003). While GSB were entirely represented by *Chlorobium phaeobacteroides* (Tonolla *et al*. 2003) and *Chromatium okenii* was the only representative of the large-celled PSB, small-celled PSB consisted of at least six populations related to the genera *Lamprocystis* and *Thiocystis* (Tonolla *et al*. 2003; Tonolla *et al*. 2005). While two populations related to the genus *Thiocystis* were found in small numbers in two years only of a monitoring study that covered a decade (Tonolla *et al*. 2005), the remaining four populations of small-celled PSB affiliated with the genus *Lamprocystis* were continuously present as prominent component of the bacterial community in the chemo-

cline with two populations accounting for 40-80% of these PSB depending on the season (Tonolla *et al*. 1999; Tonolla *et al*. 2003).

After 2000 a major change in community structure in the chemocline was observed, with numbers of PSB decreased and those of GSB increased by about one order of magnitude (Tonolla *et al*. 2005). GSB were entirely represented by *C. clathratiforme* and made up about 95% of the phototrophic sulfur bacteria. Changes in profiles of turbidity and photosynthetically available radiation, as well as in sulfide concentrations were documented and were supposedly attributed to a disruption of the chemocline, related to strong mixing events occurred in autumn 1999 and 2000. Autumnal storms were documented on the Lake Cadagno, with wind speeds up to 100 m s^{-1} and strong mixing of the upper layer of the water body till 17 m depth. Data on physical parameters measured on October 18th, 2000 showed the presence of oxygen (2 mg L^{-1}) at 17 m depth (Tonolla & Peduzzi 2006). Mixing events may have altered environmental niches and consequently affected bacterial populations structure in subsequent years. Long-term population dynamic data (years 1994 to 2003) from Tonolla and co-workers (2005) were obtained from pooled samples representing an average of environmental conditions and bacterial populations over the whole chemocline. Specific distributions of bacterial populations in a depth profile along the chemocline were up to now not considered even though PSB assigned to the genus *Lamprocystis* had previously exhibited differences in their depth distribution suggesting eco-physiological adaptations to the steep physico-chemical gradients encountered in the chemocline (Tonolla *et al*. 2003).

In this study, we were interested to expand the previous study by an additional year and to analyze the potential effects of the disturbance on bacterial populations, their distribution and possible micro-stratification along the chemocline in a detailed fine scale study. Therefore we compared the distribution of GSB and of different PSB populations in depth profiles of the Lake in October 1998 and September 2004, before and after the disturbance respectively. Because small-celled PSB, affiliated with the genus *Lamprocystis*, typically form aggregates consisting of 200 to 900 cells that are associated with SRB related to *Desulfocapsa thiozymogenes* (Tonolla *et al*. 2000; Peduzzi *et al*. 2003a, b), additional investigations dealt with population analyses of these SRB and their interaction with the aggregates.

2. MATERIALS AND METHODS

2.1. Study site

The chemocline of crenogenic meromictic Lake Cadagno, located 1923 m a.s.l. in the catchment area of a dolomite vein rich in gypsum in the Piora Valley in the Southern Alps of Switzerland (46°33'N, 8°43'E) has been the subject of many microbial analysis studies in the past (for review see Tonolla *et al*. 2004). The compact chemocline is generally found at a depth between 11 and 13 m and characterized by high concentrations of sulfate, steep gradients of oxygen, sulfide and light and a turbidity maximum that correlates to large numbers of bacteria (up to 10^7 cells mL^{-1}) (Tonolla *et al.* 1999; Tonolla *et al*. 2004).

2.2. Sampling

Water samples were collected from the chemocline of Lake Cadagno over the center of the lake, at its deepest point (21 m), at the end of the summer season (October 14th, 1998 and September 28th, 2004). The chemocline and the bacterial plume in Lake Cadagno were located at these sampling dates using temperature (°C), conductivity (µS cm^{-1}), pH, dissolved oxygen (mg L^{-1}), turbidity (FTU, formazine turbidity unit) and redox potential (mV) measurements with a YSI 6000 profiler (Yellow Springs Inc., Yellow Springs, OH, USA) (Tonolla *et al*. 1999; Tonolla *et al.* 2000). The YSI 6000 profiler as well as two LI-193SA spherical quantum sensors (LI-COR Ltd., Lincoln, NE, USA) that were used to determine PAR-light transmission conditions (%) down to the chemocline in steps of 0.1 m were fixed at the lowest part of a thin-layer pneumatic multisyringe sampler that was used to take 20 simultaneous samples of 100 mL over a total depth of 2 m, yielding in a depth resolution of 10 cm between each sample (Tonolla *et al*. 1999; Bosshard *et al*. 2000a; Bosshard *et al*. 2000b; Tonolla *et al*. 2000).

2.3. Sample analyses

For the analysis of sulfide, 12-mL-subsamples were immediately transferred to screw capped tubes containing 0.8 mL of a 4% zinc acetate solution. These solutions were stored on ice and analyzed colorimetrically (Gilboa-Garber 1971) using a Merck (Switzerland) Spectroquant® kit (Tonolla *et al*. 1999; Tonolla *et al*. 2000). For microbial analyses, 15-mL-subsamples were filtered immediately after sampling through 0.22 µm polycarbonate membrane filters (25 mm diameter; Millipore, Volketswil, Switzerland) (Glöckner *et al*. 1996) and bacteria fixed by overlaying the filters with 4% paraformaldehyde in phosphate buffered saline (PBS; 0.13 M NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.2) for 30 min at room temperature (Amann *et al*. 1990). The filters were subsequently washed twice with PBS, transferred into plastic bags with 600 µL of 50% ethanol in PBS into which bacteria were re-suspended by slightly massing the filter with thumb and forefinger (ISO Organisation 1998). Re-suspended bacterial cells were then transferred into 2 mL cryotubes and stored at -20 °C until further use (Amann *et al*. 1990; Tonolla *et al*. 1999).

Aliquots (1 µL) of re-suspended bacterial samples were spotted onto gelatin-coated slides [0.1% gelatin, 0.01% $KCr(SO_4)_2$], dehydrated in 50, 80 and 96% etha-

Tab. 1. Oligonucleotide probes for the *in situ* hybridisation assay (FISH). PSB = purple sulfur bacteria, GSB = green sulfur bacteria.

Probe	Target	Sequence (5' =>3') (% formamide in hybridization buffer)	Reference
Purple sulfur bacteria (PSB)			
Cmok453	*Chromatium okenii* (DSM 169) 16S rRNA, pos.453-479	AGCCGATGGGTATTAACCACCAGGTT (35%)	(Tonolla *et al*. 1999)
Apur453	*Lamprocystis purpurea* (DSM 4197) 16S rRNA, pos. 453-479	TCGCCCAGGGTATTATCCCAAACGAC (40%)	(Tonolla *et al*. 1999)
Laro453	*Lamprocystis roseopersicina* (DSM 229) 16S rRNA, pos. 453-479	CATTCCAGGGTATTAACCCAAAATGC (30%)	(Tonolla *et al.* 1999)
S453D	Clone 261 from Lake Cadagno 16S rRNA, pos. 453-479	CAGCCCAGGGTATTAACCCAAGCCGC (40%)	(Tonolla *et al.* 1999)
S453F	Clone 371 from Lake Cadagno 16S rRNA, pos. 453-479	CCCTCATGGGTATTARCCACAAGGCG (40%)	(Tonolla *et al.* 1999)
S453H	Clone 222 from Lake Cadagno 16S rRNA, pos. 453-478	GACGGAACGGTATTAACGCCCCGCTT (10%)	(Tonolla *et al*. 2005)
S448	Clone 249 from Lake Cadagno 16S rRNA, pos. 448-468	TATTGACCCCGCGCTTTTCTT (25%)	(Tonolla *et al.* 2005)
Green sulfur bacteria (GSB)			
GSB532	Chlorobiaceae 16S rRNA, pos. 532-546	TGCCACCCCTGTATC (10%)	(Tuschak *et al.* 1999)
Chlp441	*Chlorobium phaeobacteroides* (DSM 266), 16S rRNA, pos. 441-464	AAATCGGGATATTCTTCCTCCAC (20%)	(Tonolla *et al.* 2003)
Chlc190	*Chlorobium clathratiforme* (DSM 5477), 16S rRNA, pos. 190-211	GGCAGAACAACCATGCGATTGT (20%)	(Tonolla *et al.* 2005)
***Desulfocapsa* sp.**			
DSC441	Few *Desulfocapsa* 441-459	ATTACACTTCTTCCCATCC (30%)	(Tonolla *et al.* 2000)
DSC213	Desulfocapsa thiozymogenes 213-230	CCTCCCTGTACGATAGCT (30%)	(Tonolla *et al.* 2000)

nol for 3 minutes each, and then hybridized in 9 µL of hybridization buffer (0.9 M NaCl, 20 mM Tris/HCl, 5 mM EDTA, 0.01% SDS; pH 7.2) in the presence of 10 to 40% formamide depending on the probe (Tab. 1), and 1 µL of Cy3-labeled probe (25 ng μL^{-1}) at 46°C for 2 h (Zarda *et al*. 1997). After hybridization, the slides were washed in buffer containing 20 mM Tris/HCl, pH 7.2, 10 mM EDTA, 0.01% SDS and either 450, 318, 159, 112, 80 or 56 mM NaCl depending on the formamide concentration during hybridization (10, 20, 25, 30, 35, and 40%, respectively) for 20 min at 48 °C, subsequently rinsed with distilled water, and air-dried (Zarda *et al.* 1997). Cells were subsequently counter-stained with 0.001% DAPI (4',6-diamidino-2-phenylindole) in PBS in the dark at room temperature. After incubation for 5-7 minutes, slides were rinsed with distilled water and air-dried (Amann *et al*. 1990). Slides were mounted with Citifluor AF1 (Citifluor Ltd., London, UK) and examined by epifluorescence microscopy using filter sets F31 (AHF Analysentechnik, Tübingen, Germany; D360/40, 400DCLP, D460/50 for DAPI) and F41 (AHF Analysentechnik, HQ535/50, Q565LP, HQ610/75 for Cy3). Microorganisms were counted at 1000× magnification in 30 fields covering an area of 0.01 mm^2 each (Fischer *et al*. 1995). Numbers were expressed as mean ± standard error.

Images of 30-100 cells for each investigated species were captured with a charge-coupled device camera (CF 8/1 FMC, Kappa, Gleichen, Germany) and analyzed using the Q500MC Image Processing and Analysis System (Leica Cambridge Ltd., Cambridge, UK) (Tonolla *et al*. 2003). Biovolumes were calculated using (1:

$$V = 4/3\ \pi\ ab^2 \qquad (1$$

where V is the cell volume, a and b are the major and the minor axes, respectively, of the best fitting ellipsoid (Ramsing *et al*. 1996). The total biovolume of microbial populations in the water column was determined using cell bacterial counts multiplied by the calculated mean cell biovolume. This was converted into total microbial biomass using the calibration factor of 310 fg C μm^{-3} (Fry 1990).

3. RESULTS AND DISCUSSION

At both samplings in October 1998 and in September 2004, a subset of the physico-chemical characteristics resembled those typically found in the chemocline of Lake Cadagno, while others differed between years. Typical and similar profiles were displayed in both years for oxygen with concentrations below 1 mg L^{-1} in the upper part of the chemocline, rapidly decreasing with depth, and also for light intensity with about 1% of the transmitted light reaching the depth of 11.5 m in both years (Fig. 1). In contrast, sulfide concentrations and turbidity differed between years, which reflected results obtained in a previous study (Tonolla *et al*. 2005). While sulfide concentrations in 1998 resembled a typical sulfide profile with increasing concentrations

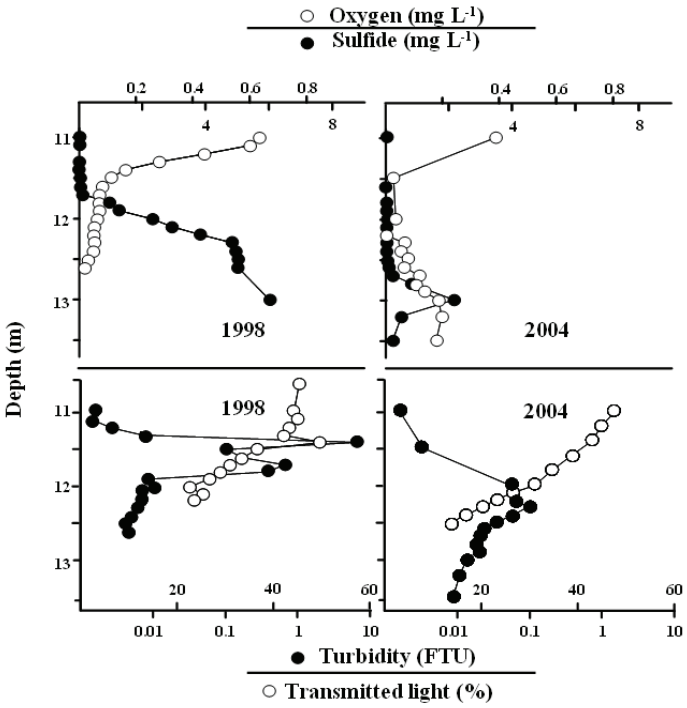

Fig. 1. Selected physico-chemical characteristics in a depth profile of the chemocline of Lake Cadagno (oxygen and sulfide concentrations (mg L^{-1}), transmitted light (%) and turbidity (mV)) at the sampling times in October 14th, 1998 (left panel) and September 28th, 2004 (right panel).

with depth reaching 6 mg L^{-1} at 13 m (Tonolla *et al*. 1998), those in 2004 were much lower with a small peak (2 mg L^{-1} at 13 m) but no marked increase with depth (Fig. 1). The pattern of sulfide in 2004 with decreasing levels around 14 m depths might be explained by the presence of a peack in iron concentrations at 14 m depth (0.18 mg L^{-1}) compared to the average concentration at other depths (0.05 mg L^{-1}). Iron might reduce the sulfide concentration by precipitating it in form of FeS (Garcia-Gil *et al.* 1990). Sulfide is produced, under anoxic conditions, by sulfate reducing bacteria, which reduce sulfate to sulfide with electrons provided by organic substances (Lüthy *et al*. 2000). Therefore, if an increase in organic matter would have occurred as consequence of the strong mixing of the lake water column, an increase in sulfide concentration *via* the production by SRB should be evident. However the huge development of GSB and their intense photooxidation metabolism might have exponentially increased the consumption of sulfide in the chemocline, leading to an overall decrease of sulfide concentration. Moreover the absence of sulfide in the chemocline described in 1998 and 2004 is consistent with literature reports, where depths of highest bacterial density are often completely devoid of sulfide during the day because of the intense photooxidation carried out by the phototrophic sulfur bacteria (Lüthy *et al*. 2000). The oxygen, present near to the water surface, usually produces a chemical oxidation of the sulfide (Lüthy *et al*. 2000), but in the pattern distribution of year 2004 some coexistence of oxygen and sulfide is documented at the upper layer of the chemocline which might be explained by the internal waves and seiches already documented in Lake Cadagno by Egli *et al*. (2004). The turbidity profile in 1998 displayed a maximum at about 11.5 m depth which was coincident to environmental conditions characterized by overlapping profiles of oxygen and sulfide, and by the presence of about 1% of the transmitted light (Fig. 1). In 2004, however, the turbidity maximum was observed more than half a meter lower than in 1998, with oxygen present at low concentration and sulfide absent except for the lower part of the chemocline. At this turbidity maximum, light transmission was about one order of magnitude lower than in 1998 (Fig. 1). The turbidity profile was also less condensed in 2004 compared to 1998 and reached a lower maximum value with about 30 FTU compared to 57 FTU.

The environmental conditions observed in 2004 are consistent with data retrieved in our previous study for the years 2000 to 2003 (Tonolla *et al*. 2005) indicating a stable shift of environmental conditions in the chemocline after the assumed disturbance in autumn 1999 and 2000 (Tonolla *et al*. 2005) and therefore suggesting long-term consequences for microbial communities in the chemocline. In our previous study, the shift in environmental conditions was accompanied by a large change in microbial community structure, as demonstrated by a slight decrease in abundance of PSB and a

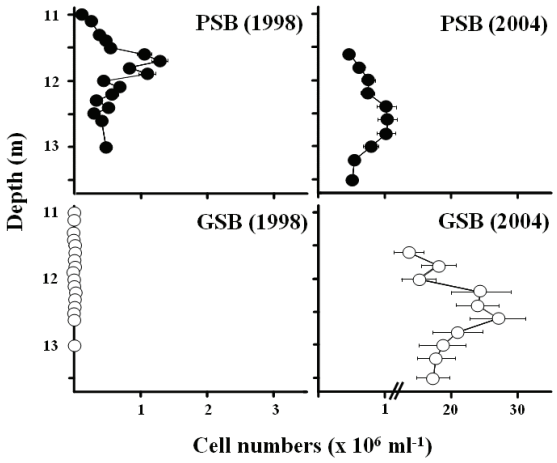

Fig. 2. Cell numbers ($\times 10^6$ mL^{-1}) of purple sulfur bacteria (PSB, the sum of values obtained for each specific probe) and green sulfur bacteria (GSB, detected with probe GSB532) in a depth profile of the chemocline of Lake Cadagno at the sampling times in October 14th, 1998 (left panel) and September 28th, 2004 (right panel).

concomitant large, one order of magnitude, increase in abundance of GSB (Tonolla *et al*. 2005). Data from 2004 confirm this shift with comparable abundances of GSB to years 2002 and 2003, and a slight increase for PSB (Tonolla *et al*. 2005). Abundances were reflected in values for biomass that were similar for PSB in 1998 and 2004 with 13 µg C mL^{-1} and 14.9 µg C mL^{-1}, respectively, while biomass of GSB was only significant in 2004 (34.3 µg C mL^{-1}, compared to 0.1 µg C mL^{-1} in 1998). Thus, the presumed disturbance and the subsequent shift in environmental conditions resulted in a three-fold increase of the total biomass of phototrophic sulfur bacteria and a stable shift in community structure from one dominated by PSB to one dominated by green sulfur bacteria.

Although environmental conditions differed between 1998 and 2004, no significant differences in the distribution profiles of populations belonging to PSB that matched the turbidity profiles for both years were obtained (Fig. 2). While they were virtually absent in 1998, in 2004 GSB distribution profile also followed the turbidity profile though with numbers about 1 order of magnitude higher than those of PSB. In 1998 GSB were observed at about 1 order of magnitude lower in numbers than PSB with approx. $5 \times 10^4 \pm 1 \times 10^4$ cells mL^{-1}), (Fig. 2). These results expand those of our previous study that had shown the replacement of PSB, as the typical and numerically most prominent bacterial group in the chemocline of Lake Cadagno (see Peduzzi *et al*. 1998; Tonolla *et al*. 2004 for review), by GSB (Tonolla *et al.* 2005). However, these data indicate no significant effect, on the distribution profiles and abundances of the overall community of PSB, of the changed environmental characteristics, such as light transmission decline, and presence of potential competitors such as GSB. Although in 2004 GSB were numerically most prominent bacterial group in the chemocline, they did not impede the development of PSB.

Similar to results for 2002 and 2003 (Tonolla *et al*. 2005), in 2004 all GSB were represented by one organism only, *Chlorobium clathratiforme*, with numbers up to 2.8×10^7 cells mL^{-1} (Fig. 2), while populations of PSB consisted of six populations of small-celled PSB (Fig. 3). Large-celled PSB represented by *Chromatium okenii* were not detected in significant numbers (5.6×10^3 averaged over the whole chemocline in 2004). Except for one population, distribution profiles of small-celled PSB showed a microstratification with population-specific distribution profiles that were generally similar between years, except that maxima in 2004 were found deeper in the chemocline, which were consistent with the turbidity profile (Fig. 3). Population D detected with probe S453D, however, exhibited a much higher maximum and tighter distribution profile in 1998 than in 2004, and overall numbers in the chemocline were lower in 2004. The reduction in numbers of this population, however, was compensated for by the presence of two additional populations that were related to the genus *Thiocystis* and detected with probes S453H and S448 (Fig. 3), which were previously detected in significant numbers in 1995 and 2001, only. These differences in population profiles are in agreement with results of our previous studies in which several populations were found to be relatively stable in abundance for years while numbers of others (i.e., populations detected with probes S453D, S453H and S448) varied significantly from year to year (Tonolla *et al*. 2005). The shift of profiles deeper into the chemocline in 2004 resulted in largely different environmental conditions with lower light transmission values and oxygen and sulfide concentrations which, in addition to the large increase in abundance of GSB, however, did not seem to affect or drive the distribution of these populations with depth.

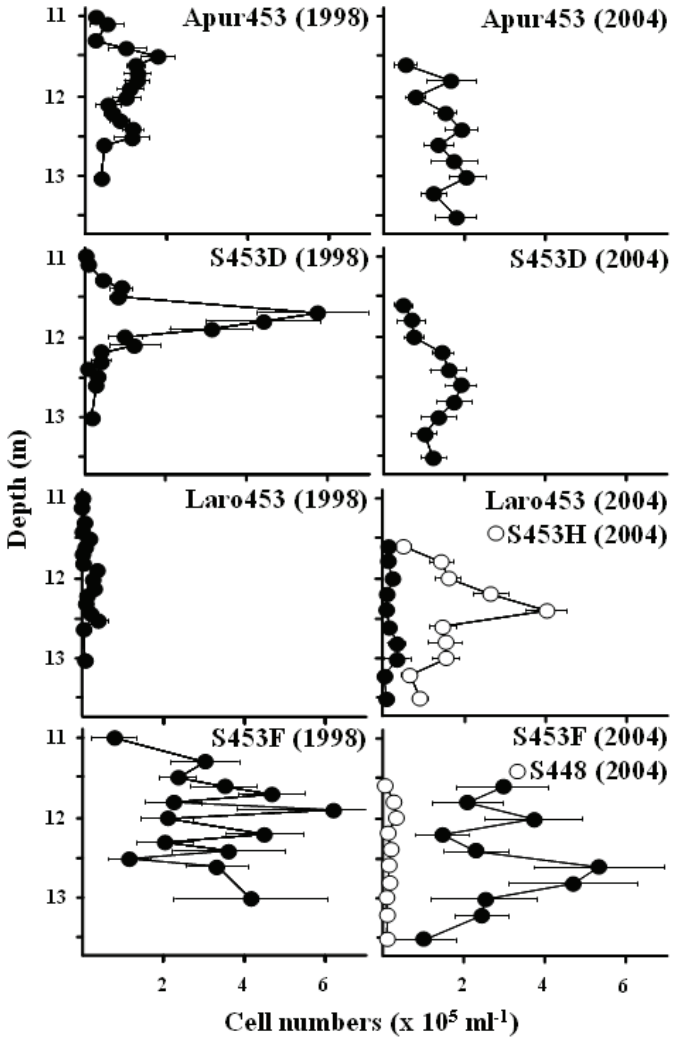

Fig. 3. Cell numbers ($\times 10^5$ mL^{-1}) of small celled purple sulfur bacteria detected with probes Apur453, S453D, Laro453, S453F, S453H and S448 in a depth profile of the chemocline of Lake Cadagno at the sampling times in October 14th, 1998 (left panel) and September 28th, 2004 (right panel).

In contrast to PSB related to the genus *Lamprocystis* (Tonolla *et al*. 2000; Peduzzi *et al*. 2003a, b), both populations related to *Thiocystis* did not form aggregates, and were not associated with SRB of the genus *Desulfocapsa*. The aggregates of PSB related to the genus *Lamprocystis*, however, had decreased in size about four-fold in the year 2004 compared to 1998, but remained associated with SRB of the genus *Desulfocapsa* (Fig. 4). A reduction in size of small-celled PSB aggregates, as consequence of reduced abundance of small-celled phototrophic sulfur bactieria, has been described in winter and spring when ice and snow cover had reduced light transmission to values similar to this study (Peduzzi *et al*. 2003b).

Smaller aggregates during winter and spring resulted in fewer bacteria related to *D. thiozymogenes* being associated to the aggregates, in addition to a five-fold reduction in cell numbers. Associated cells accounted only for about 62% of the cells during winter and spring, while during summer up to 97% of all cells of bacteria related to *D. thiozymogenes* were associated with the aggregates (Tonolla *et al*. 2000). In our study, cells of bacteria related to *D. thiozymogenes* had increased in numbers about three times in 2004 compared to 1998 (Fig. 5), resulting in an important change of the ratio between the number of *Desulfocapsa*-like cells and the purple sulfur cells forming the aggregates. Interestingly in 1998 the aggregates were composed by approximately 31% of *Desulfocapsa*-like cells and 69% of PSB cells. In contrast in 2004 the composition was 66% for *Desulfocapsa*-like and 34% for PSB. The depth distribution of *Desulfocapsa*-like cells in the chemocline remained similar between 1998 and 2004 and followed profiles of small-celled PSB since almost all *D. thiozymogenes* cells detected were associated with aggregates (up to 95% of *Desulfocapsa* cells were associated) (Fig. 5).

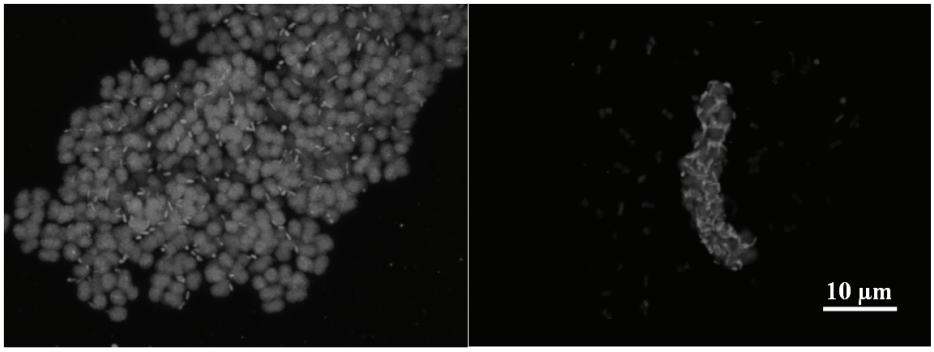

Fig. 4. Characteristic appearance of aggregates of small-celled purple sulfur bacteria and their association with sulfate-reducing bacteria of the genus *Desulfocapsa* at the sampling times in October 14th, 1998 (left panel) and September 28th, 2004 (right panel).

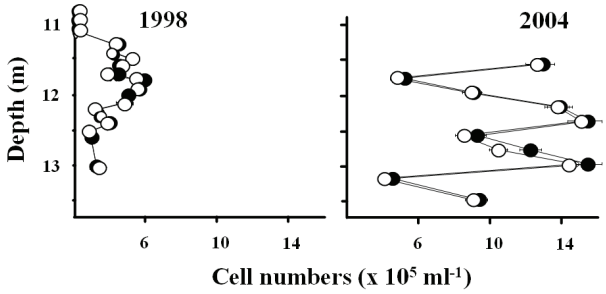

Fig. 5. Cell numbers ($\times 10^5$ mL^{-1}) of sulfate-reducing bacteria related to the genus *Desulfocapsa* in a depth profile of the chemocline of Lake Cadagno at the sampling times in October 14th, 1998 (left panel) and September 28th, 2004 (right panel). Closed circles represent all cells detected with probe DSC213 and DSC441, while open circles represent only those associated with small-celled purple sulfur bacteria.

Although the driving forces which are regulating aggregate formation and composition are still not well understood, overall, these findings suggest that the aggregate is a dynamic association responding to shifts in environmental conditions (e.g., changes in sulfide and oxygen concentration as suggested also by Overmann 1997) and to changes in global microbial community structure. Our results might indicate an increased role of SRB related to *Desulfocapsa* for small-celled PSB, and interaction between both might overcome growth obstacles introduced by the changes in environmental conditions.

The most intriguing change after 1998 is the establishment of GSB as the most prominent component of the microbial community, which might be a consequence of increased nutrient or organic carbon availability after the mixing event. Studies are needed to assess changes in nutrient and organic carbon availability in relation to the blooming of GSB in Lake Cadagno. Indeed in addition to changes in light intensity, enhanced carbon availability has been suggested to be an important factor influencing growth of GSB and therefore support seasonal blooms (Bergstein *et al*. 1979). Despite the fact that GSB need only about one quarter of the light intensity of the PSB in order to grow at comparable growth rates (Biebl & Pfennig 1978) and show different light absorption optima than PSB (Del Don *et al.* 2001), a micro-stratification with PSB growing above GSB was not obtained, which is consistent with results of previous studies on their distribution in the chemocline of Lake Cadagno (Tonolla *et al*. 2003) and on other meromictic lakes (Vila *et al*. 1998). PSB are able to use polysulfides as electron donors, which can not be exploited by GSB in the presence of hydrogen sulfide, and to assimilate a wider variety of carbon compounds than GSB (Garrity & Holt 2001). Taken together, these two considerations might explain the coexistence of PSB and GSB at the same depths in the chemocline of Lake Cadagno.

The large abundance of GSB throughout the whole chemocline and the concomitant use of sulfide might account for the low sulfide concentrations which could further favor growth of GSB over that of PSB (Guerrero *et al*. 1987; Vila *et al*. 1998). This would be comparable to periods of intensive photo-oxidation by phototrophic sulfur bacteria, a situation that is encountered in Lake Mahoney (Overmann *et al*. 1991, 1994; Overmann 1997), as well as in Lake Cadagno (Joss *et al*. 1994; Fritz & Bachofen 2000; Lüthy *et al*. 2000). Under these conditions, the interaction of small-celled PSB with SRB related to *Desulfocapsa* might help to overcome a potential sulfide limitation. This is in agreement with previous studies where concomitant growth enhance-

ment of both partners of the aggregate in mixed culture could be observed (Peduzzi *et al*. 2003a), thus suggesting synergistic interactions based on the mutual exchange of sulfur compounds. On the other hand GSB, thank to their higher affinity to sulfide compared to PSB (van Gemerden 1984), seam not to be affected by sulfide limitation even if they are not associated with *Desulfocapsa*.

PSB are metabolically versatile and are capable of both autotrophic and mixotrophic metabolism using a variety of different carbon sources (Trüper 1981; Eichler & Pfennig 1988; Schaub & van Gemerden 1994; van Gemerden & Mas 1995). *In situ* measurements of chemolithotrophic inorganic carbon fixation have been documented in the chemocline of Lake Cadagno by Camacho *et al*. (2001). Moreover mixotrophic growth on several carbon sources has also been demonstrated for an isolate representing population *F* of the small-celled PSB in the chemocline of Lake Cadagno (Peduzzi *et al*. 2003a). Populations associated with SRB might therefore benefit, under mixotrophic conditions, from the potential excretion of small organic molecules such as acetate during sulfate reduction and concomitant oxidation of organic substrates by the SRB (Eichler & Pfennig 1988; Peduzzi *et al*. 2003a).

Our analyses expand those of a previous study over a decade by an additional year and present a more detailed analysis of specific populations throughout the chemocline of Lake Cadagno. The results suggest the establishment of different, but stable, environmental conditions after a potential disturbance that supported growth and establishment of a new population of GSB without significant impact on the abundance of populations of specific PSB. The assumed stability of the latter populations might be a function of aggregate formation and their interaction with SRB.

ACKNOWLEDGEMENTS

The authors wish to thank A.P. Caminada for technical support. This work was supported by grants from the Swiss National Science Foundation (SNSF) (NF31-46855.96), the canton of Ticino and the Alpine Biology Center Foundation (Switzerland).

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Received: September 2008
Accepted: November 2008

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