Chapter 7
Lake Cadagno: Microbial Life in Crenogenic Meromixis

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7.1 Introduction

Due to their permanent stratification, meromictic lakes are interesting model ecosystems. In particular, the chemocline offers many advantages for studying the planktonic life and processes mediated by microorganisms. The physico-chemical gradients across the chemocline support the development of very intense microbial blooms (Overmann et al. 1996; Parkin and Brock 1981; Sorokin 1970; van Gemerden and Mas 1995). Along vertical gradients of light and different electron donors and acceptors, diverse physiological groups of organisms substitute each other (Guerrero et al. 1985; Jørgensen et al. 1979; Overmann et al. 1991) in distinct layers at depth intervals ranging from cm to m. These distinct layers facilitate accurate sampling and measurement of environmental characteristics. Lake Cadagno is a crenogenic meromictic lake located in the southern Swiss Alps (46°33′N, 8°43′E) at 1921 m asl, in the catchment area of a dolomite vein rich in gypsum (Piora-syncline). A permanent chemocline lies between 10 and 14 m depth, stabilized by density differences of salt-rich, high-density water constantly supplied by sub-aquatic springs to the monimolimnion and of electrolyte-poor, low-density surface water that feeds the mixolimnion. Steep sulphide gradients in the chemocline support the growth of large numbers of phototrophic sulphur bacteria (up to 10^7 cells ml^{-1}). This community includes purple sulphur bacteria (PSB, family *Chromatiaceae*) of the genera *Chromatium*, *Lamprocystis*, *Thiocystis* and *Thiodictyon* and green sulphur bacteria (GSB. family *Chlorobiaceae*) of the genus *Chlorobium*. Since the beginning of the last century, Lake Cadagno and the nearby Lake Ritom have been used as models to study water stratification, sulphidogenic bottom waters and blooms of PSB. Since the early 1980s, studies on ecological issues in Lake Cadagno significantly increased, supported by two major developments. The first was the establishment of research facilities at the shore of the lake, the Alpine Biology Center, and the second was the introduction of novel molecular techniques that allowed researchers to analyse microbial populations in Lake Cadagno unaffected by the difficulties in culturing them.

This contribution presents an overview of more than 30 years of studies on Lake Cadagno, including biogeochemical analyses, diversity surveys and ecological studies on the very diverse microbial community developing in it.

The present chapter is structured in the following major parts:

1. Hydrology, and morphology of the lake in brief, including the lake's origin and age
2. Historical perspective and description of early investigations conducted on Lake Cadagno and description of the lake's meromixis and the main physico-chemical features of the lake and its related stratification
3. The organisms inhabiting the lake, with its diversity dominated by microorganisms, particularly in relation to the sulphur cycle

The vast majority of the studies on Lake Cadagno concern the bacterial community developing in the chemocline. The chapter on the biology of the lake reviews comprehensively studies published since 1985 and results of recent studies on prokaryotic diversity (see Sect. 5.1) and on microbial eukaryotic diversity (see Sect. 5.3).

7.2 Origin, Morphology and Hydrology of Lake Cadagno

Lake Cadagno (Fig. 7.1) originated due to glacial erosion during the last glacial period, about 10,000 years ago, and the formation of a moraine acting as a small dam (Boucherle and Züllig 1988; Krige 1918; Stapfer 1991; Wirth et al. 2013). The lake is located in the southern Swiss Alps at 1921 m asl (latitude, 46°33′N; longitude, 08°43′E), in Val Piora, a region characterized by its richness in mountain lakes, waterways, wetlands and ponds (Garwood 1906; Peduzzi 2011). Lake Cadagno is small (maximum length, 830 m; maximum width, 420 m; surface area, 0.26 km^2; maximum depth, 21 m; average depth, 9.27 m) (Del Don et al. 2001), situated among pre-Triassic gneiss and mica-schists of central alpine crystalline rocks just south of the Gotthard Pass in the Canton Ticino (Switzerland). It has a catchment area of 2.71 km^2. The southern lake shore rests on late Triassic and early Jurassic rocks folded into the so-called Piora-syncline, characterized by the presence of tectonized dolomitic limestones (magnesian limestone) and gypsum deposits with a karstic hydrology. The Piora-syncline forms a vein that separates the crystalline rocks of the Gotthard massif to the north and the metamorphic

Fig. 7.1 Lake Cadagno and Piora valley. Photo taken from the west side of the lake

Pennine Lucomagno Nappe, about 10 km to the south, along the whole Piora valley (Otz et al. 2003).

Lake's sediment analysis reveals that permanent stratification, i.e. meromixis, and the presence of phototrophic sulphur bacteria must have been established very early in the history of the lake. This suggestion was first based on the analyses of organic components in the upper sediments (Putschew et al. 1995; Hebting et al. 2006) and of Cladocera (Crustacea) remains (Boucherle and Züllig 1988). Trace pigments, e.g. okenone and isorenieratene, characteristic for purple and green phototrophic bacteria, were detected in the upper 170 cm of the lake's sediment (Züllig 1985). However, the detailed longer-term history of development of meromixis and differential redox conditions in Lake Cadagno, and the timing of the transition from oxic to euxinic (i.e. sulphidic and anoxic) conditions in the water column, remained unknown until 2009, when 10.5-m-long sediment cores covering the complete lacustrine record of Lake Cadagno were investigated using an interdisciplinary approach (Niemann et al. 2012; Ravasi et al. 2012b; Dahl et al. 2013; Wirth et al. 2013). The lake history was characterized by a first oxic-intermediate-sulphidic redox-transition period shortly after glacial retreat, and lake formation was estimated at 12.5 kyr BP (before present). During the 2300-year-long intermediate period from 12.1 to 9.8 kyr BP, strongly fluctuating Mn concentrations in the sediments associated with episodic water column mixing events were recorded (Wirth et al. 2013). At the same time, efficient Mo burial (Dahl et al. 2010) and the presence of remains of phototrophic sulphur bacteria (Ravasi et al. 2012b) provided an evidence for a sulphidic environment. In fact, at the end of this period with intermediate redox conditions, ca. 9800 ± 130 years ago, a pronounced drop in Mn and a simultaneous rise in Mo concentrations in the sediments indicated efficient Mo burial in sulphide-rich water column and thus the onset of euxinic conditions in Lake Cadagno. This redox transition was causally linked to the concurrent climate warming, which led to permafrost melting and the activation of the sub-aquatic springs. The inflow of salt-rich waters to the lake bottom likely initiated the formation of a chemocline and a stable crenogenic meromixis (créno, spring; from the Greek "Krini") in the early Holocene. Recent biogeochemical data provide evidence that Lake Cadagno has remained sulphidic from the start of Holocene to date. Flood and mass movement events, however, generated underflows of turbidity-rich water that sporadically introduced oxygen-rich water to the hypolimnion, thereby only temporarily weakening the sulphidic conditions (Wirth et al. 2013). Such events may have influenced water turbidity and nutrient supply and may thus have caused shifts in the microbial community structure (see Sect. 5.1.1.2.) (Tonolla et al. 2005b; Decristophoris et al. 2009; Gregersen et al. 2009).

Water penetrating and circulating through the karstic dolomite and reappearing as underwater springs in the southern part of the lake has a rather high ionic strength (9–10 mM), and it flows downwards feeding the anoxic lower part of the water column (monimolimnion). Dye tracing experiments (Otz et al. 2003) have revealed that this groundwater originates from a relative small underground catchment area which extends for about 2 km to the southeast up to the so-called Calderoni Sinkhole. The sinkhole causes, uphill, a flow of the underground water towards the Lucomagno, i.e. in the opposite direction to the surface water flow. On the other hand, the upper part of the lake's aerobic water column (mixolimnion) is fed by surface run-off from a small drainage area of about 2 km^2, north of the lake, which encompasses the crystalline rocks of the Gotthard massive. The major source of surface input to Lake Cadagno is a small stream that drains water from Lake Stabbio, a small lake at 2351 m asl. The crystalline rocks of the watershed are rather resistant to chemical weathering, such that the water of the mixolimnion has a relatively low ionic strength and is oligo- to mesotrophic. The density difference between the two water masses in Lake Cadagno causes the development of a very stable stratification with a chemocline at about 10–14 m depth characterized by the rapid change of the concentrations of chemical components (Del Don et al. 2001; Hanselmann and Hutter 1998; Peduzzi et al. 1998).

7.3 Historical Overview: Scientific Studies and Hydroelectric Exploitation

Lake Cadagno has attracted scientists since the end of the nineteenth century. In fact, since the foundation of limnology in 1904, the lakes of the Piora region have created considerable scientific interest (Bourcart 1906; Garwood 1906; Burckhardt 1910); for a review on the historic data sets on lakes Ritom and Cadagno, see Peduzzi and Bianconi (2012). Stratification phenomena, as well as sulphur-rich deep waters and mass developments of phototrophic sulphur bacteria, are among the early observations on the lake. A monograph on the nearby Lake Ritom (Collet et al. 1918) reported results of studies carried out during the construction of the Ritom hydroelectric dam (1915–1920). Already then, the importance of the sulphur cycle and the bacteria involved therein were mentioned (Düggeli 1924). Studies on fish populations (Surbeck 1917) and earlier researches on phytoplankton and sulphur bacteria were carried out on Lake Ritom and Lake Cadagno (Bachmann 1924, 1928; Borner 1928a, b; Burckhardt 1910; Düggeli 1924). These early published works are important documents reporting the loss of meromixis in Lake Ritom following the construction of the Ritom dam. Similar results were reported for a French alpine lake, Lac de la Girotte in Savoie, in 1926 (Delebeque 1898; Barades 1926). Lake Cadagno is probably the most unique among the lakes in the entire alpine range that exhibit crenogenic meromixis. In 1948, a small dam was built raising the lake's surface water level from 1918 to 1921 m asl, and up to the year 2007, the lake was exploited for production of electricity resulting in a 3-m decrease in the lake's water level during the winter. During almost 60 years of exploitation of the lake for hydroelectric generation, it retained its permanent stratification. The lowering of the water level led to a loss of about 720,000 m^3 water, i.e. about 30 % of the lake's total water volume (2.42 million m^3). This comprised 50 % of the water volume with O_2 concentration >4 mg l^{-1} (around the minimum survival limit for Salmonidae). Aerobic organisms in the mixolimnion were subjected to a significant reduction in vital living space. In addition to that, 45,000 m^2 of littoral dried up causing, on the one hand, the death of numerous populations of sediment dwellers (e.g. Ephemeroptera, Plecoptera and Trichoptera), which were a potential source of food for fish species and, on the other hand, the loss of spawning areas for the fish reproduction (Tonolla et al. 1988). At present, the lake is no more actively being exploited for the production of electricity, and therefore its water level remains fairly constant at 1921 m asl.

Studies on Lake Cadagno intensified during the twentieth century, more so since the 1980s. The opening of the Alpine Biology Center (ABC) in Piora in 1994 in cooperation with the Universities of Geneva, Zurich, Lugano and recently the newly opened University of Applied Sciences and Arts of Southern Switzerland (SUPSI), as well as participating cantons, the municipality and the patriciate of Quinto, have led to a substantial increase in scientific studies in the region.

The presence of scientific database is an invaluable source of information for researchers, as it provides an important historical perspective and solid data sets as a basis for future research. For a complete bibliography on scientific studies on the region and on Lake Cadagno before 1998, refer to *Chronological list of publications on Lake Cadagno and Piora Valley* in Peduzzi et al. (1998), and for the period after 1998, check on the website of the Alpine Biology Center, Piora (*http://www.piora.org/*).

7.4 Lake Water Chemistry and Meromixis

The dolomite vein present in the Piora valley is in direct contact with the southern part of the Cadagno basin. The water which percolates through these easily weathered rocks becomes enriched in calcium, magnesium, carbonate and sulphate ions and enters the waterbody through sublacustrine springs. The analyses of seven underwater springs located in the southern part of the lake at depths between 8.0 and 12.5 m provided the following averages for: conductivity, 1359 (±145) μS cm^{-1}; calcium, 171.1 (±22.5) mg l^{-1}; magnesium, 74.7 (±10.1) mg l^{-1}; sulphate, 622.9 (±81.5) mg l^{-1}; and carbonate, 120.7 (±35.6) mg l^{-1} (Del Don et al. 2001). The inflow of this water contributes to the establishment of the crenogenic meromixis in the lake. The chemocline is permanent and stabilized by the difference in density between the two water strata: the upper mixolimnion and the lower monimolimnion (Del Don et al. 2001; Lehmann and Bachofen 1999; Tonolla et al. 1998b).

The concentrations of nutrients in the oxic mixolimnion (between 0 and 10–12 m depth) are low, with phosphate (PO_4_P) close to the detection limit (<0.3 μg l^{-1}), nitrate (NO_3_N) below 11.5 μg l^{-1} and dissolved inorganic carbon (DIC) ca. 10 mg l^{-1} (Tonolla et al. 1998b, 1999). The monimolimnion is anoxic and rich in reduced compounds due to the action of anaerobic sulphur bacteria. Moreover, it is a steady food source indispensable for the development of the dense community of phototrophic sulphur bacteria in the chemocline. High concentrations of sulphur compounds (HS^- up to 30 mg l^{-1}; SO_4^{2-} up to 200 mg l^{-1}) ammonium (NH_4_N up to 3.9 mg l^{-1}), phosphate (PO_4_P up to 0.132 mg l^{-1}), carbonates (HCO_3^- and CO_3^{2-} up to 50 mg l^{-1}) (Del Don 2001; Tonolla et al. 1998b) and dissolved organic carbon (DOC, up to 6 mg C l^{-1}) (Bertoni et al. 1998) are the basis for the massive development of populations of these phototrophic sulphur bacteria, which reach maximum densities of 10^7 cells per ml^{-1} during summers.

With sulphate concentrations ranging between 100 and 200 mg l^{-1} (1–2 mM), Lake Cadagno contains up to ten times more sulphate than most freshwater lakes (Hanselmann and Hutter 1998). In addition, sulphate, bicarbonate, calcium and manganese are the dominant ions in Lake Cadagno (Tonolla et al. 1998a).

For the water stability, the massive development of large-celled, flagellated phototrophic purple sulphur bacteria in the chemocline may also influence the profiles of temperature and salinity by their active movements, contributing to maintain constant values over a depth interval of about 0.5 m. The water mixing caused by the organisms at this level destabilizes the density gradient and the physico-chemical stratification (Wüest 1994).

7.5 Biology of Lake Cadagno

7.5.1 Prokaryotic Diversity

The prokaryotic diversity of Lake Cadagno has been studied by several workers in the last three decades (Züllig 1985; Peduzzi et al. 1998, 2000, 2012; Tonolla et al. 1999, 2000, 2005a). Because of the difficulty in culturing environmental microorganisms, the study of the bacterial populations was focused, as early as in the 1990s, on direct detection methods such as nucleic acid staining with DAPI or acridine orange (Bensandoun et al. 1998; Tonolla et al. 1998a). Such approaches revealed the presence of several bacterial morphotypes (e.g. *Chromatium okenii*, *Lamprocystis* sp., *Desulfocapsa* and morphotype R) and allowed an evaluation of the bacterial diversity in the lake's water column. The phylogenetic analysis of bacterial communities continued with the amplification and generation of gene clone libraries for 16S ribosomal RNA genes (Bosshard et al. 2000a; Demarta et al. 1998; Schramm et al. 2003). The microbiological analyses did not only focus on the chemocline (Tonolla et al. 1999, 2000, 2005a; Bosshard et al. 2000a, b; Decristophoris et al. 2009; Halm et al. 2009), but also on the monimolimnion, the mixolimnion (Tonolla et al. 2005b; Gregersen et al. 2009; Milucka et al. 2015) and the anoxic sediment (Schubert et al. 2011). They allowed the identification of novel bacterial species, later isolated from the chemocline of the lake (e.g. *Thiocystis chemoclinalis* sp. nov., *Thiocystis cadagnonensis* sp. nov. and *Candidatus* "*Thiodictyon synthrophicum*" sp. nov.) (Peduzzi et al. 2011; Peduzzi et al. 2012).

Recently a next-generation sequencing technology, 454 pyrosequencing, was also applied to explore the prokaryotic diversity in the layers of the water column (mixolimnion, chemocline, monimolimnion) and the superficial anoxic sediment of Lake Cadagno (unpublished data). This generated 22,700 sequences of the hypervariable V1–V3 regions (average length, 400 bp) of the 16S rRNA gene. Through PICRUSt algorithm (Langille et al. 2013), 3244 sequences (1227 sequences for mixolimnion, 621 for chemocline, 1326 for monimolimnion and 70 for anoxic sediment) could be assigned to 18 known bacterial phyla, 11 of which were dominant (>1 % relative abundance). This number is higher than the 12 phyla identified in the previous studies (Ravasi et al. 2012a). The monimolimnion noticeably differed, with 13 phyla identified by 454 pyrosequencing, compared with eight phyla previously identified. Also, microbial communities differed among the different layers in the water column and the anoxic sediment. The mixolimnion was dominated by the phylum *Verrucomicrobia* (51 %), which was less abundant in the chemocline (8 %) and monimolimnion (4 %) and absent in the anoxic sediment. Other important phyla of mixolimnion were *Actinobacteria* (14%) and *Bacteroidetes* (3 %). *Proteobacteria* were the most represented phylum in the chemocline communities (76 %); they were also well represented in the mixolimnion (31 %), monimolimnion (19 %) and anoxic sediment (18 %). Other important communities in the chemocline were *Chlorobi* (8 %), *Actinobacteria* (5 %) and *Bacteroidetes* (2 %). Other than the presence of *Proteobacteria*, the monimolimnion communities were mainly comprised of *Chlorobi* (66 %), *Actinobacteria* (6 %), *Bacteroidetes* (3 %) and *Chloroflexi* (1 %). The anoxic sediment was the most diverse habitat, with various phyla almost equally represented, in addition to the previously mentioned *Proteobacteria* (*Chloroflexi* 19 %, *Chlorobi* 15 %, *Actinobacteria* 14 %, *Cyanobacteria* 11 %, OP8 8 %, *Bacteroidetes* 7 %, *Firmicutes* 2 % and *Nitrospirae* and *Acidobacteria* 1 %). At a finer taxonomical resolution, *Alphaproteobacteria* were the dominant class of *Proteobacteria* in the mixolimnion, with the family of *Pelagibacteraceae* (aerobic oxygenic bacteria). Alphaproteobacteria also represented a good part of the *Proteobacteria* found in the anoxic sediment (*Hyphomicrobiaceae*, *Pelagibacteraceae* and *Rhodobacteraceae*). The class *Betaproteobacteria* was represented in all samples, mainly by *Comamonadaceae*. *Deltaproteobacteria* were also present in all habitats analysed, except in the mixolimnion; they were best notable in the monimolimnion, with the sulphate reducers *Desulfobulbaceae*, and in the sediment (clade OM27). *Epsilonproteobacteria* were present in chemocline and monimolimnion (*Helicobacteraceae*). *Gammaproteobacteria* were the most dominant *Proteobacteria* class in the chemocline, with the sulphur purple photosynthetic *Chromatiaceae*, and were also prominent in the monimolimnion (*Chromatiaceae*) and the sediment (*Moraxellaceae*, *Pseudoalteromonadaceae*, *Sinobacteraceae*, *Vibrionaceae*).

The diversity of archaea in Lake Cadagno has received less attention than the bacteria. Nonetheless, the few studies that were conducted have highlighted interesting patterns of distribution. Bottinelli (2008) explored the coexistence of sulphate-reducing bacteria and methanogenic archaea in the sediments of the lake.

The analysis of 16S rRNA gene sequences allowed the detection of few phylotypes, with more diversity in the deeper part of the sediments. Schubert et al. (2011) studied the archaeal community involved in methane production or degradation in upper sediments of Lake Cadagno, constructing 16S rRNA gene clone libraries from 2 to 4 cm sediment (42 clones) and 9- to 15-cm (43 clones) sediment depth. These depths represented the peak (2–4-cm sediment) and bottom (9–15-cm sediment) of the AOM (anaerobic oxidation of methane) zone. The archaeal diversity revealed in this study was also low, with only nine different phylotypes detected, based on a 97 % 16S rRNA gene sequence similarity criterion. The majority of 16S rRNA gene sequences belonged to the euryarchaeotal marine benthic group D (MBGD), found in a variety of limnic and marine habitats. In total, six distinct MBGD phylotypes were retrieved, three of which were only found in 2–4-cm sediment depth. The second archaeal lineage detected was the recently described AOM-associated archaea (AAA) clade of Euryarchaeota. As third and fourth archaeal lineages, the crenarchaeotal marine benthic groups B and C (MBGB and MBGC) were identified. Catalysed reporter deposition-fluorescence in situ hybridization (CARD–FISH) analysis revealed a high abundance of *Deltaproteobacteria*, especially of free-living sulphate-reducing bacteria of the *Desulfosarcina*/*Desulfococcus* branch of *Deltaproteobacteria* in the AOM zone. Here, loose aggregations of AAA cells were found that might be responsible for oxidation of methane in the lake sediments (Schubert et al. 2011). A more recent study by Milucka et al. (2015) on methane oxidation coupled to oxygenic photosynthesis in the chemocline of Lake Cadagno revealed that, despite the geochemical evidence for high methane-oxidizing activity, no anaerobic methane-oxidizing archaea (ANME)-1 or (ANME)-2 were detected in samples from the anoxic zone (i.e. from 10 and 13 m) by CARD–FISH with any of the used probes.

The Anoxygenic Phototrophic Sulphur Bacteria in the Chemocline

One of the main features of sulphur-rich Lake Cadagno is the diverse community of microorganisms which develops in the chemocline especially in the spring and summer months. The community, which is dominated by anoxygenic phototrophic sulphur bacteria, also contains microorganisms that acquire energy via anaerobic respiration, aerobic respiration and fermentation (Bosshard et al. 2000a). Both green sulphur bacteria (GSB) and purple sulphur bacteria (PSB) have been observed in Lake Cadagno, and a number of their strains have been successfully isolated, cultivated in the laboratory and described as new bacterial species (i.e. Eichler and Pfennig 1988; Tonolla et al. 1999; Peduzzi et al. 2011, 2012). *Thiocystis chemoclinalis*, *T. cadagnonensis* and *Candidatus* "*Thiodictyon syntrophicum*" were recently described (Peduzzi et al. 2011, 2012).

Anoxygenic phototrophic sulphur bacteria are phylogenetically diverse, but all harvest light energy using antenna complexes containing bacteriochlorophylls, and accessory pigments, such as carotenoids. The antenna complexes adsorb light radiation and transfer the captured energy to a reaction centre where it is subsequently used to generate ATP and reductant (NAD(P)H). Anoxygenic phototrophic sulphur bacteria utilize either the pheophytin–quinone-type reaction centres (PSB and *Chloroflexus* sp.) or the Fe–S reaction centres (GSB and *Heliobacteria* sp.). In addition to sulphide, some of these bacteria utilize elemental sulphur and thiosulphate as electron donors (Frigaard and Dahl 2009).They can also utilize hydrogen ($H_2(g)$) as a reductant. Many anoxygenic phototrophs can also oxidize ferrous iron to reduce CO_2 (Widdel et al. 1993; Ehrenreich and Widdel 1994; Overmann and Garcia-Pichel 2006; Hegler et al. 2008). The oxidation of reduced inorganic sulphur by sulphide-oxidizing anoxygenic phototrophic bacteria is usually coupled to CO_2 reduction to form cell biomass following a number of possible pathways (Eqs. 7.1–7.5):

$$CO_2 + 2H_2S \rightarrow CH_2O + H_2O + 2S^0 \tag{7.1}$$

$$3CO_2 + 2S^0 + 5H_2O \rightarrow 3CH_2O + 2SO_4{}^{2-} + 4H^+ \tag{7.2}$$

$$2CO_2 + H_2S + 2H_2O \rightarrow 2CH_2O + SO_4{}^{2-} + 2H^+ \tag{7.3}$$

$$CO_2 + 2S_2O_3{}^{2-} + H_2O \rightarrow CH_2O + 2S^0 + 2SO_4{}^{2-} \tag{7.4}$$

$$2CO_2 + S_2O_3{}^{2-} + 3H_2O \rightarrow 2CH_2O + 2SO_4{}^{2-} + 2H^+ \tag{7.5}$$

Taxonomy of Anoxygenic Phototrophs from Lake Cadagno

In Lake Cadagno, the cell concentration maxima of anoxygenic phototrophs (*Chromatiaceae* and *Chlorobiaceae*) in the summer are located between 10.5 and 13.5 m depth in the chemocline (Schanz et al. 1998; Bosshard et al. 2000a). The community of phototrophic bacteria in the chemocline is diverse but dominated by a few species of purple and green sulphur bacteria (Tonolla et al. 1999; Bosshard et al. 2000a). The main anoxygenic phototrophic bacteria identified in Lake Cadagno (Table 7.1) are three flagellated PSB, one large-celled, *Chromatium okenii*, and two small-celled, *Thiocystis chemoclinalis* and *T. cadagnonenensis*; four small-celled PSB *Lamprocystis purpurea*, *Candidatus* "*Thiodictyon syntrophicum*", *Lamprocystis roseopersicina* and *Lamprocystis* sp. strain D; and two GSB *Chlorobium clathratiforme* and *Chlorobium phaeobacteroides* (Bosshard et al. 2000a, b; Tonolla et al. 1999, 2003, 2005b; Peduzzi et al. 2011, 2012). The main sulphide-oxidizing anoxygenic phototrophs are grouped according to their physiology, morphology, pigment composition and membrane structure (Table 7.1).

The PSB belong to the gamma division of the *Proteobacteria*. They have BChl *a* as the main bacteriochlorophyll and contain carotenoids, such as okenone. The reaction centre is of Type II containing pheophytin and quinone. They fix CO_2 using the reductive pentose phosphate cycle. Metabolically, they can be photoautotrophs or photoheterotrophs and in some cases also chemolithotrophs growing in the absence of light (Table 7.1). Two examples of strains that can grow chemolithotrophically are *Lamprocystis purpurea* and *Candidatus* "*Thiodictyon syntrophicum*". PSB are mostly obligate anaerobes, although some may tolerate low-oxygen conditions. The four main small-celled PSB found in Lake Cadagno are between 1.4 and 4 μm in diameter and spherical to oval in shape. The best described of the group, *L. purpurea* and *Candidatus* "T. syntrophicum", utilize

Table 7.1 Characteristics of the main sulphide-oxidizing anoxygenic phototrophs in the chemocline of Lake Cadagno

Organism	Group	Phylogenic position	Electron donor	Cell size [µm]	Shape	Abs max [nm]	Photosynthetic pigments	C-fixation pathway	Other characteristics	References
Chromatium okenii	PSB	γ-*Proteobacteria*	Sulphide, thiosulphate, elemental sulphur	4.5–6 × 8–15	Rod	370, 520, 835	Purple-red colour BChl *a* okenone	Reductive pentose phosphate cycle	Motile, intracellular sulphur globules	Fischer et al. (1996), Tonolla et al. (1999)
Cand. Thiodictyon syntrophicum *(Strain F, Cad16*^{T}*)*				1.4–2.4[a]	Spherical to oval	374, 528, 582, 833	Purple-red colour BChl *a* okenone		Slime capsule, intracellular sulphur globules, gas vacuoles, immotile, aggregation, chemolithotrophic	Peduzzi et al. (2003b, 2012)
Thiocystis chemoclinalis (Strain CadH11^T*)*				2.3–3.6	Spherical to oval	525, 590, 830	BChl *a*, okenone		Motile by flagella, aggregates, chemolithotrophic growth in the presence of oxygen in the dark	Peduzzi et al. (2011)
Thiocystis cadagnonensis (Strain Cad448^T*)*				2.3–4.7	Ovoid to rod	525, 590, 830	BChl *a*, okenone		Motile by flagella, chemolithotrophic growth under oxic and micro-oxic conditions in the dark with sulphide and thiosulphate	Peduzzi et al. (2011)
Lamprocystis purpurea				1.9–2.3 × 2.0–3.2	Spherical to oval	369, 523, 580, 770, 805, 831	Purple-red colour BChl *a* okenone		Nonmotile, aggregation, intracellular sulphur globules, gas vacuoles, vesicular-type photosynthetic membranes, chemolithoautotrophic with sulphide and thiosulphate in dark	Eichler and Pfennig (1988), Fischer et al. (1996), Tonolla et al. (1999), Imhoff (2001), Peduzzi et al. (2012)
Lamprocystis roseopersicina				2.0–3.5	Spherical to oval	N/A	Pink-violet colour BChl *a* rhodopinal		Motile by flagella, gas vesicles, irregular aggregates	Tonolla et al. (1999), Bosshard et al. (2000a), Imhoff (2001)

(continued)

Table 7.1 (continued)

Organism	Group	Phylogenic position	Electron donor	Cell size [μm]	Shape	Abs max [nm]	Photosynthetic pigments	C-fixation pathway	Other characteristics	References
Chlorobium clathratiforme	GSB	Chlorobi	Sulphide	~1 × 4–7	Rod	750	Brown colour BChl *e* isorenieratene	Reductive citric acid cycle	Net-forming, immotile, cultivation-dependent gas vacuole formation	Pfennig and Cohen-Bazire (1967), Gregersen et al. (2009), Habicht et al. (2011)
Chlorobium phaeobacteroides			Sulphide, elemental sulphur	0.6–0.8 × 1.3–2.7	Rod	725	BChl *d* isorenieratene		Nonmotile	Pfennig and Cohen-Bazire (1967)

Notes: *Bchl* bacteriochlorophyll, *PSB* purple sulphur bacteria, *GSB* green sulphur bacteria

[a] Cell size depicts diameter as measured for a spherical cell, unless otherwise marked

BChl *a* and the carotenoid okenone to harvest light for photosynthesis. The small-celled PSB in Lake Cadagno are immotile and contain gas vacuoles and intracellular sulphur globules. They also form aggregates, notably together with sulphate-reducing bacteria (SRB) belonging to the *Desulfovibrionaceae* (Tonolla et al. 2003; Peduzzi et al. 2003b; review by Tonolla et al. 2004). This association with the SRB is considered to provide them with a environmental advantage by providing substrate (Descristophoris et al. 2009). The 16S rRNA gene sequence of *Lamprocystis roseopersicina* is very similar to that of *Lamprocystis purpurea*, a finding that supports the reclassification of the latter species from *Amoebobacter* to *Lamprocystis* (Imhoff 2001). The large-celled PSB in the lake, *Chromatium okenii*, also belongs to the gamma division of the *Proteobacteria* (Table 7.1). This bacterium is rod shaped and has large cells (4.5–6 × 8–15 µm). *C. okenii* are motile using flagella and they harbour intracellular sulphur globules (Fig 7.2).

The GSB belong to a separate phylum (*Chlorobi*). They have a Type I reaction centre (Fe–S) and are generally considered strict photoautotrophs fixing CO_2 using the reverse citric acid cycle. The occurrence of the concentrated pigments in chlorosomes allows the GSB to use light at very low intensities (Frigaard and Bryant 2006). The abundance of the brown-coloured GSB, *Chlorobium clathratiforme*, increased in 2000 in Lake Cadagno and thereafter formed a significant population in the chemocline (Tonolla et al. 2005b; Gregersen et al. 2009; Habicht et al. 2011). *Chlorobium clathratiforme* has irregular, rod-shaped cells with typical dimensions of ~1 by 4–7 µm (Fig 7.2). They contain BChl *e* and the carotenoid isorenieratene. The cells are net-forming and nonmotile and display cultivation-dependent gas vacuoles.

Temporal and Spatial Distribution of the Anoxygenic Phototrophic Sulphur Bacteria

The population density of anoxygenic phototrophic sulphur bacteria in the chemocline varies with depth in the anaerobic water column (upper monimolimnion), seasonally and over longer timescales. As expected from the

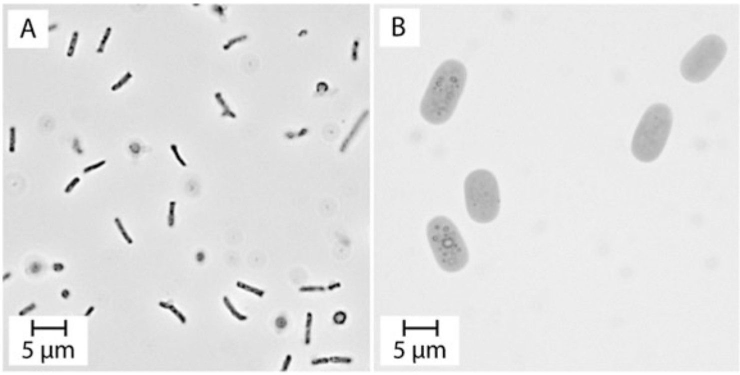

Fig. 7.2 Light microscopy images of (**a**) green sulphur bacterium *Chlorobium clathratiforme* and (**b**) purple sulphur bacterium *Chromatium okenii* showing intercellular sulphur globules

geochemical characteristics of the lake, there is a strong change in community composition between the oxic and anoxic layers (Bosshard et al. 2000b). The seasonal distribution of the anoxygenic phototroph population in the chemocline suggests that the population is adapted to changing geochemical niches (Tonolla et al. 1999). In March and June 1999, bacterial populations were evenly distributed in depth, but a microstratification of the population was observed as the season progressed and the geochemical gradient developed (Tonolla et al. 2003). A shift in dominance among the *Chromatiaceae* from *Chromatium okenii* in the spring and early summer to an increase in *Lamprocystis purpurea* in the late summer and autumn was observed by Bosshard et al. (2000a, b). However, this pattern has not been consistent in recent decades. In another seasonal study, Tonolla et al. (2003) observed high densities of *C. okenii* in the late autumn, but small-celled forms of the PSB were most abundant during spring and summer. Thus, even among the PSB populations, a number of ecophysiological factors contribute to the population composition. These results are further confounded by the motility of *C. okenii*, likely a response to light and nutrient availability.

The changes in the anoxygenic phototroph community structure in Lake Cadagno during the last decades are intriguing. Also, the relative abundance of PSB and GSB has changed dramatically over the past 15 years. A study on in situ hybridization during 1994–2003 shows that up to 2001, PSB were the most prominent phototrophic sulphur bacteria forming 70–95 % of the total bacterial population (review by Tonolla et al. 2004). In 2000, the dominance shifted from PSB to GSB, due to a dramatic increase of *Chlorobium clathratiforme* (Tonolla et al. 2005b). The bacterial biomass increased threefold, due entirely to the increase in abundance of GSB, while PSB densities remained stable possibly because of associations with SRB in the aggregates (Decristophoris et al. 2009). After this change, up to 95 % of the phototrophic sulphur bacteria community was comprised of *Chl. clathratiforme* (Tonolla et al. 2005b). Indeed, subsequent studies revealed that *Chl. clathratiforme* in Lake Cadagno is a clonal population forming up to 70 % of the bacterial 16S rRNA gene sequences obtained in the chemocline at 11–12-m depth, where the cell concentration was the highest (Gregersen et al. 2009). The population shift since 2000 could be due to a change in environmental conditions, i.e. the introduction of a new strain from outside by natural or anthropogenic means, or because of a favourable mutation in the existing population, e.g. one that allows a more effective metabolism in the dark. A study of *Chl. clathratiforme* in the water column of Lake Cadagno suggests that it may carry out the fermentation of polyglucose in the dark, effectively giving this bacterium a competitive advantage (Habicht et al. 2011).

As expected, the PSB are located higher in the anoxic water column than are GSB. Due to the differences in the light-harvesting mechanism in PSB and GSB, it seems logical that these groups of anoxygenic phototrophic bacteria would easily coexist, each inhabiting its niche determined by light intensity and possibly wavelength of light. The concentrations of chlorophyll in oxygenic phototrophs in the oxic upper layers of Lake Cadagno are low. At water depths corresponding to the depth of chemocline, the wavelength distribution of the available light is largely determined by the light absorbance by water itself. The wavelengths reaching a depth of 10 m mainly correspond to that absorbed by carotenoids and the Qx band of BChl *e*. The intensity of near-infrared radiation at wavelengths above 700 nm is much less so the characteristic long-wavelength absorbance of aggregated BChl *e* in the chlorosomes of *Chl. clathratiforme* or protein-associated BChl *a* in the PSB is unlikely to be important in the lake. Nonetheless, long-term studies of the phototrophic population reveal that the changes in light-related limnological properties of the lake, such as turbidity, light availability and light intensity over long timescales (decades), profoundly influence the population composition (Tonolla et al. 2005b).

Role of the Anoxygenic Phototrophic Sulphur Bacteria in the Primary Production of Lake Cadagno

Although the chemocline represents only a small part of the total lake volume, here about half of the total daily photoassimilation of carbon in the lake occurs (Camacho et al. 2001). Photosynthetic ^{14}C-assimilation, measured with radioisotope (^{14}C) technique, generally peaks at 1–3 m (phytoplankton) and at 10–12 m, the location of the chemocline with the anoxygenic phototrophic bacteria community. Very high values of phototrophic and chemotrophic C-assimilation are generally observed below 10 m (Bossard et al. 2001). Indeed, in a study on Lake Cadagno in September 1999, Camacho and coworkers found high rates of dark carbon fixation by chemolithotrophs in the chemocline, suggesting a high contribution of these organisms to the overall carbon cycle of the lake (Camacho et al. 2001). Recently, the rates of CO_2 assimilation of the most abundant phototrophic sulphur bacteria of the chemocline were measured using both nanoscale secondary ion mass spectrometry (nanoSIMS) (Musat et al. 2008; Zimmermann et al. 2015) and $^{14}CO_2$ quantitative assimilation in dialysis bags (Storelli et al. 2013). Thus, the strongest assimilator in the presence of light was the large-celled PSB *C. okenii*, while in the dark the major assimilator was the small-celled PSB *Candidatus* "T. syntrophicum", which was also a strong CO_2 assimilator in the light. While *C. okenii* represents only ca. 0.3 % of the total cell number, it contributes 70 % of the total uptake of carbon in the system (Musat et al. 2008), an assimilation effectiveness theorized by the authors to be based in *C. okenii*'s mobility towards substrate and optimal light conditions. Likewise, although it only contributes just about 2 % of the total bacterial populations in the chemocline, *Candidatus* "T. syntrophicum" appears to also play a greater role in CO_2 fixation in Lake Cadagno, irrespective of if there is light (Storelli et al. 2013). The most abundant population of the chemocline, GSB *Chl. clathratiforme*, showed only low CO_2 fixation rates. These results were supported by a micro-autoradiography analysis which revealed that about 10 % of the cells of *Chl. clathratiforme* from below the photic zone in Lake Cadagno fixed CO_2 in the dark, and 20–55 % of the cells from the photic zone fixed CO_2 in the light (Habicht et al. 2011). Although GSB are considered strictly phototrophic, their low rates of CO_2 photoassimilation are surprising. Another metabolic pathway probably contributes to biomass production. At present, only acetate and pyruvate are known to be used by GSB as organic carbon source in the presence of sulphide and CO_2 (Van Gemerden and Mas 1995).

Non-photosynthetic carbon assimilation is often observed in phototrophic sulphur bacteria but the pathway is not yet fully understood. In order to gain insight into the process of dark CO_2 fixation, two-dimensional differential gel electrophoresis (2D-DIGE) was used to monitor the global changes in the proteome of *Candidatus* "T. syntrophicum" strain Cad16^T in anoxic autotrophic cultures, irrespective of availability of light. Interestingly, three enzymes more abundant in the dark are part of a hypothetical anaerobic dicarboxylate/4-hydroxybutyrate (DC/HB) cycle, an autotrophic CO_2 fixation pathway found essentially in archaea (Berg 2011). The substrates needed for this process [NAD(P)H and acetyl-CoA] should be provided by the degradation of the storage globules of poly (3-hydroxybutyrate) (PHB), whose synthesis was shown to be higher in the presence of light (Storelli et al. 2014).

The Sulphate-Reducing Bacteria (SRB)

Within the chemocline, non-phototrophic bacteria are also present: these include SRB that can account for a significant fraction of the total community (Tonolla et al. 1998a). Their seasonal depth distribution was consistent with the variations of the phototrophic PSB. Over an annual cycle, however, differences in abundance of bacteria detected with probes SRB385 (detecting most *Desulfovibrionaceae*) and SRB385Db (detecting most *Desulfobacteriaceae*) were observed (Peduzzi et al. 2003a). In situ determination of sulphide turnover rates (Luthy et al. 2000) and high-resolution images of sulphide concentrations in the water column (Lehmann and Bachofen 1999) confirmed the presence of active SRB in the bacterial layer dominated by the phototrophic sulphur bacteria and introduced the possibility of a significant sulphur cycling within the chemocline coupled to a rapid turnover of sulphide (Fritz and Bachofen 2000). The vertical distribution profiles of SRB detected with probes SRB385 and SRB385Db were similar to those of the phototrophic sulphur bacteria and amounted on average 24 % of the DAPI-stained bacteria in the chemocline before the rise of the GSB *Chl. clathratiforme* population at the beginning of the twenty-first century. This percentage shows that SRB made up a significant part of the bacterial population in the chemocline of Lake Cadagno (Tonolla et al. 1998a, 1999, 2000). The in situ observations also reveal majority of the SRB consisted of one particular morphotype, which was observed in cell aggregates formed by small-celled phototrophs (Fig 7.3).

Desulfomonile tidjei in the monimolimnion of Lake Cadagno is easily recognizable as it emits a green fluorescence after acridine orange staining (Bensadoun et al. 1998) and is detectable with the two specific probes DsmA445 and DsmB455 (Tonolla et al. 2005a). Its vertical distribution was unusual, compared with other SRB, as it did not produce a maximum in abundance in the chemocline, but its distribution pattern followed that of hydrogen sulphide profiles. It accounted from 17 to 44 % of the total bacterial numbers, especially at 15–17 m depth (Bensadoun et al. 1998; Tonolla et al. 1998a, 2005a). Seasonal maximum was most frequently

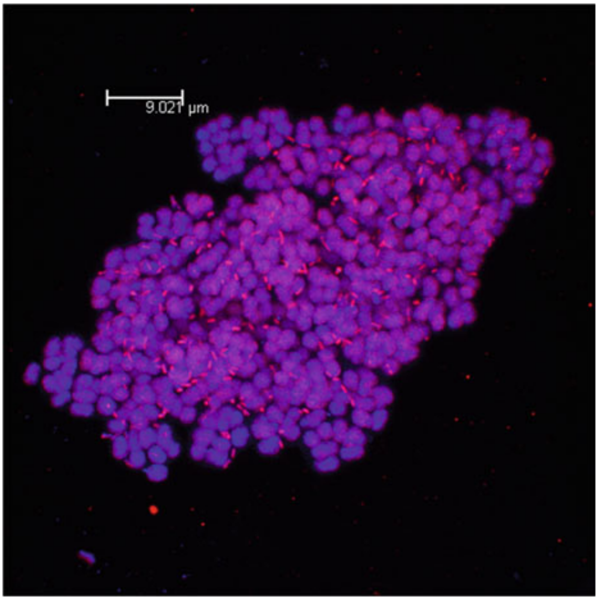

Fig. 7.3 Aggregates of small-celled purple sulphur bacteria (*in purple-violet, coccoid cells*) and their association with sulphate-reducing bacteria of the genus *Desulfocapsa* (*pink-red rod shaped cells*) in samples from the chemocline of Lake Cadagno. Confocal laser scanning microscopy image

around 30 %, the calculated biovolumes reaching values between 10 and 17 % of the total bacterial volume (Tonolla et al. 1998a, 2005a).

7.5.2 Interactions and Functions of the Microbial Populations

Long-term Alternation Between Green and Purple Bacteria
The long-term changes of GSB and PSB in Lake Cadagno were followed by analysing pigments of photosynthetic sulphur bacteria and 16S rRNA gene sequences in long-core sediment samples. The carotenoid pigments okenone and isorenieratene were extracted and identified, and their concentrations measured semi-quantitatively by chromatography (Wirth et al. 2013). Total DNA was extracted, and, for each of the sediment samples, the number of copies of 16S rRNA genes of nine targeted phototrophic sulphur bacterial populations (seven *Chromatiaceae* and two *Chlorobiaceae*) was determined by quantitative PCR (Ravasi et al. 2012b).

Out of the seven *Chromatiaceae* populations analysed using DNA sequences, six contained okenone and only one, *Lamprocystis roseopersicina*, contained rhodopinal (Peduzzi et al. 2011, 2012). During 1990–2012, *L. roseopersicina* had very low numbers in the water column and therefore constituted a minor fraction of the total *Chromatiaceae* population (Tonolla et al. 2005b). Both the investigated *Chlorobiaceae* produce isorenieratene. Therefore, as expected, records from lipid biomarkers (carotenoid pigments) and DNA analysis showed a consistent pattern (Wirth et al. 2013).

The GSB/PSB ratio was found to vary during the evolution of the lake. In the early Holocene (10.5–8 cal kyr BP), GSB weakly dominated over PSB. On the contrary, the middle Holocene (8–5 cal kyr BP) was characterized by a minor prevalence of PSB. In the period <5 cal kyr BP, flood frequencies in the lake area increased. Floods and mass movement events (i.e. mobilization, mixing and redeposition of sediments from shallower parts of the lake to the deepest lake area) generated turbiditic underflows that sporadically introduced O_2-rich water to the hypolimnion, thus briefly weakening the sulphidic conditions. Concomitantly with the rise in flood frequency, the relative abundance of GSB increased again. Here, the two isorenieratene peaks at 3.7 and 3.4 cal kyr BP seemed to correspond to flood-frequency highs with a time lag of roughly 50–100 years (e.g. at 4.3, 3.8, 3.4 and weakly at 2.5 thousand years ago). Enhanced light absorption due to higher suspended particle concentrations and algal/cyanobacterial growth in the surface and subsurface waters during floods likely reduced light availability within the chemocline. Since GSB are known to be more low-light-tolerant than PSB (Biebl and Pfennig 1978; Brocks and Schaeffer 2008), this could have triggered the observed shift in the bacterial community from a PSB- to a GSB-dominated population (Tonolla et al. 2005a; Decristophoris et al. 2009; Gregersen et al. 2009; Wirth et al. 2013). In any case, there appears to be a time lag of 50–100 years between the deposition of the most prominent flood layers and the subsequent restructurings of the phototrophic community. It is thus uncertain what exactly controlled past shifts in the microbial community structure in the water column of Lake Cadagno. Between 1.5 and 0.5 cal kyr BP, flood frequency decreased, while the Mo burial rate again increased and PSB became more abundant. However, with increasing flood activity towards the present time, the bacterial community structure has again shifted to dominance of GSB.

Microbial Cell–Cell Interactions: The Case of Lake Cadagno aggregates
We already showed that in the chemocline, up to 15 % of the cells of the total microbial community were associated in three-dimensional cell–cell aggregates (Peduzzi et al. 2003b; Decristophoris et al. 2009). The isolate Cad16 was recently proposed as *Candidatus* "*Thiodictyon syntrophicum*" sp. nov. strain Cad16^T, a provisionally novel species within the genus *Thiodictyon* (Peduzzi et al. 2012). This PSB strain Cad16^T together with the SRB *Desulfocapsa thiozymogenes* (isolate Cad626) forms stable aggregates in the lake. We will further refer to these aggregates as *Lake Cadagno aggregates*. Aggregation of cells of *Candidatus* "T. syntrophicum" and *Desulfocapsa thiozymogenes* is not mandatory as it is for other consortia (Overmann and Schubert 2002), since in their natural environment both partners can be encountered as free-living cells although numerically inconspicuous (Peduzzi et al. 2003b; Decristophoris et al. 2009).

Major community shifts and changes have recently been observed not only in the phototrophic sulphur bacteria but also in size and composition of aggregates in Lake Cadagno (see in Sect. 5.1.1.2: Temporal and spatial distribution of the anoxygenic phototrophic sulphur bacteria). Aggregates' particle size decreased, whereas in 2004, compared with 1998, the number of SRB cells in the aggregates increased about three times (Fig 7.3) (Decristophoris et al. 2009). A reduction in size of *Lake Cadagno aggregates*, due to reduced abundance of small-celled PSB, was previously described for winter and spring periods when ice and snow cover had reduced light transmission (Peduzzi et al. 2003b). Although the driving forces that are regulating aggregate formation and composition are still not well understood, overall, these findings suggest that the aggregate formation is a dynamic association responding to shifts in environmental conditions (e.g. changes in sulphide and oxygen concentration as suggested also by Overmann 1997) and to changes in global microbial community structure.

Ecological consequences of aggregate formation may be important for other members of the microbial community not directly involved in aggregation since aggregation might significantly alter competition dynamics among the phototrophic sulphur bacteria community. During periods of intense sulphide photo-oxidation, anoxygenic photosynthesis at the upper boundary of the bacterial layer is limited by the availability of reduced sulphur compounds, a situation encountered in Lake Cadagno (Luthy et al. 2000) and also Lake Mahoney (Overmann 1997) and other lakes (Shira, Shunet, Cisò, etc.) treated in this book (see Chaps. 7 and 9 of part II). Since sulphide and light occur in vertical opposing gradients with depth in the lake, phototrophic sulphur bacteria can find suitable conditions for growth only in a narrow zone of overlap between the two opposing gradients. Under these conditions, *Candidatus* "T. syntrophicum" in aggregates could have a growth advantage over non-associated phototrophic sulphur bacteria since the sulphate-reducing partner could serve as a permanent source of sulphide. Thus, an internal sulphur cycling has the potential to influence competition dynamics among the anaerobic photosynthetic community.

Peduzzi et al. (2003a, b) discussed the potential physiological interactions between the microbial partners in *Lake Cadagno aggregates*. However, from the results presented, it is evident that the role of both sulphur and carbon compounds in the interactions of the bacteria in this association needs to be elucidated in detail. Exploiting nanoSIMS provided more evidence that the metabolic interaction and exchange are not limited to sulphur compounds but most probably also involve organic metabolites (Musat et al. 2008, 2012).

7.5.3 Novel Eukaryotic Microbes in the Anoxic Water Column

Eukaryotic microbial species diversity in freshwater ecosystems is not well understood and especially so in permanently anoxic systems (Saccà 2012). Currently, only a few molecular sequencing studies exist that address this issue in meromictic freshwater lakes (Lefèvre et al. 2007; Triadó-Margarit and Casamayor 2012;

Gies et al. 2014). From a species biodiversity perspective, meromictic lakes are the only freshwater lakes suitable for obligate anoxic organisms and should, at least in theory, hold a higher biodiversity and possibly also higher endemism, the surrounding oxic environment simply acting as a barrier to dispersal.

The anaerobic eukaryotic biodiversity of Lake Cadagno was examined by constructing SSU rRNA clone libraries (Frigaard, unpublished data). Samples for the SSU rRNA clone libraries were collected from three depths: the lower oxic mixolimnion (10 m), the chemocline (11.5 m) and the anoxic monimolimnion (15 m), during 24–28 September 2006. The cell size fraction of the microorganisms analysed was in the range between 0.2 and 149 μm. Between 49 and 98 SSU rRNA sequences per depth were retrieved from the libraries made. Further details about the physico-chemical measurements, sampling and sample preparation are described elsewhere (Gregersen et al. 2009). At the time of sampling, the Chl *a* concentration in the samples from the mixolimnion, chemocline and monimolimnion was approx. 7, 45 and <1 μg L^{-1}, respectively. The ratio of Chl *c* to Chl *a* was about 0.25 in both the mixolimnion and chemocline, indicating that Chl *c*-containing algae were present. Chl *c* was undetectable in the monimolimnion.

The non-parametric richness estimator S_{Chao1} values were about twofold higher in the monimolimnion than in the mixolimnion and about fourfold higher than in the oxycline, which indicates a more complex eukaryotic community at the time of sampling in the monimolimnion than in the oxycline or mixolimnion. Cryptophytes and ciliates dominated the libraries from Lake Cadagno (Fig. 7.4). The dominance of cryptophytes in the chemocline corresponds well to the high concentration of Chl *c* found here (11 μg L^{-1}). The other common groups encountered in the samples included choanoflagellates (related to *Monosiga* and *Desmarella*) and metazoan rotifers (Monogononta), retrieved from all three libraries, and fungi and ichthyosporean sequences retrieved from the mixo- and monimolimnic samples. The absence of Chl *b* in the examined samples corresponds well to the absence of sequences from chlorarachniophytes, green algae and euglenophytes.

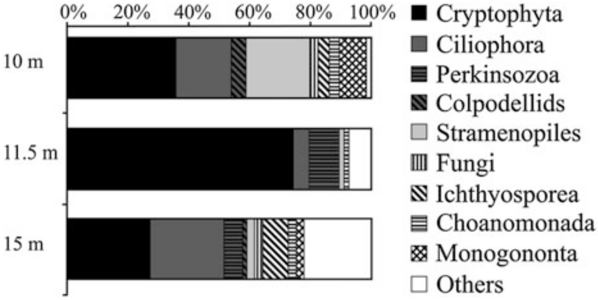

Fig. 7.4 Distribution of the main eukaryotic groups present in Lake Cadagno based on the number of clones as compared with total numbers of clones retrieved (see text for details). “Others” include sequences and OTUs (operational taxonomic units) of uncertain taxonomic origin as well as OTUs of heterolobosean and katablepharidean origin

One cryptophyte operational taxonomic unit (OTU) dominated both the oxycline and monimolimnion clone libraries based on the number of clones retrieved, but no reference sequence in GenBank matched the retrieved SSU rRNA sequences. However, the cloning generated nine nucleomorph SSU rRNA sequences identical to the nucleomorph sequence of *Cryptomonas phaseolus* (Gregersen et al. 2009). This species has been found to be sulphide tolerant and to be the dominant single cryptophyte species in the deep chlorophyll maximum in Lake Cisó, a holomictic lake in Spain that periodically experiences total anoxia with high levels of sulphide in the hypolimnion (Pedrós-Alió et al. 1987). *C. phaseolus* was also dominant in the holomictic Lake Großer Vätersee in Germany (Gervais et al. 2003), which also has sulphide-rich hypolimnic water. It forms stable populations, probably due to the lack of competition from other eukaryotes in the oxycline, one of the normal mechanisms believed to account for succession (Gasol et al. 1992).

Within the alveolates, the dominant group was the Ciliophora (ciliates). As expected, different sequences were retrieved from the oxic and anoxic samples, as ciliates in general are adapted to varying oxygen concentrations. The ciliate sequences found in the monimolimnic sample were most closely related to sequences from species known to be obligate anaerobes: *Caenomorpha uniserialis*, *Cyclidium porcatum*, *Metopus contortus* and *Trimyema compressum*. All four species have been found to harbour endosymbiotic methanogenic bacteria (Wagener et al. 1990) and also other bacteria with unknown function (Clarke et al. 1993; Bruggen et al. 1983).

In the genetic library for the monimolimnion, four sequences retrieved were related to the heterolobosean amoeba *Sawyeria marylandensis* (Fig. 7.5). The latter was isolated from an anoxic salt marsh sediment sample and was found to be microaerophilic (O'Kelly et al. 2003). Together with *Monopylocystis visvesvarai* and *Psalteriomonas lanterna*, it forms a highly supported clade within the heteroloboseans. As the Lake Cadagno OTU was found to be unique (<91 % sequence identity), a water sample from the lake was screened for possible amoeboid cells, but none were encountered. However, in both the oxycline and monimolimnic samples, a round organism was present in large numbers, feeding ferociously on the photosynthetic sulphur bacteria present in large numbers (Fig. 7.6a). Single-cell PCR of this organism revealed that it had an identical SSU rRNA with the heterolobosean OTU. Phase-contrast microscopy revealed two flagella in this organism, one long and thin and the other one short and thick (Fig. 7.6b). The general outline and presence of flagella distinguish this organism from the outline of *Sawyeria marylandensis*, and it is likely that it represents both a new genus and a new species. However, we consider that for a formal description, an ultrastructural examination needs to be conducted.

While the abundance of alveolate ciliates, choanoflagellates and fungi sequences in Lake Cadagno is similar to that found in molecular studies on protists in Lake Pavin in France (Lefèvre et al. 2007), the absence of cercozoan sequences in Lake Cadagno is surprising, unlike in the mixolimnion and in the oxycline of Lake Pavin.

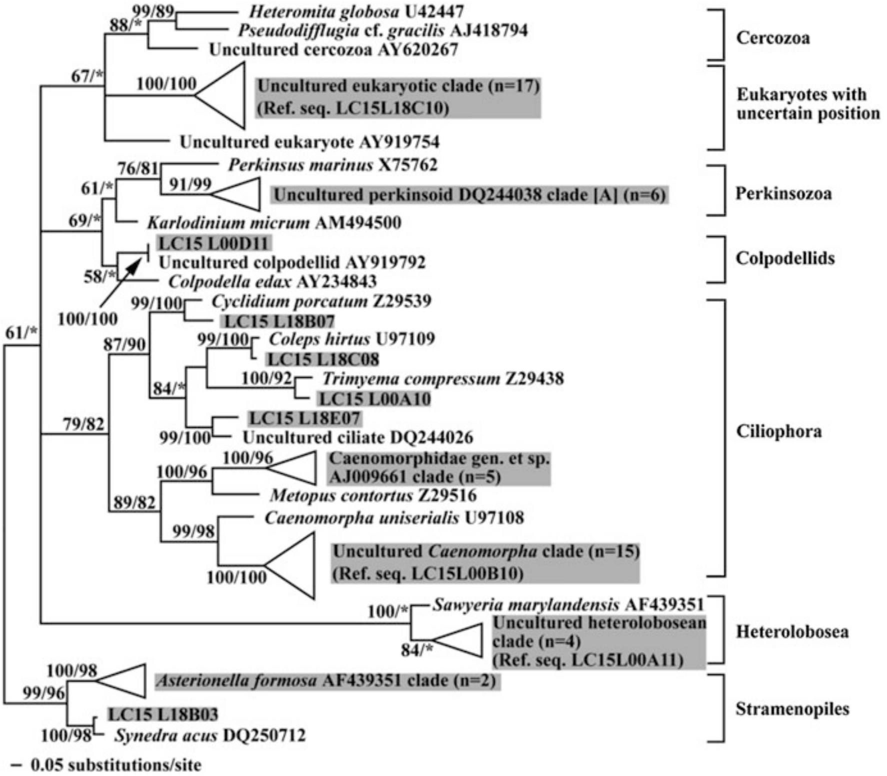

Fig. 7.5 Phylogenetic tree of a subset of monimolimnic SSU rRNA sequences (including the heterolobosean sequences) based on Bayesian analysis. Support values are Bayesian posterior probability values and neighbour-joining bootstrap support values

Lefranc et al. (2005) retrieved cercozoan sequences only from the oligomesotrophic Lake Pavin and the eutrophic Lake Aydat, in France. Thus, the absence of cercozoan organisms might be a consequence of nutrient limitation. Lake Cadagno can be characterized as an oligotrophic to oligomesotrophic lake, and this might partly explain the absence of cercozoans. However, further studies are needed to clarify whether chemical differences or population dynamics could explain the observed difference.

In conclusion, eukaryotic primary producers in the anoxic zones were dominated by *Cryptomonas phaseolus*. Eukaryotic heterotrophs were dominated by ciliates and choanoflagellates, but also included a novel clade of heteroloboseans and two novel clades of uncertain position distantly related to opisthokonts and Cercozoa. Using single-cell PCR, the novel heterolobosean clade was linked to an abundant heterotrophic flagellate in the lake that feeds on anaerobic phototrophic sulphur bacteria.

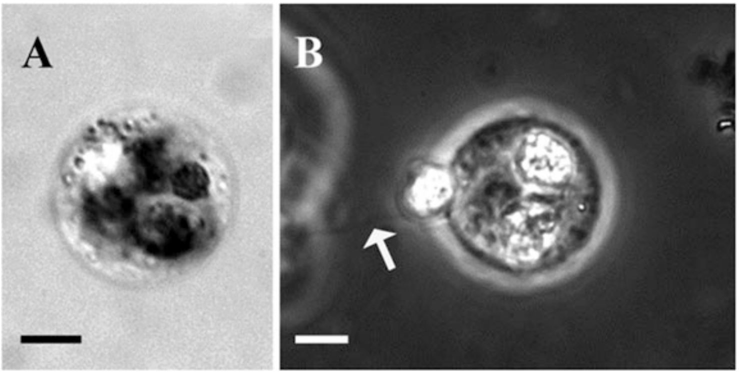

Fig. 7.6 Light micrographs of the novel heterolobosean organism observed in Lake Cadagno. (**a**) Bright-field micrograph showing the general outline of the cell. The cell interior is filled with ingested phototrophic sulphur bacteria. (**b**) Phase-contrast micrograph showing the ingestion of phototrophic sulphur bacteria. *Arrow* points to the longest flagellum; the smaller flagellum is not visible. The bacterium slides along the flagellum and ingestion occurs along close to the flagellar base. Scale bars = 10 μm

7.5.4 Phyto- and Zooplankton

Güttinger and Straub (1998) addressed the question whether the special conditions (meromicticity) of the lake had any influence on the composition of the diatom flora. As expected, live diatoms in Lake Cadagno have been found in the oxic layer only and to a depth of about 10 m. A list of diatom species observed in the water column in earlier (e.g. Schanz et al. 1988) and recent studies is given in Güttinger and Straub (1998). However, in their qualitative study, no species were found, which are not common in other alkaline alpine lakes of non-meromictic character (Güttinger and Straub 1998).

Other authors followed phytoplankton summer dynamics in Lake Cadagno (Schanz and Stalder 1998). At the beginning of summer, phytoplanktonic communities in the upper 10 m are characterized by a uniform vertical distribution of pennate diatoms (above all species of the genus *Fragilaria*, i.e. *F. crotonensis* and *F. capucina*) and centric diatoms (species of the genera *Stephanodiscus*, with *St. hantzschii*, and *Cyclotella*) as well as green algae (mostly *Scenedesmus costato-granulatus* and *Dictyosphaerium subsolitatum*). Pennate and centric diatoms become dominant later in the summer. In autumn, green algae (*Echinocoleum elegans*, *Sphaerocystis schroeterii* and *Oocystis lacustris*) dominate (Camacho et al. 2000; Schanz and Stalder 1998).

Chlorophyll *a* concentrations in the water column vary considerably, e.g. in summer 1988 between 26 mg m^{-2} in late July and 4 mg m^{-2} in early September or in summer 1986 between 18 and 65 mg m^{-2}, thus depending on the period of sampling, while phytoplankton primary production varies between 2 and 17 mg C m^{-3} h^{-1}, from 0 to 9.5 m depth (Schanz and Friedl 1993; Bossard et al.

2001). The comparison of 1987 and 1997 production rates did not show any statistically significant variation (Bossard et al. 2001; Pasini and Schanz 1998).

Dense developments of *Fragilaria capucina* and *Cyclotella comensis* are sometimes noted in the oxic–anoxic transition zone of the upper chemocline. A few centimetres deeper, the massive presence of cryptomonads *Cryptomonas erosa* and *Cryptomonas phaseolus* sometimes gives the water an intense green colour. At the same depth, often by nearly anoxic conditions, an important zooplankton community thrives all year round (Camacho et al. 2001; Tonolla et al. 1988). The dominant species are Cladocera *Daphnia longispina* complex and *Bosmina longirostris*, which often represent up to 40 % of the zooplankton density. Rotifers constitute, however, the largest share of zooplankton diversity (15 species, according to Riccardi et al. 2012), with *Conochilus* sp. (35 % of total zooplankton density) and *Asplanchna priodonta* (10 %) (Camacho et al. 2001). Other zooplankton species encountered in significant quantities are the calanoid copepod *Acanthodiaptomus denticornis* and the cyclopoid *Cyclops abyssorum*. The complete list of zooplankton species of Lake Cadagno is available in Riccardi et al. (2012).

Recently, possible responses of the biological parameters of the Lake to climate changes were investigated through the analysis of the populations of different diatom genera found in the sediments over the last 100 years (Tiffay et al. 2015). A significant shift in relative genus abundance was observed, with a dominance of small-size planktonic diatoms in recent years (1985–2012) compared with periphytic/benthic diatoms dominated in earlier years (1900–1950). Interestingly, taxa previously not observed in the Lake (Güttinger and Straub 1998, Riccardi et al. 2012) were also identified in the sediments: *Aneumastus* sp., *Craticula* sp., *Gyrosigma* sp., *Meridion* sp. and *Reimeria* sp. (Tiffay et al. 2015).

In contrast to Mahoney Lake in Canada (Overmann 1997), Lake Cadagno planktonic community in oxic layer takes advantage of the bacterial development in the chemocline for growth, and zooplankton is a link between bacterial primary production and the higher levels of the food chain (e.g. fish). In situ hybridization has showed the presence of PSB in the intestine of these predators (e.g. *Cyclops abyssorum*). This was further confirmed by the analysis of stable carbon isotopes in bacteria and zooplankton by which the contribution of the bacterial layer in providing carbon to the zooplankton through the latter's predation on bacteria was estimated to be around 50 % (Camacho et al. 2001).

7.5.5 Fish

Meromictic conditions in Lake Cadagno support high fish productivity. The massive development of anaerobic photosynthetic bacteria in the chemocline provides a supplementary form of primary production along with that of the algae. On the whole, bacterial and phytoplankton primary productions are the beginning of a food chain that ensures the development of a fairly large fish population (Tonolla et al.

1988). Fish productivity, estimated at 25–30 kg ha^{-1} (Marrer 1975; Tonolla et al. 1988), represents relatively high values for an alpine oligomesotrophic lake situated at an altitude close to 2000 m asl. In fact, the lakes of the Piora region, Lakes Ritom and Cadagno in particular, have a long history of fish management, documented as far back as the fifteenth century (Surbeck 1917). For the inhabitants of this mountain region, fish represented an important supplementary source of proteins (Peduzzi 2000). Moreover, the fish of both lakes Ritom and Cadagno were renowned for their natural salmon-like colouration supposedly due to the carotenoids of the PSB inhabiting the chemocline. The presence of fish is due to man-made introductions for exploitation of fishery. The species present are rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), lake charr (*Salvelinus umbla*), brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), bullhead (*Cottus gobio*), *Phoxinus lumaireul* and Italian chub (*Squalius squalus*), though the last two species are not caught by fishermen. Brook trout and lake trout were introduced to the lake in the 1950s and fry of the commercial fish species are introduced annually (Polli 2012).

7.6 Concluding Remarks

Lake Cadagno represents an ideal model lake for studying the role of microbes in aquatic environments. The scientific projects performed over many decades offer an important source of information supporting ongoing and future scientific projects. Despite its constant meromictic state, Lake Cadagno is unique in that it is responding to the ongoing climatic changes, and man-made changes are influencing the lake itself as well as the catchment area. In addition, continuing studies of the major bacteria involved in the sulphur cycle, as well as studies of microbial interactions and the investigation of the microbes from the mixolimnion and from the sulphidogenic sediments, will contribute to advance our knowledge of this interesting ecosystem.

Meromictic lakes such as Lake Cadagno can be used as a model for studying biogeochemical processes mediated by microorganisms and characterized by permanently anoxic water columns, as earlier reported for Lake Cadagno (Hanselmann and Hutter 1998; Putschew et al. 1995). In this perspective, of great interest are the investigations promoted by the Nordic Center of Earth Evolution focusing on the long history and evolution of life on Earth.

Since anoxic and sulphidic marine conditions may have prevailed during the Precambrian (Canfield et al. 1998; Meyer et al. 2011), Lake Cadagno has the potential to provide insights into the biogeochemical cycling of elements in the Proterozoic oceans and thus can be considered as the present-day analogue to the ancient prokaryote-dominated oceans (Canfield et al. 2010; Honeycutt et al. 2008), considering particularly the bacteria involved in the sulphur cycling (Johnston 2011). Phototrophic sulphur bacteria, especially the GSB *Chlorobium* spp., have been suggested to proliferate in ancient anoxic ocean basins (Crowe et al. 2008;

Summons and Powell 1987; Halm et al. 2009). They are, indeed, believed to have been important for the carbon fixation and chemical development of the anoxic Precambrian ocean, where they may have contributed to the genesis of banded iron formations and the stabilization of the sulphide-rich ocean during the mid-Proterozoic period, Proterozoic aeon 2500–542 million years ago (Brocks et al. 2005; Crowe et al. 2008; Habicht et al. 2011; Johnston et al. 2009).

Further, we applied in Lake Cadagno very innovative investigation techniques, which are among the most promising and powerful in the field of molecular microbial ecology to study of the lake's ecosystem. Thus, Lake Cadagno and its sulphur phototrophic bacteria and associated sulphate-reducing bacteria have proven to represent an excellent model system to test and develop novel investigation techniques in both environmental microbiology and microbial ecology. Particularly interesting are the methods described in Musat et al. (2008), Halm et al. (2009), Habicht et al. (2011) and Storelli et al. (2013) to infer or measure in situ activities of single cells of diverse groups of organisms (e.g. *Chromatium okenii*). These techniques have now the potential to be applied to more difficult environments such as some other aquatic environments, running waters, sediments and microbial mats or soils.

In the studies reviewed here, much of the attention was devoted to population dynamics of phototrophic sulphur bacteria comprising both an apparently monophyletic GSB community and a multiple and diverse PSB community. One question raised but yet unanswered is about the coexistence in the apparently same ecological niche of diverse, but closely related, populations of phototrophic sulphur bacteria. This coexistence and alternation has been present on a long timescale, since the formation of euxinic conditions in the lake, soon after its formation, about 10,000 years BP.

As already pointed out above, other functional groups in the bacterial community such as SRB or, as highlighted in a recent work (Berg et al. 2016), bacteria involved in the iron cycle have a great impact in the functioning of this ecosystem. In particular, sulphate-reducing bacteria in *Lake Cadagno aggregates* may well play a major role in competition dynamics among the phototrophic populations. Thus, the mechanisms underlying PSB–SRB aggregate formation certainly deserve more attention. The study of aggregates, with the tools and techniques described above, potentially integrates all levels of biological analysis from molecular to ecological and holds the promise to give new insights into the metabolic coupling between organisms in aquatic systems.

There are striking parallels and analogies between Lake Cadagno and other stratified meromictic ecosystems worldwide, from microbial mats to ancient oceans. The most interesting analogy is with a low sulphidic and prokaryote-dominated Proterozoic ocean (2500–542 million years ago). Research on this particular ecosystem thus holds the promise to give insights into many fascinating aspects of lake biology and biogeology like life and Earth history and into some of the growing interdisciplinary fields in environmental microbiology: the microbial community dynamics and interactions, as well as the single-cell trait-based biodiversity in microbial communities and its link to ecosystem functioning.

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