Aquat Ecol (2010) 44:497–511
DOI 10.1007/s10452-010-9332-x

Densities and distribution of flagellates and ciliates in the chemocline of saline, meromictic Lake Shunet (Siberia, Russia)

E. B. Khromechek · Y. V. Barkhatov ·
D. Y. Rogozin

Received: 21 May 2009 / Accepted: 22 June 2010 / Published online: 13 July 2010
© Springer Science+Business Media B.V. 2010

Abstract The vertical and seasonal distributions of the phytoflagellate *Cryptomonas* spp., and its most common, the planktonic ciliate predators (*Oligotrichida*, *Scuticociliatida*, *Hypotrichida* and *Prostomatida*) were investigated in chemocline region of small saline, meromictic lake Shunet (Siberia, Russia) during 2003 and 2005. The lake has a pronounced chemocline, with abundance of purple and green sulphur bacteria. Vertical distribution of the *Cryptomonas* populations near the oxic/anoxic boundary layer was studied at close intervals in water sampled using a hydraulically operated thin-layer sampler. In both summer and winter, *Cryptomonas* peaked in water stratum 5–10 cm above anoxic zone or in the anoxic zone water column in the chemocline (about 5 m). Ciliate densities and biomass were also much higher in chemocline than in mixolimnion. The range of diurnal migration of *Cryptomonas* population was not very wide, and it was restricted to layers with high light intensity. The ciliates were sometimes detected above the upper border of the anoxic zone but also several centimetres below this zone.

Handling Editor: R.D. Gulati.

E. B. Khromechek · Y. V. Barkhatov (✉) ·
D. Y. Rogozin
Institute of Biophysics, Siberian Branch of Russian
Academy of Sciences, Akademgorodok, Krasnoyarsk
660036, Russia
e-mail: barkh@ibp.ru

Keywords Chemocline · *Cryptomonas* · Ciliate · Meromictic lakes · Deep chlorophyll maximum · Diel vertical migrations

Introduction

In lakes that are strongly stratified, especially the meromictic ones, there are zones of persistently stable physicochemical conditions. Most of such strata are characterized by abundant development of specialized populations of aquatic organisms that have some common adaptive features (Reynolds 1992; Pedrós-Alió and Guerrero 1993; Pedrós-Alió et al. 1995; Tyler and Vyverman 1995). For instance, planktonic species inhabiting the anaerobic-sulphide strata of these lakes are tolerant to both high sulphide concentration and the lack of oxygen, which is advantageous to these planktonic populations in the metalimnion and hypolimnion, the zones that are rich in food resources and biogenic elements, and where predatory pressure is low and physical stability of water is high (Pedrós-Alió et al. 1995; Gervais 1998; Camacho et al. 2000). Thus, the stratified lakes contain populations that can persistently exist in such zones.

Dense, frequently stratified, populations of anaerobic photosynthesizing bacteria (Montesinos et al. 1983; Miracle et al. 1992) and specialized heterotrophic bacteria (Caldwell and Tiedje 1975; Gast and Gocke 1988) develop in the anaerobic water when light penetrates into sulphide-containing layers. Different populations of anaerobic, photosynthesizing bacteria when coexisting in a lake frequently distribute at different depth due to competition for light (Miracle et al. 1992).

The probability of occurrence of the deep chlorophyll maximum (DCM) frequently dominated by *Cyanobacteria*- and/or *Cryptophyta* in the chemocline region of these lakes is rather high. The origin and maintenance of DCM in these lakes have been attributed to different mechanisms (Cullen 1982). For example, depth-differential sinking of phytoplankton (Steele and Yentsch 1960); in situ growth of autotrophic algae (Fee 1976; Moll et al. 1984) and mixotrophic algae (Bird and Kalff 1989); and temporary residence of motile algae in deep-water layers during diel vertical migration (Salonen et al. 1984; Arvola et al. 1992); aggregation of motile algae at certain preferred depths (Galvez et al. 1988). The process that is most frequently discussed in the literature is depth-differential zooplankton grazing (Longhurst 1976). In the chemocline region, the grazing pressure on phytoplankton is generally greatly reduced since main zooplankton grazers do not migrate to depths where oxygen level is very low (Lass et al. 2000). Most rotifers prefer grazing in the chemocline, but they avoid contact with sulphide-rich water (Miracle et al. 1992). Nevertheless, many studies have demonstrated that different species of copepods can consume algae or sulphur bacteria that live under microaerobic or anaerobic conditions (Takahashi and Ichimura 1968; Kettle et al. 1987).

Stratified water bodies often contain large populations of cryptomonads in the chemocline region (Gasol et al. 1992; Massana et al. 1994; Gervais 1998; Adler et al. 2000). Ichimura et al. (1968) observed a DCM in the mesotrophic Lake Haruna (Japan) due mainly to *Cryptomonas* sp. that was photosynthetically active and adapted to poor light conditions. Gasol et al. (1992) and Massana et al. (1994) observed a dense population of *C. phaseolus* to grow and develop in the chemocline region of Lake Ciso (Spain). Gervais (1997, 1998), Gervais et al. 2003 studied dynamics of growth and behaviour of large populations of the three *Cryptomonas* species, namely, *C. rostratiformis, C. phaseolus* and *C. undulata*, in the chemocline of the eutrophic Lake Schlachtensee (Germany). Cryptophytes dominate flagellates in the Antarctic lakes Hoare and Fryxell and form deep maximum in the chemocline (Marshall and Laybourn-Parry 2002).

Diel vertical migration is an adaptive mechanism to maintain a large population. By migrating into the anoxic sulphide-rich monimolimnion during the night, cryptomonads are reported to reduce their predation losses by about 38% (Pedrós-Alió et al. 1995). Studies on distribution with depth of cryptophytes in Lake Schlachtensee (Germany) showed that the populations that inhabited the chemocline migrate daily, with daytime ascent and night-time descent but within a narrow amplitude of migration (Gervais 1997). More than 80% of *Cryptomonas* populations moved into the anoxic, hydrogen sulphide-containing water layers during night-time, and at least 40% of the population stayed in the microaerobic zone during daytime. The author attributed this migratory behaviour to better light supply and a decrease in grazing pressure (Gervais 1997). Migrations into the lower, sulphide-rich water layers, toxic to main grazers protected the *Cryptomonas* populations from predation during several hours of each diel cycle and maintained their large biomass throughout stratification period, although at cost of a considerable decrease in growth (Pedrós-Alió et al. 1995). Knapp et al. (2003) investigated migrations of *Cryptomonas* spp. populations in chemocline in Cross Reservoir (USA), a small impoundment, and also suggested that migration was the populations phototactic response to daily variations in light intensity (Knapp et al. 2003).

Specialized ciliate populations are the main grazers of algae and bacteria in the hypolimnium of meromictic lakes, in the monimolimnion, particularly in lakes with a reduced or simplified food chain. In the DCM of Lake Ciso (Spain), in the absence of *Daphnia*, ciliates were the only grazers, which daily removed from 5 to 25% of cryptophyte biomass (Pedrós-Alió and Guerrero 1993; Pedrós-Alió et al. 1995). A number of studies have reported that cryptophytes avoid predation by migrating to the anoxic metalimnetic maxima in lake (Pedrós-Alió et al. 1995; Gervais 1997). Most of ciliate species unable to live without oxygen, stay in water strata above the chemocline (Pedrós-Alió et al. 1995). Anaerobic ciliate species can live in chemocline zone, together with *Cryptomonas* and sulphur bacteria.

Among the works on protozooplankton of meromictic, lakes there are only a few studies that describe brackish lakes. Lake Shunet is one such lake in southern Siberia, which is small, saline, meromictic and fishless, with high sulphur content in the bottom water. The lake has a pronounced chemocline, with abundance of purple and green sulphur bacteria (Rogozin et al. 2009). We determined in Lake Shunet the vertical and seasonal distribution of *Cryptomonas* spp., and their most important predators, the planktonic ciliates, which form deep maximum in the chemocline in Lake Shunet. This small lake has sulphide-rich monimolimnion, and it exhibits massive development of sulphate-reducing bacteria in chemocline (Pimenov et al. 2003; Rogozin et al. 2005). Lake Shunet is second in the world (after Mahoney in Canada) in terms of high densities and biomass of purple photosynthesizing bacteria (Mahoney, Canada) (Rogozin et al. 2005, 2009). In this lake, green sulphur bacteria develop under the purple photosynthesizing bacteria layer and can attain record biomass levels (Lunina et al. 2007).

The purpose of this study was to investigate the vertical and seasonal distribution of pelagic ciliates and *Cryptomonas* as important components of the microbial loop in Lake Shunet, depending upon the vertical distribution of the main physicochemical factors (oxygen, sulphide and nutrients). We studied the species composition of phytoflagellates and ciliates and monitored their vertical distribution in the pelagic zone using standard depth intervals (1 m), with more narrow depth intervals in the chemocline zone. We hope that this information is basic for any future studies concerning the role of microbial loop in the stunted food chain—that misses certain essential microbial elements, such as cladoceran grazers and fish but has gammarids (Zadereev et al. 2010) and calanoids (Tolomeev et al. 2010) both in the littoral and open water regions.

Materials and methods

Description of lake

Lake Shunet is situated in the Khakass Republic (Russia) (54°25′N, 90°13′E). The area of the lake is 0.47 km^2, and its the maximal depth is 6.2 m. The lake water is brackish, with anions dominated by sulphate and chloride and cations by sodium and magnesium (Parnachev and Degermendzhy 2002). The salinity in mixolimnion (17–20 g l^{-1}) and monolimnion (up to 66 g l^{-1}) of the lake differs markedly. The upper boundary of the anaerobic monimolimnion is at a depth of about 5 m. The concentration of hydrogen sulphide (H_2S) in bottom water layers is as high as 300 mg l^{-1}, dropping to zero ca. 5 m i.e. above the chemocline (Rogozin et al. 2005). The ice cover persists from early November to late April or early May (Rogozin et al. 2009).

Distributions of dissolved oxygen and redox potential at different depth suggest the presence of an oxic (0–4 m) and anoxic (5–6.2 m) layers. Due to the presence of H_2S, pH in the chemocline region is reduced from 8.4 to 7.0. High mineral content of the water induces stratification of the other parameters and prevents wind-induced mixing. Based on the position of the chemocline, we divided the water column of the lake into the epilimnion (0–4 m), the mixolimnion (4–5 m) and the monimolimnion (5–6, 2 m) (Rogozin et al. 2009).

In the chemocline zone of the lake, which coincides with the upper boundary of the layers containing sulphides and hydrogen sulphide, Rogozin et al. (2005) found a 5-cm layer that contained abundant photosynthesizing, purple sulphur bacteria that were present perennially. These layers were related to *Lamprocystis purpurea* (*Chromatiaceae*) with bacterial numbers in summer of 1.5 × 10^8 cells ml^{-1} (Fig. 1). As the chemocline was located at 5 m, a relatively shallow depth, even under-ice light intensity was sufficient for the population of photosynthesizing bacteria to persist at a level of 5 × 10^6 cells ml^{-1}.

The chlorophyll maximum was registered 4–5 m depth. Based on the contents of chlorophyll and bacteriochlorophyll, Rogozin et al. (2005) suggested that the 4- to 5-m layer was a transitory layer between the zones of oxygenic and anoxic photosynthesis. The zooplankton in the lake is reduced in both species and numbers. It is dominated by only one calanoid copepod, *Arctodiaptomus salinus* and two rotifer species *Brachionus plicatilis* and *Hexarthra oxiuris* (Tolomeev et al. 2010).

Sampling

Lake Shunet was monitored from August 2003 to September 2005 to investigate its microplankton and nanoplankton in the chemocline and the water column. We sampled in the central part of the lake with a Ruttner sampler in different depth strata at 1-m

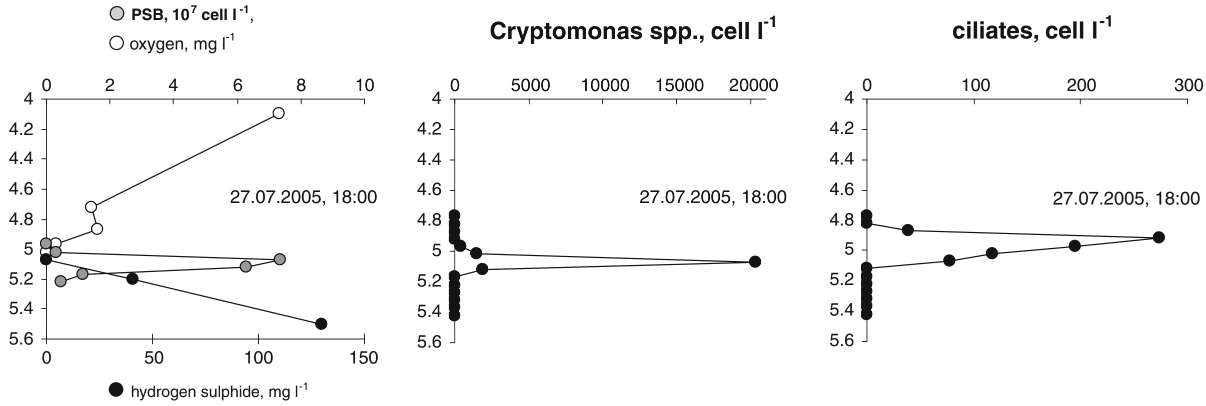

Fig. 1 The vertical distributions of the abundance of purple sulphur bacteria (PSB), oxygen, hydrogen sulphide (Rogozin and Degermendzhy 2008), cryptomonas and ciliates in the chemocline of Shunet Lake on 27 July 2005

intervals from surface to bottom. In addition, the chemolimnion was sampled—using a thin-layer multi-syringe sampler designed by Rogozin and Degermendzhy (2008). This sampler simultaneously collects 15 vertical samples at 5-cm depth intervals (Rogozin and Degermendzhy 2008) to investigate the stratification with depth of different bacteria and other organisms in the chemocline. We monitored daily the diurnal, vertical migrations of ciliates and *Cryptomonas* in the chemolimnion at 6-h intervals.

Dissolved oxygen concentration was measured using a submersible multi-parameter Hydrolab sonde (USA). pH and Eh were measured using a WTW-320 pH-millivoltmeter (Germany). Concentration of dissolved hydrogen sulphide was determined colorimetrically, using a Merck test kit (Germany).

The species composition, concentrations and biomass of both phytoflagellates and ciliates were also monitored in the samples. The organisms were counted both live and in samples fixed with Lugol's solution (1% end concentration) and glutaraldehyde (2% end concentration). Fixed phytoflagellates were counted in a Fuchs-Rosenthal chamber; 10 pseudo-replicates were used. Large live ciliates were counted in a counting chamber at magnification, and small-sized species were counted using the drop method in 20 replicate (fixed and preserved) samples of 25 µl each. Phytoflagellates were enumerated in the Fuchs-Rosenthal chamber under an MBI-11 light microscope and an Axioskop 40 fluorescence microscope (Carl Zeiss). The ciliates were identified to species following Kahl's (1930–1935), and taxonomic system of Corliss (1979). We identified the ciliate species in both fixed and live samples, using Kiselev's identification guide (Kiselev 1954).

We measured the body size (length, width) of the organisms using an ocular micrometer and from the size calculated their average body mass from data of body length and width of 50 individuals of each species. For this, the individual volume was first calculated from the appropriate geometrical form, and the biomass was estimated as 10^6 mm^3 = 1 mg of wet weight (Lohmann 1908).

Results

Lake Shunet was clearly stratified on all sampling dates (from August 2003 to September 2005). The chemocline zone, defined as the boundary between aerobic and anaerobic zones, was positioned between 4.9 and 5.1 m during the sampling period. Light intensity in the chemocline on 28 February 2003 was 0.97 µE m^{-2} s^{-1}, and during the summer sampling (5 August 2008; 04 and 27 July 2005) was 57 and 49 µE m^{-2} s^{-1}, respectively. The purple sulphur bacteria were characterized by a well-marked peak in the chemocline zone (Rogozin et al. 2009, 2010). In the lower below sulphide concentration rose steadily with increasing depth.

Seasonal dynamics of ciliates and *Cryptomonas*, 2003–2005

Lake Shunet was sampled for ciliates and *Cryptomonas* from August 2003 to September 2005 seasonally. We found a large *Cryptomonas* population in the pelagic zone (about 5 m) of Lake Shunet; in summer (during the whole period of observation—about 3 times every season), it mainly consisted of *C. salina* and in winter of *C. salina* and *C.* sp., the last being dominant.

Table 1 presents the data on *Cryptomonas* distribution in the pelagic zone of Lake Shunet in 2003 and 2005, in water samples from the central part of the lake. The *Cryptomonas* population densities were highest in 4.0–5.2 m stratum but generally either absent in the strata above and below or their concentrations were an order of magnitude lower (November 2003, February 2004 and July 2005). The population density was highest in August 2004, when *Cryptomonas* was distributed throughout the water column, including hypolimnion. The samples collected using the thin-layer sampler allowed to examine the distribution of *Cryptomonas* spp. in the chemocline region (Fig. 2). The population appeared to always aggregate at the oxygen-sulphide interface.

In both summer and winter period, *Cryptomonas* peaked in the 5- to 10-cm layer, adjacent to and in the chemocline. In spring (26 May 2004, 25 May 2005) and autumn (10 September 2005) samples, *Cryptomonas* exhibited only two minor peaks—the population was distributed rather uniformly in the 30-cm zone above and in chemocline. The *Cryptomonas* population density was high throughout the 2-year study period, and it was located in the zone of the high abundance of purple photosynthesizing bacteria (about 5 m). These bacteria were restricted to the 5- to 10-cm-thick layer of the water column in summer and winter and were distributed over 30 cm in spring and autumn. This could be attributed to wind-induced mixing of the water.

The annual maxima of *Cryptomonas* populations were observed in August 2003 and August 2004 (191.01×10^3 cells ml^{-1} and 157.44×10^3 cells ml^{-1}, respectively). In summer 2005, the size of the *Cryptomonas* spp. population was an order of magnitude lower than in 2003–2004 (Fig. 2).

Ciliates

Free-moving ciliates of four genera (*Oligotrichida*, *Scuticociliatida*, *Hypotrichida* and *Prostomatida*) developed dense populations in the lake's pelagic zone. The species composition was depth dependent:

Table 1 Abundance (cells ml^{-1}) and total biomass [mg l^{-1} (Wet weight)] of *Cryptomonas* spp. in the pelagic zone of Lake Shunet

Layer	4 November 2003		8 February 2004		26 May 2004		6 August 2004		2 July 2005	
	Abundance	Total biomass	Abundance	Total biomass	Abundance	Total biomass	Abundance	Total biomass	Abundance	Total biomass
Mixolimnion (0–4 m)	Not found	Not found	Not found	Not found	165.18	0.10	227.3	0.14	Not found	Not found
Chemolimnion (4–5.2 m)	4562.9	2.74	3046.9	1.83	7515.6	4.54	36562.5	21.91	1889.3	1.130
Monolimnion (5.2–6.2 m)	Not found	Not found	Not found	Not found	Not found	Not found	703.1	0.42	Not found	Not found

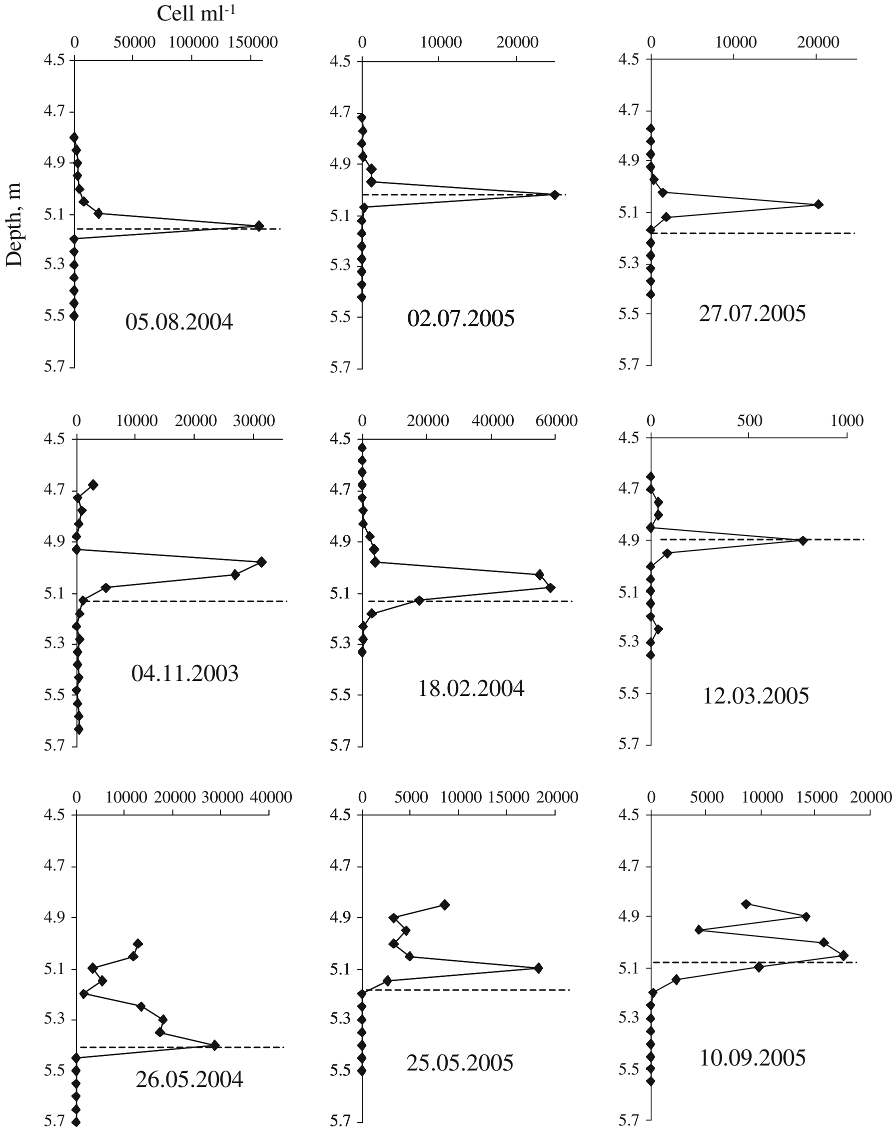

Fig. 2 Seasonal distribution of *Cryptomonas* spp. in the chemocline zone of Lake Shunet. The *horizontal lines* mark the upper boundary of the microaerobic (<0.5 mg O_2 l^{-1}) yet sulphide free chemocline

in the mixolimnion, oligotrichids (*Rimostrombidium* sp.) contributed mainly to the numbers and *Strombidium* sp. to biomass. The prostomatid *Balanion* sp., contributed about 35% to the total ciliate density in the mixolimnion (27 July, 2005). Whereas *Rimostrombidium* was absent in chemocline, the scuticociliate *Cyclidium* sp. dominated. The biomass of all the species present was similar.

The abundance and biomass of ciliates in the mixolimnion and the chemocline differed (Table 2). In November 2003 and February 2004, ciliates were absent in the upper water layers, being present in 4.5–5 stratum. Their numbers and biomass were much higher in the chemocline zone than in the mixolimnion.

The seasonal dynamics and distribution of ciliates and of *Cryptomonas* in the chemoclinezone, based on the thin-layer sampling, were similar (Fig. 3). Highest density of ciliates occurred very close to chemocline, mainly above the upper border of the anoxic zone, but sometimes they were detected in samples in somewhat deeper samples (18 February 2004; 02 July 2005 and 10 September 2005).

The ciliate maxima in the chemocline zone, observed in May 2004 and May 2005 (1.75×10^3 cells^{-1}ml and 2.23×10^3 cells/ml, respectively),

Table 2 Abundance (cells ml^{-1}) and total biomass [mg l^{-1} (Wet weight)] of ciliates in the pelagic zone of Lake Shunet

Layer	4 November 2003		18 February 2004		26 May 2004		6 August 2004		2 July 2005	
	Abundance	Total biomass	Abundance	Total biomass	Abundance	Total biomass	Abundance	Total biomass	Abundance	Total biomass
Mixolimnion (0–4 m)	Not found	Not found	Not found	Not found	13.5	0.02	8.0	0.009	35.9	0.83
Chemolimnion (4–5.2 m)	63.5	0.79	56.6	0.19	395.4	1.04	146.9	0.30	173.6	1.52
Monolimnion (5.2–6.2 m)	Not found	Not found	Not found	Not found	Not found	Not found	Not found	Not found	Not found	Not found

were caused by *Cyclidium* sp., which constituted from 96 to 98% of the total counts and about 60% of the total biomass. These ciliates were also high relatively more abundant (90% of the total abundance) in September 2005; at other times, *Cyclidium* sp. concentration varied from 20 to 60%. Larger ciliates such as *Strombidium* sp., *Prorodon* sp. and *Euplotes* sp. dominated and comprised 70–95% ciliate community biomass (Fig. 4a, b).

Diel variation in ciliates and *Cryptomonas* distribution

We monitored during the summers of 2004 and 2005 the vertical distribution and migration of *Cryptomonas* and ciliates in the chemolimnion, daily during the daytime and the night-time (Fig. 5a, b). The phytoflagellates were dominant in 5- to 5.2-m layer during both dark and light periods. The amplitude of migration for *Cryptomonas* appeared to be narrow and it did not ascend to upper layers where light intensities were higher.

Ciliates also stayed close to the chemocline day and night (Fig. 6a, b). Ciliate biomass was situated higher depth than that of the *Cryptomonas* biomass, during both night and daytime. In one case, they were located above the chemocline zone (26 July 2005), and in another case (05 August 2004) in the anaerobic zone, where *Oxytricha* sp. an anaerobic species was encountered. The ciliates did not descend below 5.1 m in 2005; and in 2004 their distribution to the total ciliate biomass at 5.2 m was the highest, due mainly to oxytrichids.

Discussion

Throughout the study period in Lake Shunet from August 2003 to September 2005, the chemocline region was co-inhabited by large populations of flagellates (*Cryptomonas*) and ciliates (*Strombidium* sp., *Cyclidium* sp., *Euplotes* sp., *Oxytricha* sp., *Prorodon* sp., *Balanion* sp.) adapted to poor light conditions, anoxia and presence of hydrogen sulphide in their environment (Fig. 1). During the 2-year study, the concentration of population varied. In 2005, the *Cryptomonas* densities decreased almost an order of magnitude compared with 2003 and 2004. During early summer months and in winter, when the

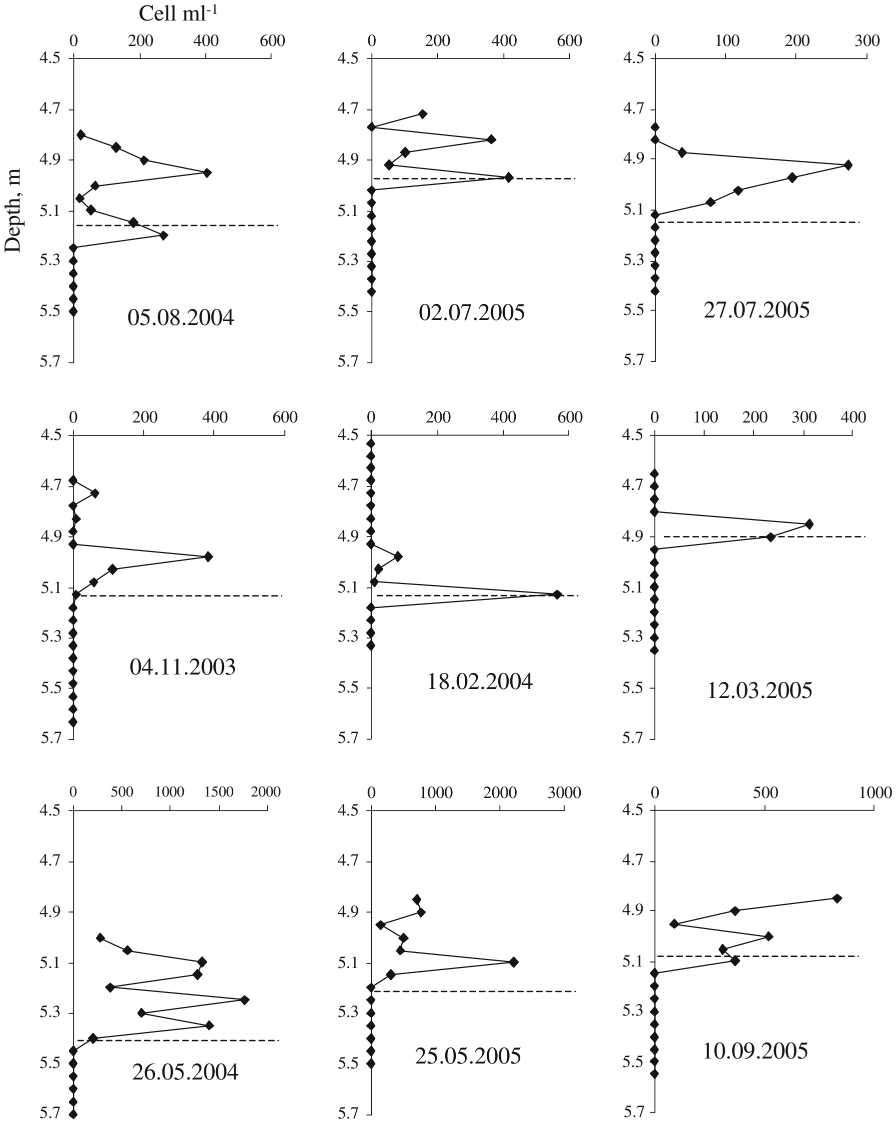

Fig. 3 Seasonal distribution of ciliates in the chemocline zone of Lake Shunet. The *horizontal lines* mark the upper boundary of the microaerobic (<0.5 mg O_2 l^{-1}) yet sulphide free chemocline

lake was covered with a layer of ice and snow, both numbers and biomass of *Cryptomonas* were highest in the narrow 5- to 10-cm chemocline region.

Annual variations in *Cryptomonas* concentration can depend on many factors, the analysis of which was not among the main aims of this study. In both spring and autumn, the population was distributed rather uniformly in the 30-cm zone adjacent to the chemocline, peaks above or in anoxic zone water column. Interestingly, the population density maximum of *Cryptomonas* spp. overlapped with that of purple sulphur bacteria. Moreover, *Cryptomonas* population did not migrate extensively but remained in the chemocline region, where light intensity was constantly low. Minor shifts (about 10 cm) in their depth of their maximum were due to changes in the chemocline depth rather than to their active migration.

The reasons why cryptophytes occur closely above the layer inhabited by photosynthesizing sulphur bacteria or even in it (Fig. 2) remain unclear. The hypothesis suggesting that the sulphide layer prevents zooplankton grazing on flagellates is the most widely accepted (Gasol et al. 1993; Garcia-Gil et al. 1993; Gervais 1997). Carpenter et al. (1993) also suggest that the highly edible cryptophytes dominate the

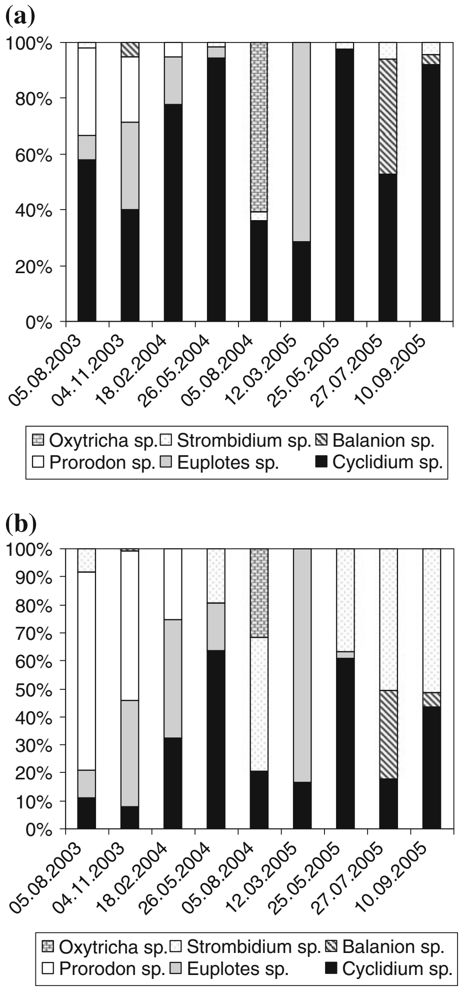

Fig. 4 Relative contribution of different ciliates groups to abundance (**a**) and total biovolume (**b**) in the metalimnion of Shunet Lake

DCM only if the water body contains a sulphide layer; otherwise, the DCM layer is dominated by green algae and *Cyanobacteria* (Carpenter et al. 1993). Another hypothesis suggests that the high number of cryptomonads in the dense aggregation of bacterioplankton is supported by the mixotrophic ability of these phitoflagellates. Many genera of phytoflagellates have the ability for mixotrophic feeding in fresh and marine aquatic ecosystems (Porter 1988; Sanders and Porter 1988; Sanders 1991).

A number of earlier works (Gasol et al. 1992; Garcia-Gil et al. 1993; Gervais 1997) also report that *Cryptomonas* populations stay in the proximity of the layer inhabited by photosynthesizing purple bacteria. It, however, remains unclear whether these bacteria play a role for the growth of *Cryptomonas*, or whether the flagellates provide any feedbacks for bacterial growth, or whether the association of these two organisms is just a coincidence. For example, in Lake Schlachtensee (Germany) (Gervais 1998) cryptophyte populations mostly stay in the anaerobic, sulphide-containing layer below the chemocline and are never observed in the summer epilimnion. Below the chemocline, purple and green sulphur bacteria coexisted in this lake (Gervais 1997). In Lake Ciso (Spain), greatest part of the *Cryptomonas phaseolus* population was reported to always remain at the oxygen–sulphide interface where light intensity was <1% of incident light, i.e. much lower than the optimum light intensity for photosynthesis. In Lake Ciso, population *C. phaseolus* as in Lake Shunet also changed its position along with the chemocline (Gasol et al. 1992).

Data on diel distribution of the organisms in the Lake Shunet show that the ciliate maxima are always located somewhat higher than those of *Cryptomonas* vis-à-vis the presence of layers rich in sulphide (Fig. 6). It seems quite likely that *Cryptomonas* is less sensitive to increases in sulphide levels than the ciliates. Moreover, because the summer and winter stratification gradients of physicochemical factors in the chemocline region of Lake Shunet vary only narrowly (5–10 cm), a short descent of motile *Cryptomonas* cells into the depths where sulphides levels are high can reduce predation by ciliates inhabiting waters layers above the *Cryptomonas.*

The occurrence of a large population of *Cryptomonas* in the chemocline region and the absence of its extended daily migrations in Lake Shunet can, in our opinion, be due to two reasons. First, it is the small depth of the lake, compared with other lakes where cryptomonads have been reported to daily migrate, and the shallow chemocline depth (5 m). Though light intensity in Lake Shunet in thermocline zone is low (about 60 μE m^{-2} s^{-1}) in summer and it is nearly dark (1 μE m^{-2} s^{-1}) in winter if the lake is covered with a layer of ice and snow, the irradiance seems to be sufficient for maintaining the *Cryptomonas* population, as confirmed by the present study. Also, the presence in chemocline of the extremely dense population of photosynthesizing sulphur bacteria (Rogozin et al. 2005, 2009, 2010) also supports the assumption of optimal light conditions in the

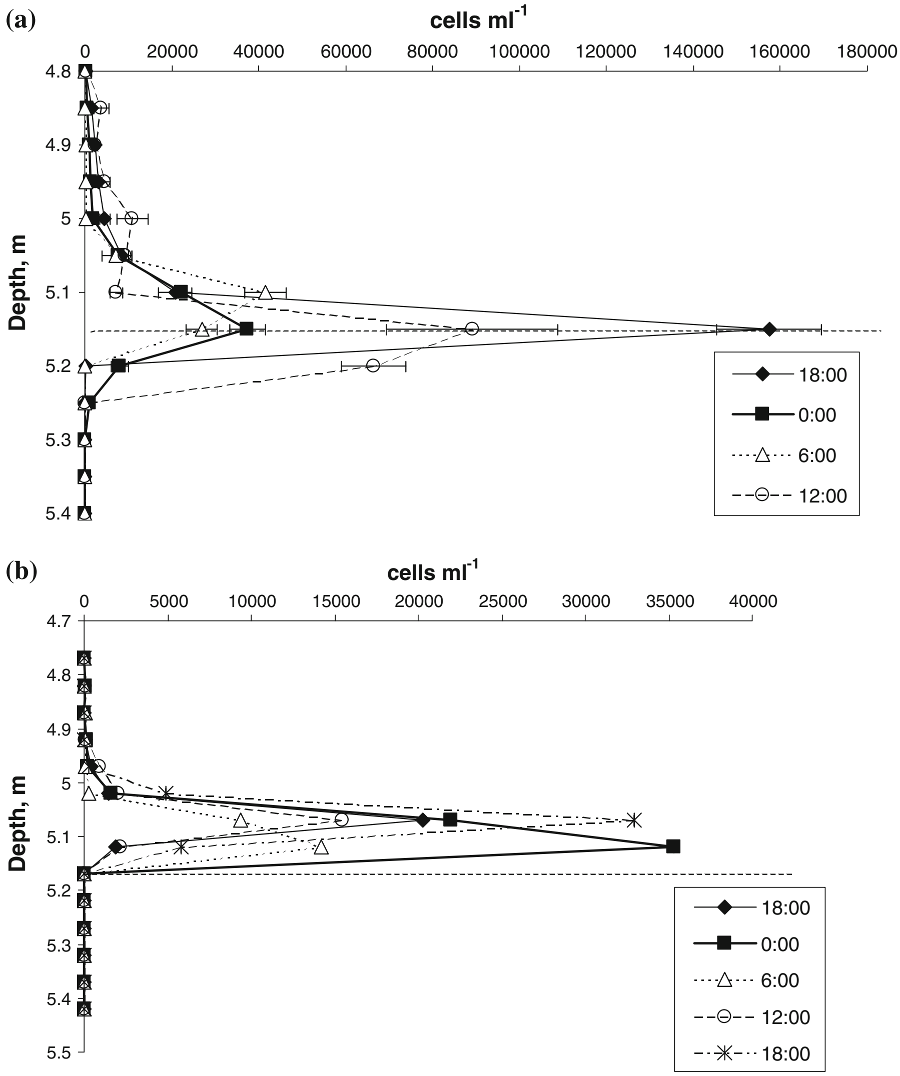

Fig. 5 Dynamics of diurnal distribution of *Cryptomonas* spp. in the chemocline zone of Lake Shunet. **a** 05.08.2004. **b** 26.07.2005. The *horizontal lines* mark the upper boundary of the microaerobic (<0.5 mg O_2 l^{-1}) yet sulphide-free chemocline

chemocline. To maintain the high density of the bacteria and flagellates, flow of nutrients must take place from the lake's bottom which is only about 1 m. Secondly, Lake Shunet does not have any large-bodied zooplankton species as *Daphnia,* which can graze upon these flagellates. However, even if *Daphnia* were present in Lake Shunet, there are published works that show that daphnids can survive in such a sulphide-rich environment, let alone graze upon the flagellates there.

Moreover, the three zooplankton species that dominate in the lake, *Arctodiaptomus salinus*, and the rotifers *Brachionus plicatilis* and *Hexarthra oxiuris* (Tolomeev et al. 2010), cannot stay for a long time in the anaerobic and microaerobic water layers (Lass et al. 2000), so that they cannot graze upon *Cryptomonas* present in the sulphide-rich layers.

Ciliates

The ciliate community in the pelagic zone of Lake Shunet clearly prefer to stay exclusively in chemocline: here where they have their maximum within a 10- to 35-cm-thick layer above or sometimes in the layer of purple sulphur bacteria, where H_2S concentrations vary from 0 to 5 mg L^{-1} of sulphide with little or no oxygen. Moreover, it appears that the ciliate populations in this region do not seem to migrate.

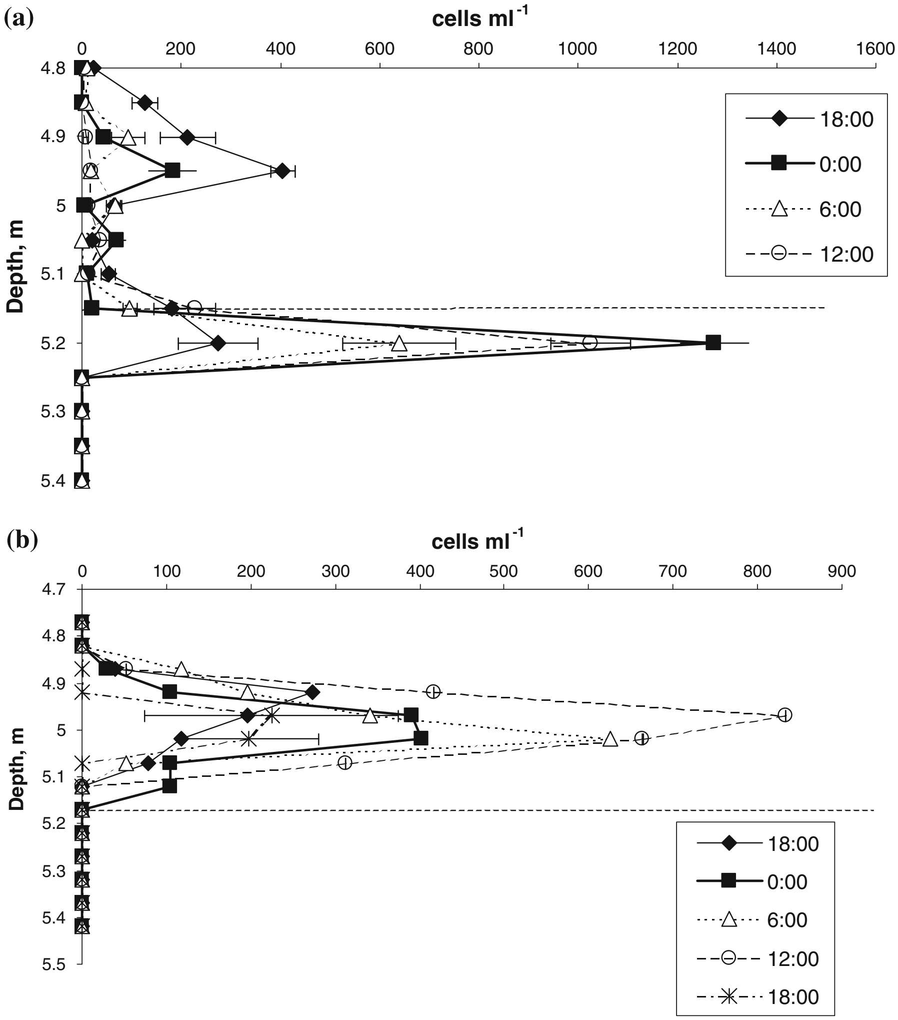

Fig. 6 Dynamics of diurnal distribution of ciliates in the chemocline zone of Lake Shunet. **a** 05.08.2004. **b** 26.07.2005. The *horizontal lines* mark the upper boundary of the microaerobic (<0.5 mg O_2 l^{-1}) yet sulphide-free chemocline

The above findings on the distribution of anaerobic ciliate communities in Lake Shunet are similar to those described for freshwater stratified reservoirs and marine systems all over the world (Fenchel et al. 1990; Laybourn-Parry et al. 1990; Zubkov et al. 1992). Both availability of food sources and physicochemical gradients of the water bodies seem to play an important role. The lower boundary of ciliate distribution is governed by high concentration of sulphide, which inhibits metabolism (Guhl et al. 1996). In the DCM of Ciso and Schlachtensee, ciliates e.g. *Coleps* sp. and *Prorodon* sp., which form metalimnetic maxima, cannot descend into the lower layers with high sulphide levels (Pedrós-Alió et al. 1995; Gervais 1998). In DCM in Lake Arcas (Spain), small scuticociliates (<30 μm in length) were concentrated immediately below the dense layer of *Chromatium weissei*, where sulphide levels decreased dramatically (Finlay et al. 1991).

Guhl et al. (1996) showed that although the ciliate communities in three stratified lakes they studied differed in their species compositions, they had similar structure of vertical distribution of ciliates in pelagic zone. These and a number of other studies (Fenchel et al. 1990; Laybourn-Parry et al. 1990; Zubkov et al. 1992; Guhl et al. 1994) suggest that the vertical distribution patterns of ciliates are an evidence of the almost steady-state conditions prevailing in the anoxic hypolimnia and that changes in these patterns follow changes in the conditions in the aquatic ecosystem.

In Lake Schlachtensee (Germany), the ciliates were most dense in either the chemocline or within about 35 cm above it, i.e. in the layers where

Cryptomonas was present (Pedrós-Alió et al. 1995). In the hypolimnetic deep chlorophyll maximum of two freshwater lakes, Arcas in Spain and Esthwaite Water in England, large ciliate as the prostomatid, *Plagiocampa*, which fed on phototrophic bacteria and *Cryptomonas*, followed the distribution of their food. While the distribution of scuticociliates, which graze on heterotrophic bacteria associated with the dense *Chromatium* layer, was limited by higher sulphide concentrations (Guhl et al. 1996).

Several studies report (Fenchel et al. 1990; Zubkov et al. 1992; Guhl et al. 1994, 1996) that the species compositions in the epilimnion and hypolimnion of stratified lakes differ. The species composition of the ciliate community in the pelagic zone of Lake Shunet is not very diverse: only a few species of the genera *Oligotrichida*, *Scuticociliatida*, *Hypotrichida* and *Prostomatida* occur regularly. We, however, also found *Oxytricha* sp. in summer 2004, when ciliate population was large. Ciliate species found in L. Shunet occupy different habitats in the pelagic zone of the lake. The *Oxytricha* sp., which was found in only August 2004 in the 5- to 10-cm anaerobic layer of the chemocline, inhabited by purple sulphur bacteria in layers containing H_2S, can be considered a typical anaerobic species. The other ciliate species occur throughout the water column but are largely concentrated immediately above the chemocline.

Closely related ciliate species seem to have wide geographical distribution in both freshwater and marine anaerobic ecosystems (Guhl et al. 1996). For instance, in stratified lakes with the DCM dominated by cryptophytes, prostomatid ciliates (*Prorodon viridis* Kahl, and other *Prorodon* spp.) frequently form large populations just beneath the oxic/anoxic boundary (Pedrós-Alió et al. 1995; Gervais 1998).

In addition to being adapted to anaerobic conditions, some of the ciliate species inhabiting the metalimnion and the hypolimnion of meromictic lakes can graze on large-sized food item. For example, predatory ciliates such as *Prostomatida* found in the chemocline of Lake Shunet (Fig. 4) can swallow food objects half their own length. Guhl et al. (1996) investigated ciliate communities in the anoxic hypolimnia of three lakes and did not find prostomatids, which were specialized on flagellates other than *Cryptomonas*. The authors also suggested that the main food source for these ciliates in the lake is the *Cryptomonas* population inhabiting hypolimnion, and the secondary food sources are sinking phytoplankton and purple sulphur bacteria (Guhl et al. 1996). Our observations showed that in Lake Shunet, during both summer and winter stratification, the large ciliates such as *Strombidium*, *Prorodon* and *Euplotes* peaked closely above the chemocline, where they could feed on phytoflagellates and probably also purple bacteria (Fenchel 1968; Pedrós-Alió et al. 1995; Gervais 1998).

Scuticociliates < 25 µm in length, which were abundant in the hypolimnion and the chemocline of Lake Shunet, cannot feed on purple bacteria and *Cryptomonas* as their cells are too large to pass through the filtering apparatus of these ciliates' (Foissner and Berger 1996; Guhl et al. 1996). These ciliates apparently feed on heterotrophic bacteria, which develop abundantly in organic-rich chemocline layers of Lake Shunet. Elevated concentrations of small *Cyclidium* sp., a ciliate, during spring and autumn must be related to the increase in the abundance of these heterotrophic bacteria during lake mixing in spring and summer.

In Lake Shira, a larger meromictic brackish lake (39.5 km^2 in area with a maximum depth of 24 m), situated 8 km away from Lake Shunet, the chemocline is located at a depth of 11–16.5 m (Rogozin et al. 2009). In the chemocline zone of this lake, there is also a peak of autotrophic organisms, including purple sulphur bacteria (Kopylov et al. 2002; Pimenov et al. 2003; Lunina et al. 2007; Rogozin et al. 2005, 2009). Production of purple sulphur bacteria in L. Shira is significantly lower than in L. Shunet, due to much less favourable conditions in the chemocline: low illumination (0.2–4.9 µmol PAR m^{-2} s^{-1}) and low temperatures throughout the year, caused by the greater depth of chemocline (Rogozin et al. 2009). Also, *Cryptomonas* density in the chemocline of L. Shira was lower, and ciliate numbers and diversity were also much poorer than in L. Shunet (Kopylov et al. 2002). Only two ciliate spp, *Strombidium* sp. and *Cyclidium* sp., were encountered in the pelagic zone of the lake at the depth of 6–11 m; their abundance ranged from 10 to 30 ind. l^{-1} (Kopylov et al. 2002).

Lake Shunet differs from L. Shira not only in higher temperatures and light intensity (0.1–67.0 µmol PAR m^{-2} s^{-1}) in the chemocline zone with more pronounced stratification, which results in

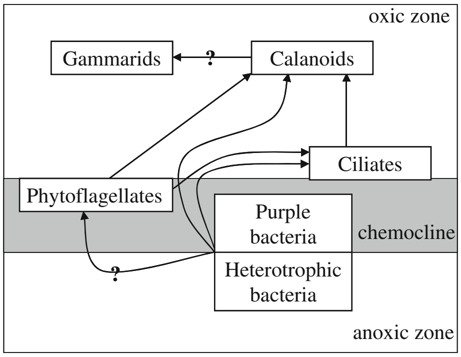

Fig. 7 Flow chart of the speculative trophical links in microbial loop of Lake Shunet chemocline zone

the stability of hydrophysical conditions (temperature, sulphide level, salinity, etc.) and restricts the abundance of purple bacteria from spreading. High sulphide concentration in the chemocline prevents zooplankton grazing in these layers, inhabited by flagellates, bacteria and ciliates.

Conclusions

From the data seasonal dynamics, species composition, vertical distribution and diel migrations of protozooplankton in Lake Shunet, we conclude the lake possesses all the basic features of meromictic lakes. Important among these are the perennial presence in a steady-state of a large *Cryptomonas* spp. population close to the chemocline, coexisting with a dense population of photosynthesizing purple sulphur bacteria and ciliates adapted to anaerobic conditions. All these organisms peak in an extremely narrow zone above and in the chemocline in the layer which lacks oxygen, is poorly lighted and contains high concentration of sulphides. Ciliates exhibit diurnal vertical migration, only within a narrow amplitude ($\sim$5 cm) or such a migration is altogether absent.

Not only bacteria and single-cell organisms tend to concentrate in thin layers but also larger species such as gammarids (Degermendzhi et al. 2010), which can use the nutrients produced by the microbial loop (Fig. 7).

Acknowledgments We would like to thank to Dr. Ramesh D. Gulati (Handling editor) for his critical reading of and comments on this manuscript and for his advice. We also want to thank the reviewers, whose comments have been valuable and useful. This work was supported by Russian Foundation for Basic Research (RFBR) and Krasnoyarsk Region Science Foundation Grant No. 09-04-98042-r_sibir_a, by RFBR Grant no. 09-05-00915-a, 10-04-10142-k, by the Netherlands Organization for Scientific Research (NWO) and RFBR Grant no. 047.017.012. The work was partially supported by Integration project of SB RAS No. 95.

References

Adler M, Gervais F, Siedel U (2000) Phytoplankton species composition in the chemocline of mesotrophic lakes. Arch Hydrobiol Spec Issues Advanc Limnol 55:513–530

Arvola L, Salonen K, Kankaala P, Lehtovaara A (1992) Vertical distributions of bacteria and algae in a steeply stratified humic lake under high grazing pressure from *Daphnia longispina*. Hydrobiologia 229:253–269

Bird DF, Kalff J (1989) Phagotrophic sustenance of a metalimnetic phytoplankton peak. Limnol Oceanogr 34:155–162

Caldwell DE, Tiedje JM (1975) The structure of anaerobic bacteria communities in the hypolimnia of several Michigan lakes. Can J Microbiol 21:362–376

Camacho A, Vicente E, Miracle MR (2000) Ecology of a deep-living *Oscillatoria* (= *Planktothrix*) population in the sulphide-rich waters of a Spanish karstic lake. Arch Hydrobiol 148:333–355

Carpenter SR, Morrice J, Soranno PA, Elser JJ, MacKay NA, StAmand A (1993) Primary production and its interactions with nutrient dynamics. In: Carpenter SR, Kitchell JF (eds) The trophic cascade in lakes. Cambridge University Press, Cambridge, pp 225–251

Cullen JJ (1982) The deep chlorophyll maximum: comparing vertical profiles of chlorophyll A. Can J Fish Aquat Sci 39:791–803

Degermendzhi AG, Zadereev ES, Gulati RD, Rogozin DY, Prokopkin IG, Barkhatov YV, Tolomeyev AP, Khromechek EB, Mooij WM, Janse J (2010) Biological and physical mechanisms involved in formation of the vertical stratification of saline lakes Shira and Shunet, (South Siberia, Russia). Aquat Ecol (this Vol.)

Fee EJ (1976) The vertical and seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, northwestern Ontario: implications for primary production estimates. Limnol Oceanogr 21:767–783

Fenchel T (1968) The ecology of microbenthos II. The food of marine benthic ciliates. Ophelia 5:73–121

Fenchel T, Kristensen LD, Rasmussen L (1990) Water column anoxia: vertical zonation of planktonic protozoa. Mar Ecol Prog Ser 62:1–10

Finlay BJ, Clarke KJ, Vicente E, Miracle MR (1991) Anaerobic ciliates from sulphide-rich solution lake in Spain. Eur J Protistol 27:148–159

Foissner W, Berger H (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshw Biol 35:375–482

Galvez JA, Niell FX, Lucena J (1988) Description and mechanism of formation of a deep chlorophyll maximum due to *Ceratium hirundinella* (O.F. Müller) Bergh. Arch Hydrobiol 112:143–155

Garcia-Gil LJ, Borrego CM, Bafieras L, Abella CA (1993) Dynamics of phototrophic microbial populations in the chemocline of a meromictic basin in Lake Banyoles. Int Rev Ges Hydrobiol 178:283–294

Gasol JM, Guerrero R, Pedrós-Alió C (1992) Spatial and temporal dynamics of a metalimnetic *Cryptomonas* peak. J Plankton Res 14:1565–1579

Gasol JM, García-Cantizano J, Massana R, Guerrero R, Pedrós-Alió C (1993) Physiological ecology of a metalimnetic *Cryptomonas* population: relationships to light, sulphide and nutrients. J Plankton Res 15:255–275

Gast V, Gocke K (1988) Vertical distribution of number, biomass and size-class spectrum of bacteria in relation to oxic/anoxic conditions in the central Baltic Sea. Mar Ecol Prog Ser 45:179–186

Gervais F (1997) Diel vertical migration of *Cryptomonas* and *Chromatium* in the deep chlorophyll maximum of a eutrophic lake. J Plankton Res 19:533–550

Gervais F (1998) Ecology of cryptophytes coexisting near a freshwater chemocline. Freshw Biol 39:61–78

Gervais F, Siedell U, Heilmann B, Weithoff G, Heisig-Gunkell G, Nicklisch A (2003) Small-scale vertical distribution of phytoplankton, nutrients and sulphide below the oxycline of a mesotrophic lake. J Plankton Res 25:273–278

Guhl BE, Finlay BJ, Schink B (1994) Seasonal development of hypolimnetic ciliate communities in a eutrophic pond. FEMS Microbiol Ecol 14:293–306

Guhl B, Finlay B, Schink B (1996) Comparison of ciliate communities in the anoxic hypolimnia of three lakes: general features and the influence of lake characteristics. J Plankton Res 18:335–353

Ichimura S, Nagasawa S, Tanaka T (1968) On the oxygen and chlorophyll maxima found in the metalimnion of a mesotrophic lake. Bot Mag Tokyo 81:1–10

Kettle WD, Moffett MF, DeNoyelles F Jr (1987) Vertical distribution of zooplankton in an experimentally acidified lake containing a metalimnetic phytoplankton peak. Can J Fish Aquat Sci 44:91–95

Kiselev IA (1954) Pyrophitic algae. Identification guide of freshwater algae of the USSR, issue 16. Sovetskaya Nauka, Moscow

Knapp CW, deNoyelles F, Graham DW, Bergin S (2003) Physical and chemical conditions surrounding the diurnal vertical migration of *Cryptomonas* spp. (Cryptophyceae) in a seasonally stratified Midwestern reservoir (USA). J Phycol 39:855–861

Kopylov AI, Kosolapov DB, Degermendzhy NN, Zotina TA, Romanenko AV (2002) Phytoplankton, bacterial production and protozoan bacterivory in stratified, brackish-water Lake Shira (Khakasia, Siberia). Aquat Ecol 36:205–217

Lass S, Boersma M, Spaak P (2000) How do migrating daphnids cope with fish predation risk in the epilimnion under anoxic conditions in the hypolimnion. J Plankton Res 22:1411–1418

Laybourn-Parry J, Olver J, Rees SC (1990) The hypolimnetic protozoan plankton of a eutrophic lake. Hydrobiologia 203:111–119

Lohmann H (1908) Untersuchungen zur Feststellung des vollstandigen Gehaltes des Meeres an Plankton. Wiss Meeresunters 10:129–370

Longhurst AR (1976) Interactions between zooplankton and phytoplankton profiles in the eastern tropical Pacific Ocean. Deep-Sea Res 23:729–754

Lunina ON, Bryantseva IA, Akimov VN, Rusanov II, Rogozin DY, Barinova ES, Lysenko AM, Pimenov NV (2007) Seasonal changes in the structure of the anoxygenic photosynthetic bacterial community in Lake Shunet, Khakassia. Microbiology 76:368–379

Marshall W, Laybourn-Parry J (2002) The balance between photosynthesis and grazing in Antarctic mixotrophic cryptophytes. Freshw Biol 47:2060–2070

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

Miracle MR, Vicente E, Pedros-Alio C (1992) Biological studies of Spanish meromictic and stratified karstic lakes. Limnetica 8:59–77

Moll RA, Brahce MZ, Peterson TP (1984) Phytoplankton dynamics within the subsurface chlorophyll maximum of Lake Michigan. J Plankton Res 6:751–766

Montesinos E, Guerrero R, Abella C, Esteve I (1983) Ecology and physiology of the competition for light between *Chlorobium limicola* and *Chlorobium phaeobacteroides* in natural habitats. Appl Environ Microbiol 46:1007–1016

Parnachev VP, Degermendzhy AG (2002) Geographical, geological and hydrochemical distribution of saline lakes in Khakassia, Southern Siberia. Aquat Ecol 36:107–122

Pedrós-Alió C, Guerrero R (1993) Microbial ecology of Lake Cisó. In: Jones JG (ed) Advances in microbial ecology. Plenum Press, New York, pp 155–209

Pedrós-Alió C, Massana R, Latasa M, García-Cantizano J, Gasol JM (1995) Predation by ciliates on a metalimnetic *Cryptomonas* population: feeding rates, impact and effects of vertical migration. J Plankton Res 17:2131–2154

Pimenov NV, Rusanov II, Karnachuk OV, Rogozin DY, Bryantseva IA, Lunina ON, Yusupov SK, Parnachev VP, Ivanov MV (2003) Microbial processes of the carbon and sulfur cycles in Lake Shira (Khakasia). Microbiology 72:221–229

Porter KG (1988) Phagotrophic phytoflagellates in microbial food webs. Hydrobiologia 159:89–97

Reynolds CS (1992) Dynamics, selection and composition of phytoplankton in relation to vertical structure in lakes. Arch Hydrobiol Spec Issues Advanc Limnol 35:13–31

Rogozin DY, Degermendzhy AG (2008) Hydraulically-operated thin-layer sampler for sampling heterogeneous water columns. J Sib Fed Univ 1:111–117

Rogozin DY, Pimenov NV, Kosolapov DB, Chan'kovskaya YV, Degermendzhy AG (2005) Thin-layer vertical distributions of purple sulfur bacteria in chemocline zones of meromictic lakes Shira and Shunet (Khakassia). Dokl Biol Sci 400:54–56

Rogozin DY, Zykov VV, Chernetsky MY, Degermendzhy AG, Gulati RD (2009) Effect of winter conditions on distributions of anoxic phototrophic bacteria in two meromictic lakes in Siberia, Russia. Aquat Ecol 43:661–672

Rogozin DY, Trusova MY, Khromechek EB, Degermendzhy AG (2010) Microbial community of chemocline of meromictic lake Shunet (Khakassia, Russia) during summer stratification. Microbiology 79:253–261

Salonen K, Jones RI, Arvola L (1984) Hypolimnetic phosphorus retrieval by diel vertical migrations of lake phytoplankton. Freshw Biol 14:431–438

Sanders RW (1991) Trophic strategies among heterotrophic flagellates. In: Patterson DJ, Larsen J (eds) The biology of heterotrophic flagellates. Clarendon Press, Oxford, pp 21–38

Sanders RW, Porter KG (1988) Phagotrophic phytoflagellates. Adv Microb Ecol 10:167–192

Steele JH, Yentsch CS (1960) The vertical distribution of chlorophyll. J Mar Biol Assoc UK 39:217–226

Takahashi M, Ichimura S (1968) Vertical distribution and organic matter production of photosynthetic sulfur bacteria in Japanese lakes. Limnol Oceanogr 13:644–655

Tolomeev AP, Sushchik NN, Gulati RD, Makhutova ON, Kalacheva GS, Zotina TA (2010) Fatty acid composition and feeding spectra of *Arctodiaptomus salinus* (Calanoida, Copepoda) in two salt lakes in South Siberia (Russia, Khakasia). Aquat Ecol (this Vol.)

Tyler PA, Vyverman WG (1995) The microbial market place—trade-offs at the chemocline of meromictic lakes. Progr Phycol Res 11:325–370

Zadereev ES, Tolomeyev AP, Drobotov AV, Emeliyanova AYu, Gubanov MV (2010) The vertical distribution and abundance of *Gammarus lacustris* in the pelagic zone of the meromictic lakes Shira and Shunet (Khakassia, Russia). Aquatic Ecol (this issue)

Zubkov MV, Sazhin AF, Flint MV (1992) The microplankton organisms at the oxic-anoxic interface in the pelagial of the Black Sea. FEMS Microbiol Ecol 101:245–250