

Differences in the ultrastructure of two selected taxa of phytoplankton in a thermally stratified Lake Holzmaar (Germany)

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Abstract: This paper presents the results of ultrastructural studies and ecological aspects of some phytoplankton species belonging to the groups of cyanobacteria (*Planktothrix rubescens*, *Synechocystis aquatilis*) and green algae (*Desmodesmus grahneisii*). Specimens were collected during summertime from the mesotrophic and stratified Lake Holzmaar (Western Germany) as planktonic forms from the pelagic zone. The highest cyanobacterium concentration was detected in the metalimnion, where the alkaline pH, low phosphorus and high nitrogen contents were recorded. *P. rubescens* was characterized by straight filaments up to 1000 µm long and 5.4-8 µm wide and numerous aerotopes in cells. The accompanying algae were identified by ultrastructural analysis and photographic documentation was provided. In the case of *D. grahneisii*, chloroplast was situated in the parietal part of cell, with one large, oval pyrenoid and, in addition, the granular and spiny cell wall was an important taxonomical criterium for the *Desmodesmus* genus. This is in contrast with the cyanobacterium *S. aquatilis*, in which a homogeneous content with visible chromatoplasma was mostly distributed throughout the cell. This algal association was stable in the epilimnion characterized by the presence of high temperature, pH values (>8), nitrate nitrogen and oxygen concentrations.

Key words: *Planktothrix rubescens*, *Synechocystis aquatilis*, *Desmodesmus grahneisii*, ultrastructure, autecology

1. Introduction

Planktothrix rubescens (De Candolle ex Gomont) Anagnostidis et Komárek is a stenotherm species living in cold water. It is largely distributed in middle European (Reynolds 1984) southern sub-alpine lakes (Garibaldi *et al.* 2003), and is common in the lakes of North-Eastern Switzerland (Walsby *et al.* 1998; Kurmayer & Jeüttner 1999). Planktonic cyanobacteria were previously classified as *Oscillatoria rubescens* De Candolle, and later described as *Planktothrix rubescens* (Anagnostidis & Komárek 1988). This species has a very distinctive biology and high diversity of gas vesicle genes. During summer stratification, it is usually located within the metalimnion (Konopka 1980; Feuillade & Davies 1994; Scheffer *et al.* 1997; Omlin *et al.* 2001; Messyasz *et al.*

2003, 2005; Legnani *et al.* 2005; Lenard 2009) where it is photosynthetically active.

In deep and very strongly stratified lakes, the species composition of phytoplankton is closely related to environmental conditions changing with season. Many species of phytoplankton are adapted to ecological changes by their specific growth strategy. The cyanobacterial *Planktothrix* blooms typically occur in deep lakes. Lake Holzmaar is characterised by intense development of small phytoplankton cells in the layer of 0-5 m depth, where blooms of *P. rubescens* during summer have been already studied (Messyasz *et al.* 2003, 2006). Cyanobacteria were dominant in the epilimnion zone of the lake except summer months, where green algae (Chlorophyta) were more abundant and represented 34.57% of all identified species. The

most frequent species among the summer phytoplankton in the pelagic zone were: *Cosmarium* spp., *Desmodesmus* spp., *Scenedesmus* spp., *Golenkinia radiata*, *Lagerheimia subsalsa*, *Tetraedron minimum*, *Pandorina morum* and *Chlamydomonas passiva* (Messyasz *et al.* 2003, 2005). The green algae *Desmodesmus grahneisii*, *D. brasiliensis* and *D. serratus* which are the R type of strategy (fast reproducing) were compared with cyanobacterial species *Synechocystis aquatilis* representing also the R type strategy, preferring more or less turbulent waters (Reynolds 2006). *Planktothrix rubescens*, forming little changing density peaks in the metalimnion layer during summer in deep mesotrophic lakes, is classified according to Reynolds (1996) in the R functional group of phytoplankton. The main aim of our study was to examine some phytoplankton species (e.g. *Desmodesmus grahneisii*, *Synechocystis aquatilis*) accompanying *Planktothrix rubescens* using light and transmission electron microscopy to show differences in their cells ultrastructure with a special focus on the ecological requirements of these phytoplankton species.

2. Material and methods

2.1. Study area

Lake Holzmaar is a small, water-filled, crater situated in the volcanic field of the West Eifel region in western Germany, 95 km south of Cologne (Fig. 1). The studied lake is an example of a deep crater lake (maximum depth 20 m) and with almost round shape. Lake Holzmaar is stratified thermally from April to October with the metalimnion zone between 6 and 8 m. Some relevant physical and chemical factors (temperature, pH, oxygen and nutrients concentrations) are presented in Table 1. The rim of the crater and the near shore areas are forested with stands of beech, oak and spruce (Raubitschek *et al.* 1999).

2.2. Sample preparations

The phycological material, including phytoplankton, was collected every two weeks from July to September (2002-2004) from a single station situated above the deepest point of the lake and preserved with Lugol's

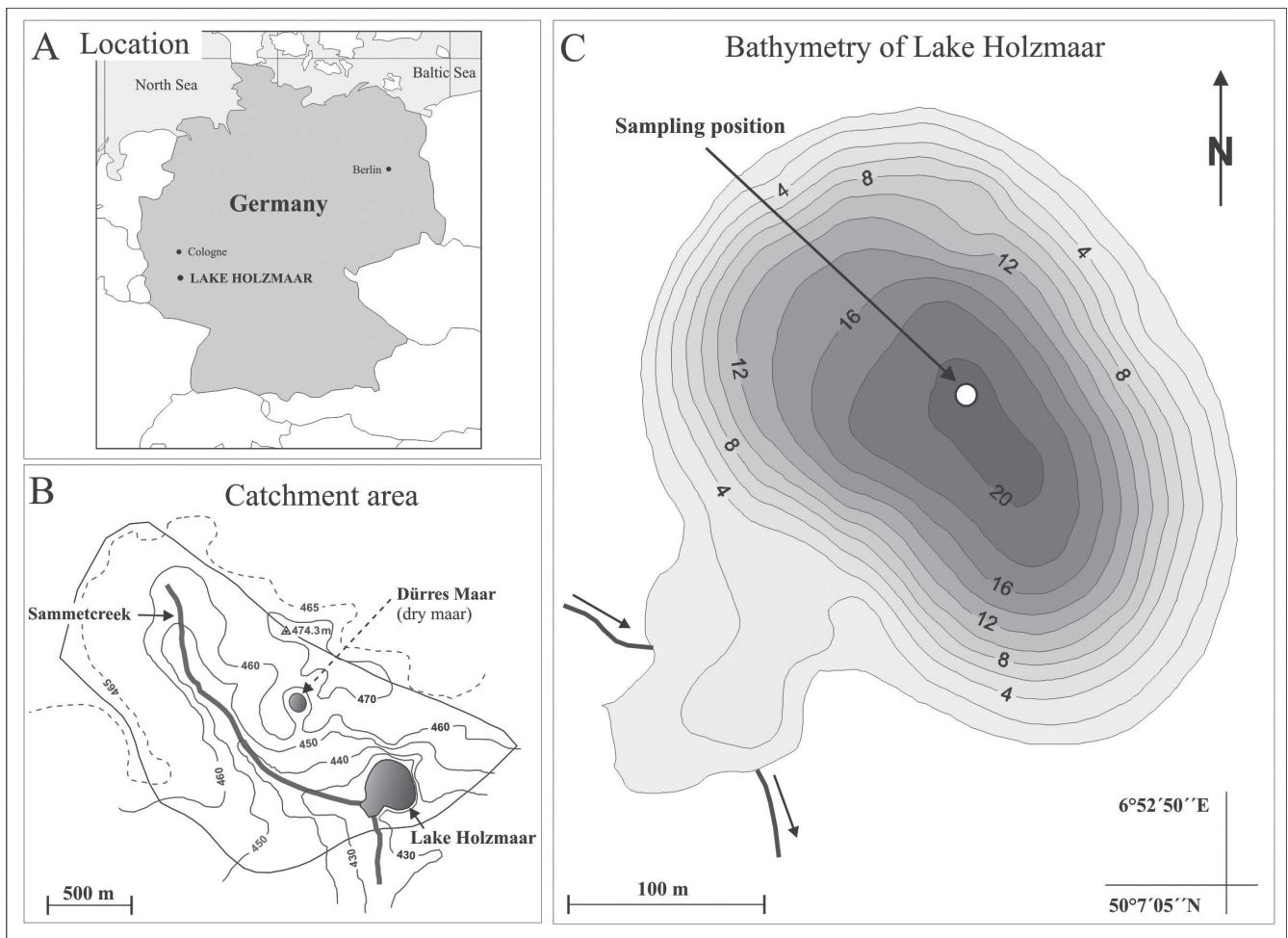


Fig. 1. Map of investigated area of Lake Holzmaar in the West Eifel Volcanic Field: A – location, B – catchment area, C – bathymetric map of the lake (changed after Scharf & Oehms 1992)

solution. The samples were studied both with the light and electron microscope. For the transmission electron microscopy (TEM), the cultures were maintained on the standard Bristol agar medium at 20°C under a 16/8 h light/dark cycle at 3000 $\mu\text{Em}^{-2}\text{s}^{-1}$ provided by 40 W cool fluorescent tubes. Cells were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH=7.2) for two hours, washed several times in buffer and postfixed in 1% osmium tetroxide in the same buffer. The cells were dehydrated by a graded acetone series and embedded in Spurr's resin. Ultra-thin sections were stained with uranyl acetate and lead citrate (Reynolds 1963). Observations and photographs were carried out on a TESLA BS 500 transmission electron microscope. Specific identification was done with the light microscope, according the cited monographs (Komárek & Fott 1983, Anagnostidis & Komárek 1988; Komárek & Anagnostidis 1989, 1999; John *et al.* 2011). Taxonomical classification of algae was adopted after Van Den Hoek *et al.* (1995).

3. Results

3.1. Morphology and ultrastructure

In our study we found *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis et Komárek (after Starmach 1966; Suda *et al.* 2002) which is the planktonic cyanobacterium, forming single trichomes or distinct bundles. Its trichomes were straight, gradually tapering at ends. Filaments were generally from 470-1000 μm long. Cells were 5.4-8 μm wide and 2-4 μm long, in the shape of one-third to one-half as long as wide, and possessing granules and gas vesicles inside. In light microscope, they usually paled purple-pink color. Another characteristic feature was that cells at the end of trichomes slightly capitates (Fig. 2), with convex calyptra at the top. Thylakoids were arranged perpendicularly to the longitudinal side of cells. The even distribution of gas vesicles in the protoplast was

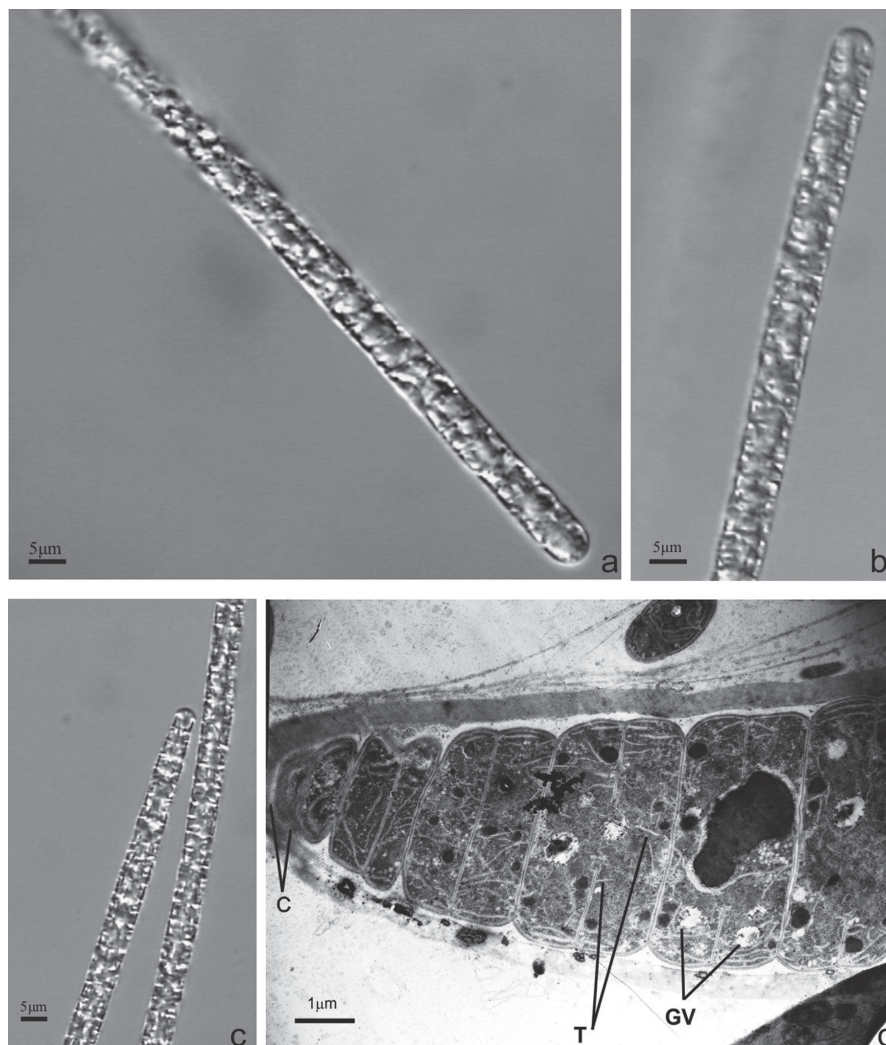


Fig. 2. Microscopic observations of cyanobacterial filaments of *Planktothrix rubescens* in the studied lake Lake Holzmaar

Explanations: photographs were performed using a LM, a-c – single trichomes and a TEM, d – longitudinal ultra-thin section of cyanobacterium, a part of a filament with terminal cell; C – calyptra, T – thylakoids, GV – gas vesicles

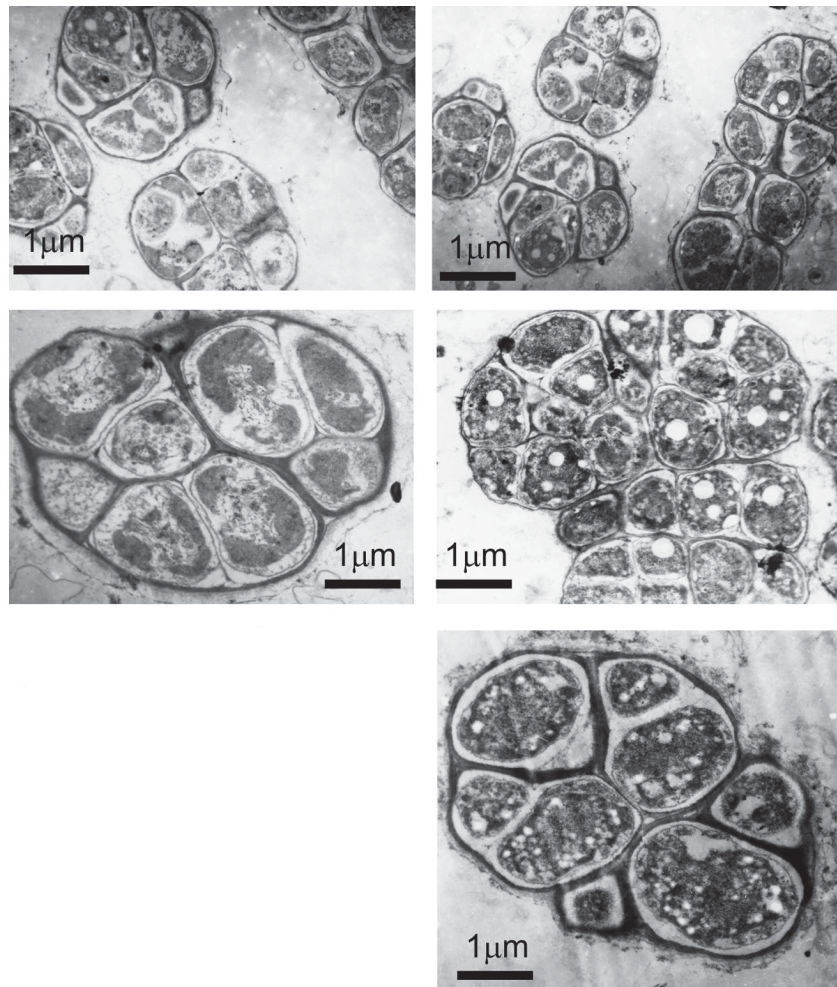


Fig. 3. Transmission electron microscopy (TEM) of *Synechocystis aquatilis* in the studied Lake Holzmaar

characteristic in young cells, while in the older cells, they were more densely packed.

Small cyanobacterium *Synechocystis aquatilis* Sauvageau was associated with *P. rubescens* filaments in both the epi- and metalimnion zones. This species had spherical or widely oval cells which were solitary or agglomerated. Its widely oval cells from 2.9 to 6.4 μm in diameter, after division, were took hemispherical form with more or less visible and colorless mucilaginous envelopes (Fig. 3). Transmission electron microscopy studies revealed the interior of the cell in a pale blue-green color containing homogeneous content or with several distinct granules, sometimes with visible chromatoplasma.

Single *Planktothrix* trichomes were observed in the epilimnion where representatives of small green algae of genus *Desmodesmus* formed quantitatively large populations during summer. Morphology of *Desmodesmus grahneisii* (Heyning) Hegewald (Fig. 4) was taken into account that cells are ovoid to ellipsoid (cell length, 5-13 μm ; width, 3-7 μm), with rounded apices (usually 2, 4 or 8-celled). The cell wall was granular, spiny or toothed. Electron microscopically investigations have

shown the presence of parietal chloroplast with a single pyrenoid in each cell.

Another green alga that was found as an accompanying species occurring in the epilimnion of Lake Holzmaar was *Desmodesmus serratus* (Corda) An, Friedl & Hegewald. *Desmodesmus serratus* was a taxon with coenobia of 2, 4 or 8 linearly arranged cells, which were 2.5-7 μm wide and 7.8-20 μm long. Moreover, oval-elongated cells possessed a row of short spines on the lateral walls, with 1-4 teeth on rounded or sometimes almost truncate apices. The cell wall was smooth except for four teeth in longitudinal rows or occasionally scattered.

Also together with *Desmodesmus* spp., the development of numerous large forms of green algae such as *Pandorina morum* (O.F. Müller) Bory was recorded in the epilimnion at the end of the summer. Spherical coenobia of *Pandorina morum* consisted of 8-16 cells compressed into dense spherical aggregates. The cup-shaped chloroplast contained a pyrenoid up to 2.2 μm in diameter. The most characteristic feature of this species was the eyespot occurring in the anterior cell end.

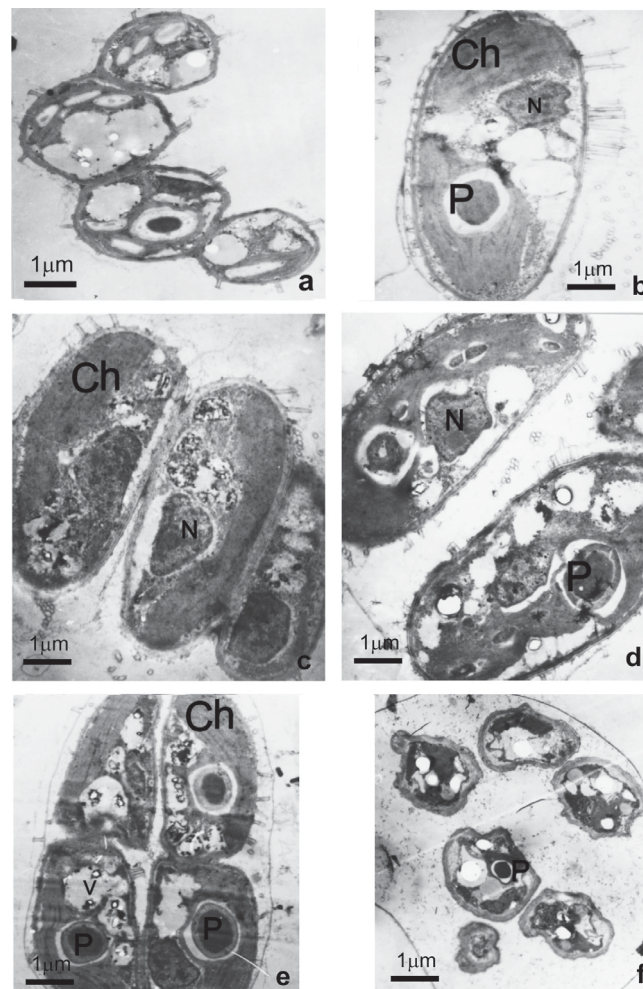


Fig. 4. Transmission electron microscopy (TEM) of vegetative and reproductive stages of *Desmodesmus grahneisii* in the studied Lake Holzmaar. Explanations: a – four-celled colony (spines up to 10 μm), b-d – vegetative cells with nucleus, pyrenoids, chloroplast and spines, e-f – reproductive stages; ch – chloroplast, n – nucleus, p – pyrenoid, v – vacuoles

3.2. Ecology of selected species

Filamentous cyanobacterium *Planktothrix rubescens* was forming dense water bloom in the metalimnion of Lake Holzmaar during summer (Table 2) characterized by a sharp peak of its biomass as well as the considerable value of chlorophyll a and the oxygen contents (Table 1). In the metalimnion, orthophosphate concentration in water was very low ($>10 \mu\text{g}\cdot\text{l}^{-1}$) in contrast with the nitrate nitrogen whose value exceeded $10 \text{mg}\cdot\text{l}^{-1}$. The pH range was found between 7.6 and 9.1. In the begin-

ning of September, *P. rubescens* concentration started gradually to increase, also in the epilimnion.

In the case of the epilimnion, cyanobacterium *Synechocystis aquatilis* development was related to low light intensity ($\text{SD} < 1.5 \text{m}$) and alkaline pH (8-10.5) during July and August (Tables 1-2). Only small differences were observed between epilimnion and metalimnion zones with regard to the orthophosphate concentrations. An exception was the epilimnion where the maximum concentrations of nitrate nitrogen ($18 \text{mg}\cdot\text{l}^{-1}$) were

Table 1. Basic limnological data for Lake Holzmaar observed during July-August in 2002-2004

Parameter	Pelagic zone (epilimnion)	Thermocline (metalimnion)
Stratified dimictic lake	0-5.0 m	6.0-8.0 m
Transparency (SD)	0.9-4.0 m	-
Chlorophyll-a $\mu\text{g}\cdot\text{l}^{-1}$	4.0-24.0	32.0-80.0
pH	8.0-10.5	7.6-9.1
Nitrate nitrogen $\text{mg}\cdot\text{l}^{-1}$	10.7-18.0	10.7-22.4
orthophosphate $\text{mg}\cdot\text{l}^{-1}$	0.001-0.010	0.001-0.006
Oxygen concentration $\text{mg}\cdot\text{l}^{-1}$	7.0-22.3	0.1-35.2

Table 2. The average biomass ($\text{mg}\cdot\text{l}^{-1}$) of particular phytoplankton species occurring from July to September in 2002-2004

Taxon	July		August		September	
	0-4 m (n=18)	6-8 m (n=12)	0-4 m (n=18)	6-8 m (n=12)	0-4 m (n=18)	6-8 m (n=12)
<i>Planktothrix rubescens</i>	0.782	10.716	1.635	8.512	3.990	8.131
<i>Synechocystis aquatilis</i>	0.035	0.001	0.023	0.001	0.012	0.001
<i>Desmodesmus brasiliensis</i>	0.138	0.002	0.139	0.005	0.006	0.001
<i>Desmodesmus grahneisii</i>	1.446	0.295	3.242	0.090	0.541	0.567
<i>Desmodesmus serratus</i>	0.007	0.0001	0.016	0.0001	0.003	0.001
<i>Pandorina morum</i>	0.068	0.041	0.289	0.258	0.836	0.169

observed only in the beginning of July, while later this value was much lower.

Green alga *Desmodesmus grahneisii* and species from the *Desmodesmus* spp. formed typical aggregates at the depth of 0-4 m (Table 2) and contributed to a great value of chlorophyll a, even though still smaller than in the metalimnion zone (Table 1). These depths were also characterized by the presence of high pH values (>8) and oxygen concentrations. The aggregation phenomenon was observed each year during almost the same period, i.e. from late July to beginning of September, and within a narrow temperature range, 19-25°C. Every single time, intense green algae development in the epilimnion zone was positively correlated with the decrease in the transparency of water. The quantitative analyses showed that alga *Pandorina morum* constituted a considerable part of the green algae biomass during the summer. However, this alga was growing under stable water conditions and was mainly noted in the epilimnion zone of Lake Holzmaar in August and September (Table 2).

4. Discussion

Planktothrix rubescens is one of the most studied organisms among the filamentous cyanoprokaryotes. This is because of its early seasonal occurrence and the striking visual appearance of blooms. It is planktonic species in standing waters, occasionally forming blooms as purplish floating masses and there are only a few modern records (John *et al.* 2011). The main morphological features of *P. rubescens* include solitary trichomes or filaments that are able to move by gliding forwards and backwards (trembling), thylakoids arranged perpendicularly to the longitudinal side of cells, even distribution of gas vesicles in the protoplast and lack of false branching of trichomes. This alga is a phycoerythrin-rich cyanobacterium and reaches its maximum growth rate at light intensities typical of the metalimnion (Skulberg *et al.* 1994). High water transparency in Lake Holzmaar was the factor which generated *P. rubescens* concentrating in the depth between 6 and 8 m. Its adaptations such as length of filaments and graz-

ing resistance contributed to its supremacy over other algal species.

Reynolds (1984, 2006) screened *P. rubescens* domination to assess the diversity of accompanying algal communities. He designated these epilimnion assemblages of algae, and inferred that higher water temperature and the concentration of nitrogen compounds (especially nitrates) constitute niches for specific species with small cells. Such a system, an example of which is Lake Holzmaar, contained only one species of cyanobacteria as a dominant one in the metalimnion, but several species of algae in the epilimnion. The results indicate that the concentration of chlorophyll a was higher in the metalimnion than in the epilimnion zone. As a general principle, the presence of few *P. rubescens* filaments and its better survival than other algae in the epilimnion were associated with the presence of gas vesicles. Samples from 0-4 m were considered to have many species with a similar number of cells, based on the phytoplankton analysis. Our results showed that the largest amount of *Chlorophyta* biomass and much smaller in the case of chroococcal cyanobacteria was observed in the epilimnion during summer in Lake Holzmaar. Electron microscopic investigations of *S. aquatilis* showed the presence of cell aggregation in the process of division indicating favorable habitat conditions for the growth of this cyanobacterium population. These results are in accordance with the ecological description of this planktonic species as inhabiting mildly eutrophic waters (John *et al.* 2011). However, the ability to form numerous natural populations in aquatic ecosystems is rather rare for *S. aquatilis* and, thus, detailed studies are carried out, primarily, under laboratory conditions. The observation of Komárek & Anagnostidis (1999) that in several strains of this cyanobacterial genera the cell walls contain a special "S-layer" with a characteristic hexagonal (p6) substructure is also valid to *Synechocystis aquatilis*. The function and taxonomic significance of this layer is not clear. Fundamental differences in the internal cell structure between *Synechocystis* and *Desmodesmus* resulted from characteristics typical for prokaryotic cells, such as the nucleus and lack of pyrenoid.

The genus *Desmodesmus* (formerly *Scenedesmus* p.p.) has been known and investigated for nearly 200 years (Schubert & Wilk-Woźniak 2003). Meyen described in 1829 *Scenedesmus* which included non-spiny and spiny colonies. Hegewald and Silva (1988) described three subgenera: *Scenedesmus*, *Desmodesmus* and *Acutodesmus*. However, in 1999, using molecular analysis based on ITS-2 rDNA sequence, the non-spiny and spiny forms were found to be separate genera. The spiny forms are now called *Desmodesmus* sp. (An *et al.* 1999) and the non-spiny forms retain the original name, *Scenedesmus* sp. Hegewald (2000) transferred 32 species and 22 varieties to *Desmodesmus* from the subgenus *Scenedesmus*.

Desmodesmus grahneisii is known from Southern Hungary (Schmidt *et al.* 2003), Slovakia (Hindák & Hindáková 2000) and recently was found in Spain (Fanés *et al.* 2009). This species was also found in Lake Holzmaar and the low water transparency in August corresponds to a massive development of small green algae with it as a dominant. There was a significant correlation between *Desmodesmus* spp. development and phosphate concentration. When the cell density was the highest, the phosphate content was considerably decreased. Besides, green alga *Desmodesmus serratus* is a common small species whose strains under the light microscope differ in the formation of a mucilage envelope (Fawley *et al.* 2011). This species is known from many locations in the plankton of various water bodies and is classified as cosmopolitan and eutrophic (John *et al.* 2011). No habitat differences are immediately apparent for our *Desmodesmus* species from Lake Holzmaar. All new species are known from eutrophic water bodies, and some of the species have also been isolated from mesotrophic and dystrophic habitats. Fawley *et al.* (2011) suggested that variability present in one lineage of the genus *Desmodesmus* and the importance of analyzing this variability result from using sequence data from multiple loci and morphology. Cells in the colony of *D. grahneisii* and *D. brasiliensis* occurred in coenobia of

two cells and with four cells in the case of *D. serratus*. The species differed in the number and type of spines on the cells and the texture of the wall.

It may be added that the volvocalean green alga *Pandorina morum* with large colonies was also observed in all investigated summer seasons when it was associated with *Staurastrum paradoxum* and *Peridiniopsis elpatiewskyi*. Colony forming *Pandorina morum* is generally known as a widely distributed cosmopolitan species, often found in stratified, shallow lakes rich in nitrate. Furthermore, it occurs in mesotrophic water ecosystems as well as in eutrophic water bodies (John *et al.* 2011).

High peaks of the phytoplankton biomass are a distinctive feature of Lake Holzmaar in metalimnetic waters in the summer period (Messyasz *et al.* 2003, 2005). It was related to *Planktothrix rubescens* dominating in phytoplankton community. We confirmed that representatives of cyanobacterium *Planktothrix rubescens*, which avoids the intensive light and prefers deeper layers of water, occurs also in mesotrophic reservoirs such as Lake Holzmaar. The strong thermal water stratification in deep Lake Holzmaar additionally enables the intense development of small cyanobacteria (*S. aquatilis*) and green algae species (*Desmodesmus* ssp.) during the entire summer period and shows their availability to rapid colonization of free space in the euphotic zone of lake. So numerous populations of both species are rarely found in lakes and thus the results of our studies of Lake Holzmaar enrich the information on their ecological and ultrastructural characteristics. In conclusion, it can be stated that specimens of *P. rubescens*, *S. aquatilis* and *D. grahneisii* collected from Lake Holzmaar exhibited cells to demonstrate intense divisions allowing their populations to increase in size rapidly. Further studies are needed to accurately determine the factors influencing the repeated occurrence of these species at different depths in the water column during summer stratification.

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