

### 3. ATTACHED PUBLICATIONS

3.1. **NEDBALOVÁ, L.**, VRBA, J., FOTT, J., KOHOUT, L., KOPÁČEK, J., MACEK, M. & SOLDÁN, T. (2006): Biological recovery of the Bohemian Forest lakes from acidification. *Biologia* **61**/Suppl. **20**: S453–S465.

## Bio lo g ical r ecover y o f the Bo hem ian Fo r es t

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**Abstract:** A limnological survey of eight small, atmospherically acidified, forested glacial lakes in the Bohemian Forest (Šumava, Böhmerwald) was performed in September 2003. Water chemistry of the tributaries and surface layer of each lake was determined, as well as species composition and biomass of the plankton along the water column, and littoral macrozoobenthos to assess the present status of the lakes. The progress in chemical reversal and biological recovery from acid stress was evaluated by comparing the current status of the lakes with results of a survey four years ago (1999) and former acidification data since the early 1990s. Both the current chemical lake status and the pelagic food web structure reflected the acidity of the tributaries and their aluminium (Al) and phosphorus (P) concentrations. One mesotrophic (Plešné jezero) and three oligotrophic lakes (Černé jezero, Čertovo jezero, and Rachelsee) are still chronically acidified, while four other oligotrophic lakes (Kleiner Arbersee, Prášilské jezero, Grosser Arbersee, and Laka) have recovered their carbonate buffering system. Total plankton biomass was very low and largely dominated by filamentous bacteria in the acidified oligotrophic lakes, while the mesotrophic lake had a higher biomass and was dominated by phytoplankton, which apparently profited from the higher P input. In contrast, both phytoplankton and crustacean zooplankton accounted for the majority of plankton biomass in the recovering lakes. This study has shown further progress in the reversal of lake water chemistry as well as further evidence of biological recovery compared to the 1999 survey. While no changes occurred in species composition of phytoplankton, new ciliate species was found in one lake. In several lakes, this survey documented a return of zooplankton (e.g., Cladocera: *Ceriodaphnia quadrangula* and Rotifera: three *Keratella* species) and macrozoobenthos species (e.g., Ephemeroptera and Plecoptera). The biological recovery has been delayed for ~20 years after chemical reversal of the lakes.

**Key words:** Acidification, lake recovery, phytoplankton, zooplankton, bacteria, ciliates, macrozoobenthos.

### Introduction

Small glacial mountain lakes with low ionic strength water usually host less complex pelagic communities compared to larger water bodies. Such headwater lake ecosystems are greatly vulnerable to atmospheric acidification (e.g., KALFF, 2002), which has caused a dramatic reduction of biodiversity followed by disruption of food web structure in many lake districts since the middle of the 20<sup>th</sup> century (e.g., SCHINDLER, 1988). Deposition of acidifying pollutants has significantly declined in Europe and North America, particularly due to reduced sulphur (S) emissions since the 1980s, leading to a partial reversal of surface waters from acidification (STODDARD et al., 1999). A corresponding biological

recovery of these areas has, however, been significantly delayed or uncertain (SKJELKVALE et al., 2003; JEFFRIES et al., 2003; WRIGHT et al., 2005).

Like the whole region of Central Europe, the Bohemian Forest (Šumava, Böhmerwald) was exposed to heavy atmospheric pollution during the last century, followed by a significant drop in both S and nitrogen (N) depositions during the last two decades (KOPÁČEK et al., 2001, 2002). Due to severe acidification, the Bohemian Forest lakes became unique ecosystems, with bacterioplankton and/or phytoplankton largely dominating their pelagic biomass, the complete absence of fish, and significantly reduced or even extinct crustacean zooplankton (VRBA et al., 2003a, b). According to their acidification status in 1999, eight Bo-

Table 1. Location and main characteristics of the Bohemian Forest lakes and their catchments.

Lake		CN	CT	RA	PL	KA	PR	GA	LA
Latitude	N	49°11'	49°10'	48°58'	48°47'	49°08'	49°05'	49°06'	49°07'
Longitude	E	13°11'	13°12'	13°24'	13°52'	13°09'	13°24'	13°07'	13°20'
Elevation	m a.s.l.	1008	1027	1071	1087	918	1079	935	1085
Lake area	ha	18.8	10.7	5.7	7.6	9.4	4.2	7.7	2.6
Catchment area	km <sup>2</sup>	1.24	0.89	0.58	0.67	2.79	0.65	2.58	1.02
Max. depth	m	40	35	13	19	9	17	16	3
Lake volume	10 <sup>6</sup> m <sup>3</sup>	2.92	1.86	0.18	0.61	0.25	0.35	0.45	0.05

Explanations: Codes and names of the lakes: CN – Černé jezero; CT – Čertovo jezero; PL – Plešné jezero; PR – Prášilské jezero; RA – Rachelsee; GA – Grosser Arbersee; KA – Kleiner Arbersee; LA – Laka. For locations see Fig. 1; for more detailed information on lakes and their catchments see VRBA et al. (2000) and JANSKY et al. (2005).

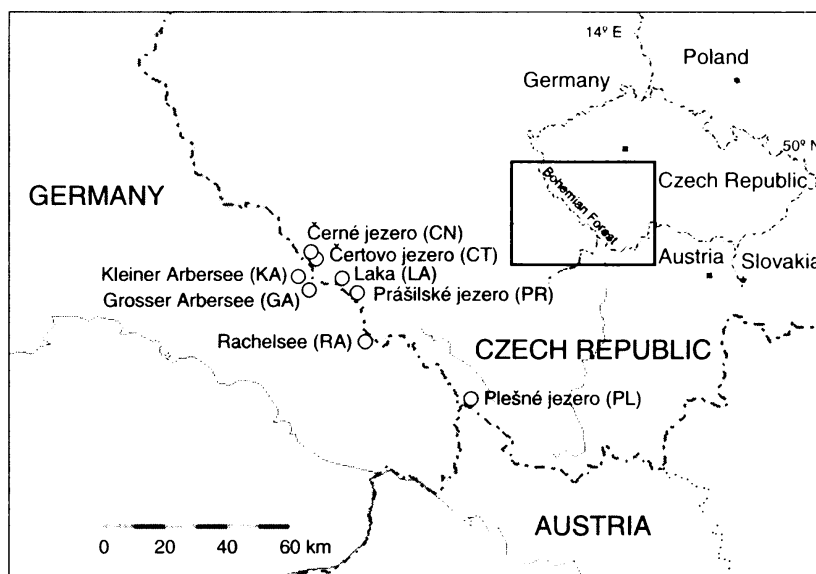


Fig. 1. The map of location of the Bohemian Forest lakes (their codes used throughout the text are in parentheses).

hemian Forest lakes were divided into three categories: (i) strongly acidified lakes (Černé jezero, Čertovo jezero, Rachelsee, Plešné jezero), (ii) moderately acidified lakes (Prášilské jezero, Kleiner Arbersee), and (iii) slightly acidified lakes (Grosser Arbersee, Laka) (VRBA et al., 2000; VRBA et al., 2003a).

This study presents both current species composition and carbon biomass of the plankton, together with relevant chemistry of the Bohemian Forest lakes. In addition, we present the first comparison of littoral macrozoobenthos assemblages in all the lakes. Our main objective is to compare the new (2003) data with a former survey from 1999 and older acidification data since the early 1990s, in order to evaluate biological recovery of the lakes from acid stress.

#### Material and methods

Eight small, glacial lakes (see Tab. 1 for lake names and their codes used further in the text) in the Bohemian Forest (C Europe) were studied. The lakes are situated along the border between the Czech Republic and Germany (Fig. 1) in steep, forested (Norway spruce) catchments with acid sensitive bedrock (mica-schist or granite); for detailed morpho-

logical data and land use history, see VESELÝ (1994), WEILNER (1997), VRBA et al. (2000) and JANSKY et al. (2005).

Samples of lake water were taken at the deepest points of the lakes during 10 days in early September 2003. The samples for chemical analyses were taken from the epilimnion (0.5 m depth) and were immediately filtered through a 200  $\mu\text{m}$  polyamide sieve. The samples for microbial analyses were sampled with a van Dorn sampler (1 m length, 6.4 L volume) from specific depths (5–6 samples along the vertical profile in the deep lakes, except for the shallow KA and LA with 3 and 2 samples, respectively). The samples were fixed with either formaldehyde (for bacteria) or acid Lugol's solution (for phytoplankton and ciliates). Large zooplankton were sampled by several vertical hauls with a quantitative net (200  $\mu\text{m}$  mesh size) of the Apstein type, while small zooplankton were sampled with the van Dorn sampler from the same depths as microorganisms, and concentrated by a plankton net (40  $\mu\text{m}$  mesh size); both samples were preserved by formaldehyde. Temperature and dissolved oxygen were measured with a DataSonde 4 (Hydrolab, USA) at 0.5-m intervals.

Main tributaries were sampled near their inlets to the lakes and the water discharges were estimated by means of a bucket and stopwatch. Samples were filtered immediately through a 40  $\mu\text{m}$  polyamide sieve to remove coarse particles

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re-suspended from the streambed during sampling. Each inlet sample was analysed separately. The chemistry and discharge of the inlets were used to calculate the discharge-weighted mean chemical composition of total water input to each lake.

In the laboratory, water samples were filtered with either membrane filters (pore size of 0.45  $\mu\text{m}$ ) for the determination of ions (ion chromatography) and silica (Si) or with glass-fibre filters (pore size of 0.4  $\mu\text{m}$ ) for other analyses, except for samples for pH, acid neutralizing capacity (ANC, determined by Gran titration), and total concentrations of aluminium (Al), phosphorus (P), carbon (C), and N, which were not filtered beyond the field pre-filter. Dissolved organic C (DOC) and particulate C ( $C_{\text{part}}$ ) were analysed with a LiquiTOC analyser (Foss/Heracus) for the filtrate, and by combustion of the glass-fibre filter for the retained particulate organic matter, respectively. Soluble reactive P (SRP), total P (TP; after perchloric acid digestion), and dissolved reactive Si were determined by molybdate methods. When the SRP concentration was below the detection limit of 1.5  $\mu\text{g L}^{-1}$ , a half of this value was used as the result in subsequent data evaluation. Total organic N (TON) was calculated as the difference between the Kjeldahl N and  $\text{NH}_4\text{-N}$ , determined by Kjeldahl digestion and ion chromatography, respectively. Fractionation of Al, i.e., total reactive Al ( $\text{Al}_T$ ), dissolved Al ( $\text{Al}_D$ ), and organically bound Al ( $\text{Al}_{\text{org}}$ ), were analysed in non-filtered samples, filtered samples, and cation exchange treated samples after their filtration, respectively (DRISCOLL, 1984). Ionic Al ( $\text{Al}_i$ ) was obtained as the difference between  $\text{Al}_D$  and  $\text{Al}_{\text{org}}$ . Particulate Al ( $\text{Al}_{\text{part}}$ ) was the difference between  $\text{Al}_T$  and  $\text{Al}_D$ . More details on analytical methods are given by KOPÁČEK et al. (2006a).

Macrozoobenthos were collected by using metal cups or sampling nets for 10–15 min mostly by means of the “kicking technique” (KERSHAW & FROST, 1978), i.e., by digging in the stony and sandy bottoms in the littoral zone (up to ~0.5 m water depth) of the lakes. In parallel, attention was also paid to submerged aquatic plants (sweeping by metal cups) and to the surface of larger stones in the littoral zone. Adults collected flying or sitting on the vegetation were not considered since determining the share of those emigrating from running waters near the lakes was impossible.

Bacterioplankton were counted and sized after DAPI staining on black Poretics filters using an epifluorescence microscope (Olympus BX-60 or AX-70) and an image analysis system (Lucia D 3.52 or G/F 4.11, Laboratory Imaging, www.lim.cz). We quantified sole unicellular bacteria separately, and used the line intercept method for quantification of the total length of bacterial filaments (NEDOMA et al., 2001).

Phytoplankton counting was done in Utermöhl's sedimentation chambers on an inverted microscope (Nikon Diaphot). A pre-sedimentation of samples (5 $\times$ ) was usually necessary except for the PL samples. Total biovolume of filamentous cyanobacteria was estimated in sedimentation chambers using the line intercept method (NEDOMA et al., 2001). Cell volumes were estimated by shape assimilation to known geometric forms (STRASKRABOVÁ et al., 1999). Concentration of chlorophyll-*a* (Chl-*a*) was determined spectrophotometrically on Whatman GF/C filters after acetone extraction (LORENZEN, 1967); values were not corrected for phaeopigments.

Samples for ciliate analysis were postfixed with Bouin's fluid. The quantitative protargol staining was applied

(MONTAGNES & LYNN, 1993); samples were dehydrated in ethanol, phenol-xylol and xylol series, and neutral Canada balsam-mounted. The whole filter area was inspected at 500 $\times$  to 1250 $\times$  on microscopes equipped with Nomarski (DIC) contrast (Olympus, Nikon, or Leica). FOISSNER (1994), FOISSNER et al. (1999) and literature cited therein were used for identification. For all observed specimens, individual cell volume was calculated (using simple shape models) from dimensions measured in the protargol stained samples.

The zooplankton samples were fixed by formaldehyde (4% final concentration), and counted on an inverted microscope (Nikon Diaphot). The length of individual species was measured to estimate their biovolume (rotifers, nauplii) or dry weight (most crustaceans).

Finally, biovolumes or dry weight of the plankton were converted to carbon biomass (in  $\mu\text{g C L}^{-1}$ ) according to conversion factors published in STRASKRABOVÁ et al. (1999) (phytoplankton and zooplankton), VRBA et al. (2003b) (unicellular bacteria and filaments), and JEROME et al. (1993) (ciliates). All plankton data from samples taken on a vertical profile were averaged as volume-weighted biomass with respect to the morphometry of the particular lake.

## Results

### Water chemistry

All the lakes, except the shallow LA, were thermally stratified in September 2003, with depleted concentrations of dissolved oxygen above the bottom; pronounced bottom oxygen deficits ( $< 1 \text{ mg O}_2 \text{ L}^{-1}$ ) were found in CT, PR and PL (data not shown). Secchi depth varied from 1.0 m to 9.8 m (Tab. 2). On the basis of the current acidity of their tributaries, the lakes were divided into the two following groups: Group 1, strongly acidified lakes (RA, CN, CT, PL), with inlet water pH  $< 4.6$  and a depleted carbonate buffering system (negative ANC values in the lakes); and Group 2, moderately acidic lakes (PR, KA, GA, LA), with inlet water pH  $> 5.1$  and positive in-lake ANC values. In both groups there were pH gradients between the tributaries and the epilimnion due to in-lake alkalinity generation; thus the carbonate buffering system was re-established in the lakes of Group 2 (Tab. 2). The pH gradients caused changes in Al speciation, hydrolysis of ionic Al forms and formation of  $\text{Al}_{\text{part}}$  precipitate in all lakes. The tributaries delivered higher  $\text{Al}_T$  concentrations to the lakes of Group 1. The most pronounced decrease in  $\text{Al}_T$  concentration between the tributary and epilimnion (in-lake Al retention) was observed in the lakes with the strongest pH gradients (RA and PL, Tab. 2). The PL inlets delivered the highest TP concentration; however, its epilimnetic concentration was by 30% lower compared to its tributaries, indicating high TP retention in this lake (KOPÁČEK et al., 2006b). Besides PL, only lakes RA and PR had detectable SRP concentrations from their tributaries, whereas their epilimnetic SRP concentrations were below the detection limit. Both C : P and N : P molar ratios in seston suggested the most

Table 2. Selected water characteristics of the Bohemian Forest lakes in September 2003.

		CN		CT		RA		PL		KA		PR		GA		LA	
		In.	Sur.	In.	Sur.	In.	Sur.	In.	Sur.	In.	Sur.	In.	Sur.	In.	Sur.	In.	Sur.
Z <sub>S</sub>	m	ND	9.8	ND	5.3	ND	8.5	ND	1.0	ND	1.7	ND	4.5	ND	4.0	ND	2.5
pH		4.47	5.01	4.56	4.68	4.36	5.14	4.54	5.26	5.12	5.81	5.16	5.37	5.61	6.11	5.75	6.22
ANC	mmol L <sup>-1</sup>	-37	7	30	-17	52	-6	-29	0	0	23	1	5	14	34	26	58
Si	mg L <sup>-1</sup>	2.7	1.8	3.0	1.5	2.8	2.0	5.2	2.9	2.8	1.1	2.7	1.4	3.3	1.9	3.3	1.9
TP	μg L <sup>-1</sup>	2.3	1.6	3.6	3.1	3.1	3.1	12.3	8.5	3.3	10.7	7.7	7.0	2.5	5.6	7.0	5.8
SRP	μg L <sup>-1</sup>	<1	<1	<1	<1	2.4	<1	9.6	<1	<1	<1	2.0	1.6	<1	<1	<1	1.9
NH <sub>4</sub> -N	μg L <sup>-1</sup>	<5	31	6	35	9	171	6	8	<5	17	5	10	<5	12	<5	27
NO <sub>3</sub> -N	μg L <sup>-1</sup>	800	686	462	434	1850	1200	937	47	366	53	447	153	458	141	785	261
TON	μg L <sup>-1</sup>	99.5	125	81.5	215	237	49	135	438	152	332	191	192	143	174	101	219
TOC	mg L <sup>-1</sup>	ND	1.69	ND	2.55	ND	1.48	ND	6.3	ND	5.46	ND	4.09	ND	3.16	ND	4.28
DOC	mg L <sup>-1</sup>	2.1	1.3	3.0	1.7	3.7	0.9	4.1	2.4	2.5	3.5	3.3	3.7	2.2	2.6	2.3	3.2
SO <sub>4</sub> <sup>2-</sup>	mg L <sup>-1</sup>	4.57	3.19	3.85	3.67	3.77	2.48	4.34	3.37	3.15	2.44	1.83	1.69	2.65	2.33	1.42	1.28
Na <sup>+</sup>	mg L <sup>-1</sup>	0.99	0.71	1.17	0.59	0.70	0.55	1.25	0.83	1.00	0.78	0.94	0.64	1.05	0.78	1.24	1.20
K <sup>+</sup>	mg L <sup>-1</sup>	0.28	0.44	0.23	0.31	0.37	0.70	0.38	0.31	0.19	0.22	0.22	0.29	0.23	0.22	0.36	0.40
Ca <sup>2+</sup>	mg L <sup>-1</sup>	0.55	0.70	0.36	0.51	0.94	0.88	0.96	0.68	0.79	0.79	0.45	0.45	0.92	0.90	0.83	0.70
Mg <sup>2+</sup>	mg L <sup>-1</sup>	0.43	0.39	0.33	0.29	0.52	0.47	0.21	0.15	0.35	0.31	0.32	0.26	0.37	0.32	0.41	0.38
Al <sub>T</sub>	μg L <sup>-1</sup>	417	217	267	322	754	164	613	361	173	169	131	167	125	102	94	103
Al <sub>i</sub>	μg L <sup>-1</sup>	297	149	144	181	564	90	419	24	35	2	12	24	15	8	13	4
Al <sub>part</sub>	μg L <sup>-1</sup>	14	57	14	126	27	64	26	305	12	68	6	37	4	34	<1	35
Chl- <i>a</i>	μg L <sup>-1</sup>	ND	0.8	ND	2.8	ND	0.6	ND	14.3	ND	17.9	ND	4.2	ND	5.2	ND	6.3
Seston C : P	mol mol <sup>-1</sup>	ND	904	ND	878	ND	830	ND	1392	ND	904	ND	205	ND	517	ND	1160
Seston N : P	mol mol <sup>-1</sup>	ND	101	ND	88	ND	43	ND	111	ND	60	ND	33	ND	33	ND	66

Explanations: In. - discharge-weighted means of the main inlets ( $n = 2-3$  per lake); Sur. - surface samples ( $n = 1$ ); Z<sub>S</sub> - transparency; ANC - acid neutralising capacity; Si - reactive silica; TP - total phosphorus; SRP - soluble reactive phosphorus; TON - total organic nitrogen; TOC - total organic carbon; DOC - dissolved organic carbon; Al<sub>T</sub> - total reactive aluminium; Al<sub>i</sub> - ionic aluminium; Al<sub>part</sub> - particulate aluminium; chl-*a* - chlorophyll-*a*, ND - not determined; for lake codes, see key in Table 1.

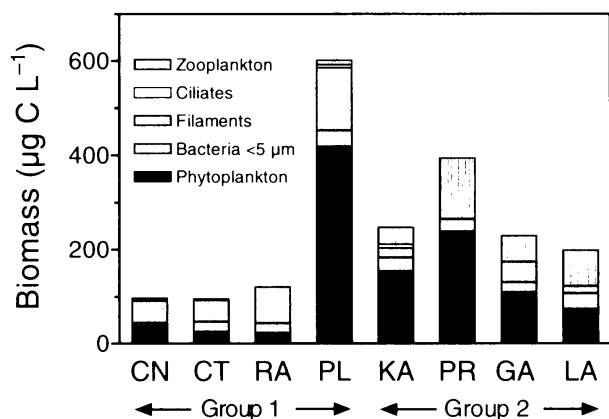


Fig. 2. Comparison of plankton biomass in the Bohemian Forest lakes in September 2003, based on volume-weighted means of phytoplankton, bacteria < 5 μm, heterotrophic filaments, ciliate, and zooplankton biomass; biomass of heterotrophic nanoflagellates was negligible. For lake codes, see key in Table 1. Groups 1 and 2 refer to strongly acidified and moderately acidic lakes, respectively (see text).

severe P limitation in PL, followed by other lakes, and only moderate P limitation in PR (Tab. 2).

#### Composition of plankton biomass

Both quantitative and qualitative composition of the volume-weighted plankton biomass differed largely among the Bohemian Forest lakes in September 2003. Generally, heterotrophic filaments and unicellular bac-

teria, phytoplankton, and partly zooplankton constituted major parts of the total biomass; heterotrophic flagellates (data not shown) and ciliates were relatively unimportant (Fig. 2). CN, CT and RA were characterised by low values of total plankton biomass ( $\sim 100 \mu\text{g C L}^{-1}$ ) with a high proportion of heterotrophic microbial biomass (> 50%). On the other hand, higher values of the total biomass were found in the lakes of Group 2 ( $\sim 200-400 \mu\text{g C L}^{-1}$ ), where phytoplankton together with zooplankton constituted more than 70% of total biomass. The highest biomass ( $\sim 600 \mu\text{g C L}^{-1}$ ), with a unique structure (70% phytoplankton), was characteristic for PL.

A high proportion of heterotrophic microbial biomass was typically formed by long filaments (> 100 μm); unicellular bacteria dominated only in PR (Fig. 2). Bacterioplankton did not show any clear trend in vertical distribution in most lakes; only in PR and PL, we observed a marked hypolimnetic peak of unicellular bacteria, associated with the most pronounced oxygen depletions in these lakes. The volume-weighted heterotrophic microbial biomass was lowest in PR ( $27 \mu\text{g C L}^{-1}$ ), whereas the highest values were in PL ( $167 \mu\text{g C L}^{-1}$ ) and RA ( $98 \mu\text{g C L}^{-1}$ ).

The number of phytoplankton species identified in the lakes during the survey in 2003 was rather low (15-27 taxa, Tab. 3). The lakes did not differ significantly in the taxonomic composition of phytoplankton, but the proportion of biomass from particular species was quite different. All the lakes except PL were dom-

Table 3. Phytoplankton taxa in the lakes in September 2003.

	CN	CT	RA	PL	KA	PR	GA	LA				
Cyanobacteria												
<i>Limnothrix</i> sp.	x	x	x	xx	x	-	x	x				
<i>Pseudanabaena</i> sp.	x	x	x	xx	x	-	x	x				
<i>Synechococcus nidulans</i> Nägeli	-	-	-	-	-	-	xx	-				
Dinophyceae												
<i>Gymnodinium uberrimum</i> (Allman) Kofoid et Sweezy	xx	x	xx	-	xx	xx	xx	-				
<i>Gymnodinium</i> sp.	x	x	x	x	-	x	x	x				
<i>Katodinium bohemicum</i> (Fott) Litvinenko	x	x	x	x	-	x	x	x				
<i>Katodinium planum</i> (Fott) Loeblich III	x	-	x	xx	-	-	-	-				
<i>Peridinium umbonatum</i> Stein (syn.: <i>P. inconspicuum</i> Lemmermann)	xx	xx	xx	x	xx	x	xx	xx				
Cryptophyceae												
<i>Cryptomonas erosa</i> Ehrenberg	x	xx	xx	x	xx	x	x	xx				
<i>Cryptomonas gracilis</i> Skuja	-	-	-	x	x	x	x	-				
<i>Cryptomonas marssonii</i> Skuja	x	-	x	x	x	x	x	x				
<i>Cryptomonas reflexa</i> Skuja	-	-	-	-	xx	-	xx	xx				
Chrysophyceae												
<i>Bitrichia ollula</i> (Fott) Bourrelly	x	-	x	x	x	x	x	x				
<i>Dinobryon</i> spp.	x	xx	x	x	x	x	x	xx				
<i>Mallomonas</i> sp.	x	-	x	-	-	-	x	x				
<i>Ochromonas</i> sp. (large)	x	xx	x	x	x	x	xx	xx				
<i>Ochromonas</i> sp. (small)	-	x	-	x	-	x	x	-				
<i>Spiniferomonas</i> sp.	x	x	x	x	x	x	x	x				
<i>Synura echinulata</i> Korschikov	x	x	x	-	x	x	x	xx				
Xanthophyceae												
<i>Isthmochloron trispinatum</i> (W. et G. S. West) Skuja	-	x	-	-	-	-	x	-				
Chlorophyta												
<i>Arthrodesmus incus</i> (Brébisson) Hassall	-	-	-	x	x	-	x	-				
<i>Carteria multifilis</i> (Fresenius) Dill + <i>C. radiosa</i> Korschikov	x	xx	xx	x	-	x	x	x				
<i>Chlamydomonas</i> sp.	x	x	x	x	-	x	x	x				
<i>Chlorogonium fusiforme</i> Matwienko	x	-	x	x	-	-	x	-				
<i>Chloromonas angustissima</i> (Ettl) Gerloff et Ettl	x	x	x	x	-	x	x	-				
<i>Koliella</i> cf. <i>corcontica</i> Hindák	xx	x	x	x	-	x	-	-				
<i>Monoraphidium dybowskii</i> (Woloszynska) Hindák et Komárková-Legnerová	-	x	x	xx	-	-	x	-				
<i>Tetraedron minimum</i> (A. Braun) Hansgörg	-	x	x	-	-	-	x	-				
<i>Cosmarium</i> sp.	-	-	-	-	xx	-	x	-				
Total	number	of	present	taxa	20	19	22	21	15	17	27	16

Explanations: x – present species; xx – important component of phytoplankton biomass; – absent species; for lake codes, see key in Table 1.

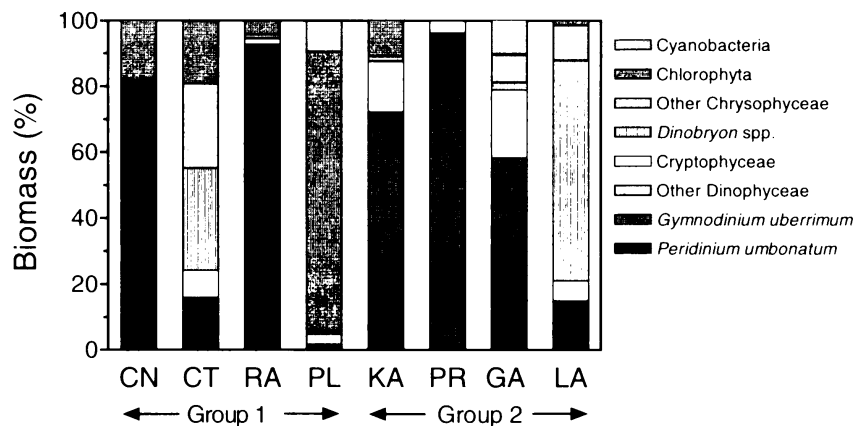


Fig. 3. Relative contribution of different species or taxonomic groups to the total biomass of phytoplankton in the Bohemian Forest lakes (volume-weighted means), September 2003. For lake codes, see key in Table 1. Groups 1 and 2 refer to strongly acidified and moderately acidic lakes, respectively (see text).

inated by flagellates from Dinophyceae (mainly *Peridinium umbonatum* and/or *Gymnodinium uberrimum*), and Chrysophyceae (colonial mixotrophic *Dinobryon*

spp.). In PL, the coccal green alga *Monoraphidium dybowskii* prevailed both in terms of abundance and biomass (Fig. 3). Concerning vertical distribution, a

Table 4. Recent list of all ciliate taxa in the Bohemian Forest lakes.

	Ecology	CN	CT	RA	PL	KA	PR	GA	LA
Gymnostomatea									
<i>Askenasia</i> spp.	R, M		x		x			n	-
<i>Belonophrya pelagica</i> André, 1914	R							-	x
<i>Lagynophrya</i> sp.	R		x		x		x		-
<i>Mesodinium</i> sp.	R				x			-	-
<i>Monodinium</i> sp. (minute)	R					n			-
<i>Pelagolacrymaria</i> sp.	R	n						x	-
<i>Spathidium</i> sp.	R					m		-	m
Prostomatida									
<i>Balanion planctonicum</i> Foissner, Berger et Kohmann, 1994	A		x		x		x		x
<i>Coleps</i> spp.	R, A		x			x	x	m	m
<i>Holophrya</i> spp., <i>Prorodon</i> spp.	A	n	x	m	x	m	x	n	-
<i>Urotricha globosa</i> Scheviakoff, 1892; <i>Urotricha</i> sp. (<10 µm)	A	x	x	x	x	x	x	x	m
<i>Urotricha furcata</i> Scheviakoff, 1892; <i>U. pseudofurcata</i> Krainer, 1995	A	x	x	x	x	x	x	n	m
Hymenostomata									
<i>Deziotricha</i> sp.	F				x			-	-
Hymenostomata g. sp.	F	n	x		x	x		x	-
<i>Lembadion</i> spp.	F	n				x	x	x	-
Scuticociliatida									
<i>Cinetochilum margaritaceum</i> Perty, 1849	F				x			n	-
<i>Cyclidium glaucoma</i> Müller, 1773	F		n					x	-
Scuticociliatida (minute)	F		x	x	x	x	x	x	x
Peritrichia									
<i>Vorticella</i> spp.	F				m				m
Spirotrichea									
<i>Caenomorpha</i> sp.	anox.		m					-	-
<i>Codonella cratera</i> Imhof, 1885	F				n			-	-
<i>Epakrella</i> sp.	anox.				n			-	-
<i>Halteria grandinella</i> Dujardin, 1841; <i>Halteria</i> sp.	F	n	x		x	x	x	x	x
<i>Limnostrombidium pelagicum</i> Krainer, 1995	F, M		n		m		m	-	m
<i>Pelagostrombidium fallax</i> , Krainer, 1991; <i>P. mirabile</i> Krainer, 1991	F, M		x		x		x	-	-
<i>Rimostrombidium brachykinetum</i> Krainer, 1995;	F	n						-	m
<i>R. hyalinum</i> Petz et Foissner, 1992									
<i>Rimostrombidium velox</i> Jankowski, 1978	F, M	n						-	-
<i>Stentor</i> sp.	F					m			-
<i>Stichotricha secunda</i> Perty, 1849	F, M						m		-
<i>Tintinnidium</i> sp.	F					n			n
<i>Trachelius ovum</i> Ehrenberg, 1838	anox.					m		-	-
Litostomatea									
<i>Litonotus</i> sp.	R								m
Colpodida									
<i>Cyrtolophosis elongata</i> Kahl, 1931	R, A					n		x	-
<i>Cyrtolophosis mucicola</i> Stokes, 1885	R							x	x
Total number of present taxa (x + n)		9	13	3	15	10	10	13	6
Total number of recent taxa (x + n + m)		9	14	4	17	14	12	14	14

Explanations: x – present species; n – new species, never found in former surveys; m – missing species in this survey, September 2003, but found in former surveys (MACEK, 2002); – absent species; anox. – anoxic species. Feeding mode: A – algivorous species; F – filter feeding species; M – mixotrophic species; R – raptorial species. For lake codes, see key in Table 1.

distinct hypolimnetic peak of phytoplankton was observed only in RA (11 m), while in the other lakes, the maximum phytoplankton biomass was in the mixing (epilimnetic) layer. The highest phytoplankton biomass was observed in PL (volume-weighted mean: 419 µg C L<sup>-1</sup>), followed by PR (238 µg C L<sup>-1</sup>), KA (154 µg C L<sup>-1</sup>), GA (109 µg C L<sup>-1</sup>), and LA (73 µg C L<sup>-1</sup>). Extremely low (23–39 µg C L<sup>-1</sup>) phytoplankton biomass was characteristic for RA, CN, and CT (Fig. 2).

Three to 15 ciliate species were found in each lake in this survey (Tab. 4). The lakes were characterised by the dominance of prostomes; algivorous hunting prostomes (genera *Urotricha*, *Holophrya* and *Prorodon*) were found in all lakes, mostly dominating both in num-

ber and biomass. Though picoplanktivorous or omnivorous ciliates were not scarce, they only seldom dominated the ciliate assemblages, as was the case with *Halteria* spp. in KA. Bottom oxygen depletions or anoxia resulted in a higher contribution of hymenostomes and odontostomatids (*Epakrella* sp.) to the ciliate assemblage, mainly in CT and PL. PL showed the highest ciliate abundance in the water column (mean of 10 cells ml<sup>-1</sup>; ~25 cells ml<sup>-1</sup> in the epilimnion) which reflected the high abundance of prostomes (*Urotricha* spp.). There were also abundant ciliates in the whole water column of KA (mean of 4.4 cells ml<sup>-1</sup>; *Halteria* sp., *Cyrtolophosis elongata*). Lower ciliate numbers (2–3 cells ml<sup>-1</sup>) were observed in particular layers of

Table 5. List of zooplankton species recorded from open water (vertical net hauls) in the Bohemian Forest lakes in September 2003.

	CN	CT	RA	PL	KA	PR	GA	LA
Rotatoria:								
<i>Brachionus urceolaris</i> (O.F.Müller, 1773) var. "sericus"	x	-	-	x	-	-	-	-
<i>Collotheca pelagica</i> (Rousselet, 1893)	-	-	-	x	-	-	-	-
<i>Keratella serrulata</i> (Ehrenberg, 1838)	-	x	x	x	-	x	x	-
<i>Keratella hiemalis</i> Carlin, 1943	-	-	-	-	-	-	x	x
<i>Keratella ticinensis</i> (Callerio, 1920)	-	-	-	-	-	-	-	x
<i>Keratella valga</i> (Ehrenberg, 1834)	-	-	-	-	x	-	-	-
<i>Lecane stichaea</i> Harring, 1913	-	-	-	-	-	-	-	x
<i>Microcodon clavus</i> Ehrenberg, 1830	x	-	x	x	-	-	x	-
<i>Polyarthra major</i> Burckhardt, 1900	-	-	-	-	xx	-	x	x
<i>Polyarthra remata</i> Skorikov, 1896	x	x	-	-	xx	x	x	xx
<i>Synchaeta tremula</i> (O.F.Müller, 1786)	-	-	-	x	-	-	-	-
<i>Synchaeta pectinata</i> Ehrenberg, 1832	-	-	-	-	-	-	-	x
Cladocera:								
<i>Ceriodaphnia quadrangula</i> (O.F.Müller, 1785)	xx	-	-	-	xx	-	x	-
<i>Chydorus sphaericus</i> (O.F.Müller, 1776)	-	-	-	-	x	-	-	-
<i>Daphnia longispina</i> (O.F.Müller, 1776)	-	-	-	-	-	xx	-	-
Copepoda:								
<i>Acanthocyclops vernalis</i> (Fischer, 1853)	-	xx	-	x	-	-	-	xx
<i>Cyclops abyssorum</i> Sars, 1863	-	-	-	-	-	xx	xx	-
<i>Heterocope saliens</i> (Lilljeborg, 1863)	-	-	-	xx	-	-	-	-
Total number of species:	4	3	2	7	5	4	7	7

Explanations: x – present; xx – relatively important; - absent; for lake codes, see key in Table 1.

some other lakes, e.g., in the epilimnion of CT (prostomes) and below the thermocline in PR, CN and GA (mainly prostomes). The maximum ciliate contribution to plankton biomass accounted for ~3% in KA (volume-weighted mean:  $7.0 \mu\text{g C L}^{-1}$ ) and CN ( $3.1 \mu\text{g C L}^{-1}$ ), and ~1% in PL ( $5.9 \mu\text{g C L}^{-1}$ ; Fig. 2).

Metazoan zooplankton were represented by a low number of species (2–7), and differed according to the acid status in the Bohemian Forest lakes (Tab. 5). While CT, RA, CN, and PL belonged to Group 1 (strongly acidified lakes) with a low share (< 2%) of zooplankton biomass (between 0.1 and  $10.2 \mu\text{g C L}^{-1}$  in RA and PL, respectively), higher zooplankton biomass in the other lakes of Group 2 (Fig. 2) reflected their current chemistry (Tab. 2). The highest biomass ( $129 \mu\text{g C L}^{-1}$ ) of zooplankton (dominated by Crustacea) was found in PR.

Typical pelagic species of crustacean zooplankton were absent in the most acidic lakes CT and RA, where only rotifers (*Polyarthra remata*, *Microcodon clavus*, *Keratella serrulata*) and the tycho planktonic crustacean *Acanthocyclops vernalis* (Copepoda) and/or nauplii were found. Another rotifer, *Brachionus urceolaris* (in its ecomorph "sericus", typical for acid waters – see KOSTE, 1978) was encountered in CN and PL; all five species of rotifers occurring in PL (Tab. 5) were acid-tolerant. They have been observed in the lake since 1990, but their abundance increased by two orders of magnitude between 1992 and 1997 (Fig. 4). PL differed from all other lakes by the presence of the large, acid-tolerant copepod *Heterocope saliens*. On the other hand, the crustacean *Ceriodaphnia quadrangula* (Cladocera) was found in the open

water of CN (but was more frequent in the stony littoral).

The pelagial of PR was inhabited by the crustaceans *Daphnia longispina* (Cladocera) and *Cyclops abyssorum* (Copepoda), which survived the peak of acidity in the 1980s. *Ceriodaphnia quadrangula*, not encountered in the previous surveys of PR, was abundant in the littoral zone among *Carex* (not listed in Tab. 5). The same species, however, was found in the open water of KA. GA and PR were the only two Bohemian Forest lakes where the planktonic copepod *Cyclops abyssorum* currently occurred. LA was remarkably poor in planktonic crustaceans; the only crustacean found in open water was *Acanthocyclops vernalis*. A rotifer species (*Keratella hiemalis*) was newly found in GA and LA, whereas *Keratella ticinensis* and *Keratella valga* occurred exclusively in LA and KA, respectively.

#### Composition of macrozoobenthos

Recent littoral macrozoobenthos samples exhibited from 10–12 (RA, GA, KA) to 32 (shallow LA) species of the insect orders Ephemeroptera (4 species), Odonata (8 species), Plecoptera (11 species), Megaloptera (2 species), Neuroptera (1 species), and Trichoptera (16 species; Tab. 6). Species composition of both Ephemeroptera and Plecoptera exhibited pronounced changes compared to the 1990s. The most acid-tolerant mayfly species, *Leptophlebia vespertina*, occurred in all the lakes. Moreover, two other species, *Ameletus inopinatus* and *Siphonurus lacustris* (first record in CT), returned to most lakes and *S. alternatus* survived in LA (Tab. 6). This survey also confirmed returning of four extinct stonefly species (*Leuc-*



Table 6. Recent list of littoral macrozoobenthos species in the Bohemian Forest lakes.

	CN	CT	RA	PL	KA	PR	GA	LA
Ephemeroptera:								
<i>Ameletus inopinatus</i> Eaton, 1887	n	n	-	n	-	n	-	n
<i>Leptophlebia vespertina</i> (L., 1758)	x	x	n	x	n	x	n	x
<i>Siphonurus alternatus</i> (Say, 1824)	-	-	-	-	-	-	-	x
<i>Siphonurus lacustris</i> (Eaton, 1870)	n	n	-	n	n	n	-	n
Odonata:								
<i>Aeschna cyanea</i> (Müller, 1764)	n	x	n	n	n	x	n	x
<i>Aeschna juncea</i> (L., 1758)	-	n	n	-	-	m	n	x
<i>Cordulia aenea</i> (L., 1758)	-	-	-	-	n	-	-	-
<i>Enallagma cyathigerum</i> (Charpentier, 1840)	-	-	-	-	-	m	-	x
<i>Lestes sponsa</i> (Hansemann, 1823)	-	-	-	-	-	x	-	x
<i>Leucorrhinia dubia</i> (Van der Linden, 1824)	-	-	-	-	-	-	-	n
<i>Pyrrosoma nymphula</i> (Sulzer, 1776)	x	-	-	n	n	x	n	x
<i>Somatochlora metallica</i> (Van der Linden, 1825)	n	-	-	-	-	m	-	m
Plecoptera:								
<i>Amphinemura triangularis</i> (Ris, 1902)	x	x	-	x	-	-	-	x
<i>Leuctra aurita</i> Navás, 1919	x	n	-	x	-	n	-	x
<i>Leuctra braueri</i> Kempny, 1898	-	n	-	-	-	-	-	-
<i>Leuctra digitata</i> Kempny, 1899	x	x	-	x	-	x	-	x
<i>Leuctra fusca</i> (L., 1758)	n	n	-	n	-	n	-	-
<i>Leuctra handlirschi</i> Kempny, 1898	-	-	-	n	-	-	-	n
<i>Leuctra nigra</i> (Olivier, 1811)	-	n	-	n	-	-	-	n
<i>Nemoura cinerea</i> (Retzius, 1783)	n	n	n	n	-	n	-	n
<i>Nemurella pictetii</i> (Klapálek, 1900)	n	n	x	n	n	n	n	n
<i>Protonemura auberti</i> Illies, 1954	-	-	-	n	x	n	-	n
<i>Protonemura hrabei</i> Raušer, 1956	x	x	-	x	-	n	-	x
Megaloptera:								
<i>Sialis fuliginosa</i> Pictet, 1836	-	-	-	-	-	-	-	n
<i>Sialis lutaria</i> (L., 1758)	x	n	n	x	-	-	x	-
Neuroptera:								
<i>Sisyra fuscata</i> (F., 1793)	-	-	-	-	-	-	-	n
Trichoptera:								
<i>Agripnia varia</i> (F., 1793)	x	-	-	-	-	-	-	-
<i>Apatania fimbriata</i> (Pictet, 1834)	-	-	-	-	-	-	-	x
<i>Halesus</i> sp.	n	-	-	-	-	-	-	n
<i>Limnephilus griseus</i> (L., 1758)	-	x	n	-	-	-	-	-
<i>Limnephilus rhombicus</i> (L., 1758)	x	x	-	x	x	-	-	x
<i>Limnephilus stigma</i> Curtis, 1834	-	-	n	x	x	x	x	x
<i>Molanna nigra</i> (Zetterstedt, 1840)	-	-	-	-	-	x	-	-
<i>Molanmodes tinctus</i> (Zetterstedt, 1840)	-	-	-	x	-	-	-	-
<i>Mystacides azurea</i> (L., 1761)	x	x	-	-	-	-	-	-
<i>Odontocerum albicorne</i> (Scopoli, 1763)	-	x	-	-	-	-	-	x
<i>Oligotricha striata</i> (L., 1758)	-	-	n	x	x	x	x	x
<i>Phryganea bipunctata</i> Retzius, 1783	x	x	-	x	-	x	-	x
<i>Phryganea striata</i> Curtis, 1834	-	-	-	-	n	-	n	-
<i>Plectrocnemia conspersa</i> (Curtis, 1834)	m	x	-	x	-	x	-	x
<i>Polycentropus flavomaculatus</i> (Pictet, 1834)	m	x	n	x	n	x	n	x
<i>Psilopteryx psorosa</i> (Kolenati, 1860)	-	-	-	-	-	x	-	-
Total number of present species (x + n)	19	22	10	23	12	20	10	30
Total number of recent species (x + n + m)	21	22	10	23	12	23	10	31

Explanations: x – present species; n – new species, never found in former surveys; m – missing species in this survey, September 2003, but found in former surveys (NOVAK, 1996; HOLUSA, 1996, 2000; SOLDAN et al., 1998; CHVOJKA & NOVAK, 2001; SCHAUMBURG, 2000; VRBA et al., 2003a); – absent species; for lake codes, see Table 1.

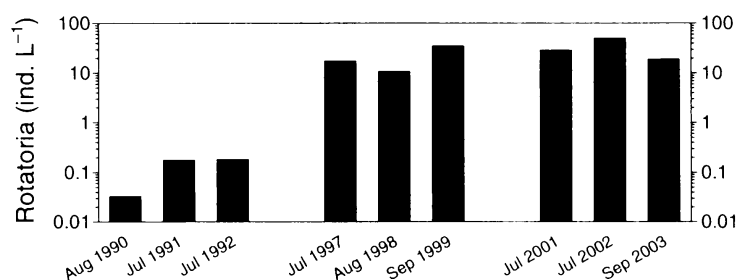


Fig. 4. Summer abundance of open-water rotifers (volume-weighted means, note logarithmic scale) in Plešné jezero (PL), 1990–2003.

*tra fusca*, *L. handlirschi*, *L. nigra*, and *N. cinerea*) to the Bohemian Forest lakes on the whole, and several other species to some of the lakes (cf. *n* in Tab. 6). In addition, for all the lakes studied, we recorded a few new species of the Odonata (*Somatochlora metallica* in CN and *Cordulia aenea* in KA), Megaloptera (*Sialis fuliginosa* in LA), Neuroptera (*Sysira fuscata* in LA), and Trichoptera (*Halesus* sp. and *Phryganea striata*; see Tab. 6).

## Discussion

### *Species composition of macrozoobenthos*

Unlike in the plankton composition, the first recent comparative survey of macrozoobenthos in all Bohemian Forest lakes showed surprising differences between the southern (RA, KA, GA) and northern (CN, CT, PL, PR, LA) lakes (cf. Fig. 1). The latter showed roughly double the species diversity of benthic insects than the former; the highest number of species occurred in the shallow LA (Tab. 6) with vast submersed vegetation. We assume that environmental conditions of the northern lakes may be more suitable overall for boreal species of macrozoobenthos; the Bohemian Forest represents the southern border of distribution of several boreal species (SOLDÁN et al., 1996). The species composition of Odonata in the lakes corresponds to that recorded in recent surveys in the region (HOLUŠA, 1996, 2000). The only species of Neuroptera was found in LA for the first time; it was known solely from KA a century ago (KLAPÁLEK, 1903). One of either species of Megaloptera have recently been found in six lakes (Tab. 6), *Sialis lutaria* was reported from CN and GA already by FRIČ & VÁVRA (1897) and KLAPÁLEK (1903), respectively. Also, BITUŠÍK & SVITOK (2006) analysed recent Chironomid assemblages of all Bohemian Forest lakes from this survey that have suggested different grouping of the lakes.

### *Plankton species composition and biomass*

Phytoplankton of the Bohemian Forest lakes are composed of a rather low number of species with a dominance of flagellates from Dinophyceae and Chrysophyceae (Tab. 3). Namely *Peridinium umbonatum*, *Gymnodinium uberrimum* and *Dinobryon* spp., which are characteristic for acidified lakes elsewhere (e.g., ALMER et al., 1978; HÖRNSTRÖM, 1999). Despite differences in acidification status and nutrient availability, the list of phytoplankton species is surprisingly similar for all the lakes. However, these differences markedly affect the relative contribution of particular species to the total phytoplankton biomass (Fig. 3). The high proportion of large *Gymnodinium uberrimum* in lakes with filtering zooplankton is probably the effect of selective grazing (Fig. 3, Tab. 5; VRBA et al., 2003b). On the other hand, this effect does not play major role in the occurrence of another large acid-tolerant dinoflagellate, *Peridinium umbonatum* (Fig. 3, Tab. 5; HAVENS & DE

COSTA, 1985). The unique phytoplankton structure and biomass in PL (Figs 2, 3) is probably caused by increased P availability in this lake (Tab. 2; cf. ROSÉN, 1981; VRBA et al., 2003a), combined with high tolerance of the dominant green coccal alga *Monoraphidium dybowskii* to increased concentration of reactive Al (HÖRNSTRÖM et al., 1995).

Generally, the ciliate taxa found in the Bohemian Forest lakes do not markedly differ from those reported from other mountain lakes. Very minute *Urotricha* sp. (resembling *U. globosa*) numerically dominated within this specialised algivorous genus. In contrast to many lakes, *U. furcata*, *U. pseudofurcata*, and large urotrichas were less abundant as were the genera *Holophrya* and *Prorodon*; even *Balanion planctonicum* occurred in negligible numbers, contrary to its dominance in many oligo- to mesotrophic lakes (e.g., MÜLLER, 1989; SALBRECHTER & ARNDT, 1994; WILLE et al., 1999). Little is known about effects of the acid stress on the ciliates so far, but a recent study of the pH effect on three *Urotricha* species (WEISSE & STADLER, 2006) suggests that the current pH in some Bohemian Forest lakes may still be lower than the pH optima of prostomatids.

Heterotrophic *Coleps* spp. were observed in the decaying phytoplankton above the bottom as usual (e.g., SALBRECHTER & ARNDT, 1994). The composition of benthic/microaerobic ciliates above the bottom of some Bohemian Forest lakes was very distinct from other water column samples; large hymenostomes, such as *Dexiotricha* sp. or *Lembadion* sp., non-symbiotic *Stentor* sp., *Trachelius ovum*, and anaerobic *Caenomorpha* sp. were observed in the late 1990s (MACEK, 2002). In the present survey, however, hymenostomes were very scarce and anaerobic odontostomatids were found only in PL. Overall, the above-bottom layers were apparently less anoxic in 2003 (cf. MACEK, 2002). A very high abundance of colpodids (*Cyrtolophosis elongata*) was found in KA. This ciliate is common in mosses and its occurrence in the surface layer was very likely due to floating sphagnum islands (cf. WEILNER, 1997). The picoplanktivorous *Halteria* sp. was the dominant ciliate in deeper layers of KA.

The common rotifers currently occurring in the open water of most Bohemian Forest lakes are *Brachionus urceolaris* "sericus", *Collotheca pelagica*, *Keratella serrulata*, *Microcodon clavus*, *Polyarthra remata*, and *Synchaeta tremula* (VRBA et al., 2003a; this study). FOTT et al. (1994) found *Microcodon clavus* and *Polyarthra remata* to be the only open-water zooplankters in CN and CT in the period 1989–1992; a similarly poor zooplankton composition has characterised RA up to the present. A major change took place in CN, where *Ceriodaphnia quadrangula* has re-appeared in the open water in 1997, where it was not detected in the period 1980–1996.

Three rotifer species of the genus *Keratella* have been recently encountered only in the recovering lakes with positive ANC at present: *K. hiemalis* (GA, LA),

*K. ticinensis* (LA), and *K. valga* (KA). All of them are known from acidified sites elsewhere: the first two from Norwegian lakes (WÆRVÅGEN & NILSSEN, 2003), the last one from drinking water reservoirs in the Jizera Mountains, the Czech Republic (Z. HOŘICKÁ, pers. commun.). It is possible that with the ongoing chemical reversal, these species will invade other Bohemian Forest lakes as well.

Although LA now has the carbonate buffering system and is fishless, the lake was remarkably poor in planktonic crustaceans in the 2003 survey, while *Ceriodaphnia quadrangula* and *Daphnia longispina* (Cladocera) were recorded previously in low numbers (VRBA et al., 2003a). The reason for poor zooplankton in LA has most probably been the phantom-midge larva, *Chaoborus obscuripes* (van der Wulp, 1859), but quantitative data on its abundance are missing. These larvae, which took over the niche of top predators after the extinction of fish, are efficient predators upon rotifers and juvenile crustaceans. After the onset of chemical reversal they cause a biological resistance to the successful biological recovery in zooplankton (NILSSEN & WÆRVÅGEN, 2003; WÆRVÅGEN & NILSSEN, 2003). A full establishment of crustacean zooplankton can be expected only after the re-appearance of the indigenous fish, i.e., brown trout, in the lake.

#### Structure of plankton biomass

The quantitative structure of pelagic food webs is very diverse in the Bohemian Forest lakes (Fig. 2) due to differences in their acidification status and nutrient loading (cf. Tab. 2). In the most acidified and oligotrophic CN, CT, and RA, bacterioplankton dominate the plankton biomass. The importance of bacteria in pelagic food webs is a typical feature of oligotrophic clear-water mountain lakes sensitive to acidification (CALLIERI & BERTONI, 1999; STRAŠKRABOVÁ et al., 2000). The predominance of bacteria in strongly acidified Bohemian Forest lakes is associated with very low P availability, caused by the combination of its low input and in-lake immobilisation (KOPÁČEK et al., 2000). Bacteria are superior competitors to phytoplankton for P under low P (CURRIE & KALFF, 1984). Furthermore, the growth of both bacteria and phytoplankton may be limited due to inactivation of their extracellular phosphatases at high concentrations of Al<sub>i</sub> (BITTL et al., 2001). Another common feature of CN, CT, and RA is very low zooplankton biomass as a result of an absence or very low abundances of planktonic Crustacea, which leads to the persisting dominance of the microbial loop in pelagic food webs of these lakes (VRBA et al., 2003a).

In contrast to the chronically acidified lakes, plankton biomass in the lakes with higher pH and/or better P availability is characterised by a low (< 30%) proportion of bacterioplankton. Phytoplankton and zooplankton were the main components of biomass in PR, KA, GA, and LA, where crustacean zooplankton survived the period of the most severe anthropogenic acidifica-

tion (FOTT et al., 1994; SCHAUMBURG, 2000). On the other hand, phytoplankton dominates the plankton of PL due to high SRP input from its catchment (Tab. 2). The prevalence of phytoplankton in a strongly acidified lake was also observed in the High Tatra Mts (FOTT et al., 1999), but it is rather unusual.

Overall, a high occurrence of filamentous bacteria is typical for all four chronically acidified lakes (Group 1) with the entire absence of crustacean herbivorous filtrators, whose grazing reduces the occurrence of filaments in most of the other lakes (VRBA et al., 2003b; this study, Tab. 4 and Fig. 2).

The levels of total plankton biomass reflect the actual nutrient status of the lakes, which is the result of a complex interplay between pH and phosphorus and aluminium concentrations in tributaries and lake water (for details, see VRBA et al., 2006). Natural P immobilisation by Al<sub>part</sub> has been recently recognised as a common and important mechanism in any water body with elevated Al input and a pH gradient between its inlet and outlet (KOPÁČEK et al., 2000). During the 2000–2005 period, this mechanism immobilised ~24% of annual TP input to CT in lake sediments (KOPÁČEK et al., 2006a) but as much as ~50% of the relatively high TP input in PL (KOPÁČEK et al., 2004, 2006b). Consequently, the severest P limitation of the plankton occurred in the oligotrophic acidified lakes (CT, CN, RA), as well as in the mesotrophic PL. On the other hand, the combination of moderate TP with low Al<sub>T</sub> inputs in PR likely released its plankton from such severe P stress (cf. the lower seston C : P ratio in PR; Tab. 2). We assume that the interplay of both Al and P inputs with in-lake alkalinity generation (pH gradient) seriously affect the stoichiometry of resources and consumers, as well as the whole ecosystems (STERNER & ELSER, 2002). Hence, the stoichiometry may determine structure and dynamics of the lake plankton, overall food web structure, and even the potential for biological recovery of the studied lakes from acid stress.

#### Biological recovery

Reversal of water chemistry has been well documented in each Bohemian Forest lake after >80% and 35% reduction in S and N deposition, respectively (KOPÁČEK et al., 2001, 2002). The most significant change in chemistry of the lakes has been the rapid decrease in Al<sub>T</sub> concentrations (by ~50% during the last two decades; cf. VESELÝ et al., 1998; Tab. 2, this study). In theory, such a large decrease in atmospheric deposition should release the ecosystem from acid stress. The biotic response of the lake ecosystems, however, has been lagging behind the chemical recovery.

This study has shown further progress in reversal of lake water chemistry, as well as the evidence of biological recovery in most Bohemian Forest lakes compared to the 1999 survey (VRBA et al., 2003a). In particular, the current chemical status of KA and PR (Tab. 2) and

## Biological recovery of Bohemian Forest lakes

the increase in the zooplankton biomass in the whole Group 2 are remarkable (Fig. 2).

OLSSON & PETTERSSON (1993) reviewed contradicting hypotheses concerning oligotrophication during acidification. The internal P cycle and P availability for phytoplankton in the Bohemian Forest lakes remains obviously disrupted as a consequence of acidification (KOPÁČEK et al., 2000; BITTL et al., 2001). During this survey, we did not observe any significant general change in phytoplankton biomass in comparison with the 1999 data. We could not confirm the increasing trend in phytoplankton biomass following chemical reversal which was previously reported for PL (from 1994 to 1998, VRBA et al., 2003a, 2006). This is most likely the result of complex conditions accompanying current changes in lake water chemistry. Although  $Al_i$  concentration decreased, suggesting a possible reduction of its inhibitory effect on extracellular phosphatases (BITTL et al., 2001), P immobilisation by  $Al_{part}$  still remains very important at the current pH in PL (KOPÁČEK et al., 2004, 2006b).

The recovery of phytoplankton species diversity and a shift towards circum-neutral assemblages have been observed following the chemical reversal of lakes from acidification in Europe and Northern America (e.g., HÖRNSTRÖM, 1999; FINDLAY, 2003). In the present study, however, we have not observed any shift in the phytoplankton species composition accompanying the chemical reversal in any of the Bohemian Forest lakes, compared to the previous data (VRBA et al., 2003a). The majority of phytoplankton species living in the harsh environment of the acidified headwater lakes is probably able to adapt to the changing pH and related factors. In the most intensively studied CN, the qualitative phytoplankton structure has remained surprisingly stable despite drastic changes in lake water chemistry within the last seven decades (FOTT et al., 1994; VRBA et al., 2003a). Most likely, we cannot expect significant changes in phytoplankton diversity of the lakes even with improving lake water chemistry in the future, and the response of the assemblage will be limited to changes in biomass and relative proportion of species due to the varying importance of bottom-up and top-down control.

We could not as yet confirm a trend in the recovery of the ciliate community in any of the studied lakes except in CN. The occurrence of other than prostomatid taxa in this lake was surprising, in particular that of *Rimostrombidium velox*, a typical ciliate of large lakes (Tab. 4). Also, the present numbers of prostomes in CT and PL are their highest epilimnetic abundances ever observed (cf. MACEK, 2002). On the other hand, ciliate (in particular oligotrich) numbers in PR and LA were lower in 2003 than in 1999 (Tab. 4). This is not so surprising because, except for some seasonal peaks, bacterivorous and/or omnivorous fine filter-feeding ciliates were quite scarce in the Bohemian Forest lakes (MACEK, 2002).

The first signs of recovery in zooplankton (with respect to the early 1990s) are the re-appearance of *Ceriodaphnia quadrangula* to CN and the increase in abundance of rotifers in PL (Fig. 4). Despite the two-order-of-magnitude increase in rotifer abundance, the share of zooplankton biomass to the total pelagic biomass remains very low in PL (Fig. 2). The obstacle to a recovery of crustacean zooplankton in LA seems to be the predatory impact of *Chaoborus* larvae.

Overall, this survey is the first evidence of the return of some insect species that were extinct during acidification of the lakes and still absent a decade ago (cf. Tab. 3 in VRBA et al., 2003a). In particular the two mayflies (*A. inopinatus* and *S. lacustris*), although originally known from most of the Czech lakes (CN, CT, PL, PR, LA), has newly re-appeared in all of them, contrary to the late 1990s (VRBA et al., 2003a). Similarly, many additional species of Plecoptera have returned to some lakes (cf. CT, PL, PR, LA in Tab. 6, this study, with Tab. 3 in VRBA et al., 2003a). Of the 47 species of Trichoptera ever found in the Bohemian Forest lakes and adjacent running waters, only six species (*Agripnia varia*, *Apatania fimbriata*, *Halesus* sp., *Limnephilus griseus*, *Limnephilus stigma*, and *Phryganea striata*) might represent those recovering from acid stress, whereas the other listed in Table 6 probably survived the period of lake acidification.

On the other hand, some invertebrate top predators currently present in the lakes due to the absence of fish, such as water bugs (*Glaenocorisca propinqua* (Fieber, 1860), Heteroptera; M. PAPÁČEK, pers. commun.), phantom midges (*Chaoborus obscuripes*, Diptera), Odonata, and Megaloptera (cf. Tab. 6) larvae, may prevent full recovery of zooplankton.

This study suggests that the biological recovery has been delayed for almost two decades after their chemical reversal, similarly to other acidified lake districts (e.g., SKJELKVALE et al., 2003; JEFFRIES et al., 2003; WRIGHT et al., 2005). YAN et al. (2003) suggested conceptual frameworks for the biological recovery of aquatic ecosystems from acid stress. They considered ecological, i.e., biotic responses, such as availability and/or dispersal of colonists, population size and growth, or community interactions. We can presume that the unassisted dispersal rate of extinct species will be very low for the lakes studied due to their remoteness. We further assume that both population growth and food web structure reflect current resource and consumer stoichiometry (see above, STERNER & ELSER, 2002). We also suppose that ecosystem resistance (see above, NILSSEN & WÆRVÅGEN, 2003; WÆRVÅGEN & NILSSEN, 2003) may prevent full biological recovery of the lakes until fish return. Further research of these unique ecosystems from acid stress may help in both understanding the mechanisms and verifying the conceptual frameworks of biological recovery from acid stress.

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## Phytoplankton of a mountain lake (Ľadové pleso, the Tatra Mountains, Slovakia): Seasonal development and first indications of a response to decreased acid deposition

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**Abstract:** A one-year study of the phytoplankton of remote mountain lake Ľadové pleso (2057 m. a.s.l.), situated in the High Tatra Mountains, was carried out in 2000–2001. Seasonal development of the species structure and depth distribution of biomass, as well as chlorophyll-*a* concentrations, and chemical characteristics of the lake water were examined. The phytoplankton was made up of a low number of nanoplanktonic unicellular species, mainly flagellates from Cryptophyceae and Chrysophyceae. *Plagioselmis lacustris*, *Cryptomonas* cf. *erosa* and *Ochromonas* spp. were the most important with regards to both abundance and biomass. The phytoplankton showed a marked seasonal development. During most of the ice-covered period, abundances of phytoplankton remained very low. In regard to the vertical distribution, both species composition and total biomass were clearly stratified during most sampling dates. The amount of chlorophyll-*a* per unit biovolume varied considerably, and significantly higher values were found on sampling dates with complete or partial ice cover. Our data on pH and phytoplankton were compared with a previous study in 1990–1991 in order to evaluate possible changes associated with the decrease of acid deposition. The extent of episodic acidification of the lake water has diminished both in time and space, and lake water pH has slightly increased. We observed a shift in species composition, and an important increase of total phytoplankton abundance. This could be considered as a first sign of biological response to changing chemical conditions.

**Key words:** Phytoplankton, high mountain lake, community structure, seasonal development, vertical distribution, pH, episodic acidification, Slovakia.

### Introduction

Systematic limnological studies of glacial lakes in the High Tatra Mountains (Mts) started in the 1980s, and were primarily aimed at quantifying the effects of acid deposition on these remote ecosystems (STUHLÍK et al., 1985). According to their acidification status, the lakes were divided into three categories (FOTT et al., 1994; KOPÁČEK & STUHLÍK, 1994). The first extensive study focusing on phytoplankton in lakes of the High Tatra Mts was done by JURÍŠ & KOVÁČIK (1987). A decrease of phytoplankton species richness with increasing altitude and decreasing pH was observed by LUKAVSKÝ (1994). Due to their difficult accessibility, the research of these lakes was mostly limited to single observations. The only two studies focusing on seasonal development of phytoplankton are presented by DARGOCKÁ et al. (1997), and FOTT et al. (1999). Due to the significant decrease of acid atmospheric deposition,

mountain lakes in many acid sensitive regions have recently exhibited pronounced changes in their chemical characteristics resulting in a partial reversal from anthropogenic acidification (MOSELLO et al., 2002; VRBA et al., 2003). In the High Tatra Mts, lake water recovery from acidification started in the early 1990s, and over the last decade, the proportion of acidified lakes (ANC < 20  $\mu\text{eq L}^{-1}$ ) decreased from 37% to 20% (KOPÁČEK et al., 2006).

Since the 1980s, Ľadové pleso was included in many regional surveys, which provided information on lake water chemistry and biology of a large set of lakes covering period from the peak of acid deposition to the beginning of lake recovery (STUHLÍK et al., 1985; KOPÁČEK & STUHLÍK, 1994; KOPÁČEK et al., 2004, 2006). Although Ľadové pleso was classified as a non-acidified one (pH > 6.2, ANC > 25  $\mu\text{eq L}^{-1}$ , concentration of  $\text{Ca}^{2+}$  > 100  $\mu\text{eq L}^{-1}$ ), the lake water chemistry was also affected by acid deposition, which caused an important



decrease in its preacidification acid neutralising capacity (STUHLÍK et al., 1985). The first detailed seasonal study of Ladové pleso was performed in 1990–1991. Upper layers of the water column were seriously influenced by episodic acidification that occurred during melting periods (DARGOCKÁ et al., 1997; KNESLOVÁ et al., 1997). Ten years later, this lake was chosen as the Tatra Mts key site of the research project EMERGE, which enabled a complex investigation of annual cycle of lake water chemistry and biota.

This study describes seasonal development and vertical distribution of chemical characteristics and phytoplankton of Ladové pleso in 2000–2001. Our objective was to compare gathered data with the previous study of DARGOCKÁ et al. (1997), in order to estimate possible impact of decreased atmospheric deposition on this lake.

### Material and methods

Ladové pleso (49°18'41" N, 20°16'29" E) is located in the Velká Studená dolina valley on the southern slope of the High Tatra Mts (Slovakia). The lake is 18 m deep, its area is 1.7 ha, and it has no surface inflow and outflow (seepage lake). The lake is oligotrophic, but the amount of plankton can reach quite high values in comparison with the other Tatra lakes probably due to its seepage character (FOTT et al., 1987). The main morphometrical and chemical characteristics are summarised in KOPÁČEK et al. (2004).

Sampling was carried out by a Van Dorn sampler from the sampling point above the deepest part of the lake, both under the ice cover and during the ice free period. Samples for chemical analyses were taken biweekly, vertical profiles of phytoplankton and chlorophyll-*a* concentrations were studied 9 times from September 2000 to October 2001. The sampling depths were 0–3–5–8–13 m and 0.5 m above bottom. During the winter cover period biological analyses of surface and bottom samples were also done on four other dates (15 March, 6 April, 11 May and 20 June). Vertical stratification of temperature, pH, specific conductivity, and concentration of dissolved oxygen was measured biweekly during the whole study period using the Multiprobe H20 and data logger Surveyor 3 (Hydrolab, USA).

Laboratory analyses of pH and acid neutralising capacity (ANC) were performed on the automatic titrator TIM 900 (Radiometer, France) using the measuring electrode pHG201 (Radiometer, France) and the reference electrode (Russel, UK). The measuring system was always calibrated by two buffers (pH 4 and 7), and the samples were equilibrated to laboratory conditions prior analysis. ANC was determined by Gran titration (MACKERETH et al., 1978). The phytoplankton samples were fixed immediately by acid Lugol's solution. The counting was done after presedimentation (5×) in Utermöhl's sedimentation chambers on an inverted microscope (Nikon Diaphot), and cell volumes were estimated by shape assimilation to known geometric forms (STRÁŠKRABOVÁ et al., 1999). Chlorophyll-*a* concentrations were determined fluorometrically (TD-700 laboratory fluorometer, Turner Designs, USA). 50 mL of sample was gently filtered through glass fibre filters (Whatman GF/F, UK), the filters were stored in well closed glass

tubes filled with 90% acetone and kept in freezer until analysis. Extraction was performed after addition of methanol in 65°C according to PECHAR (1987). The whole procedure is described in FOTT et al. (1999).

Data on long-term trends (1980–2004) of pH and ANC come from STUHLÍK et al. (1985), KOPÁČEK & STUHLÍK (1994), STUHLÍK & KOPÁČEK (unpubl. data), and KOPÁČEK et al. (2006). Autumn values from the lake surface were used for evaluation of long-term trends.

### Results

#### *Physical and chemical characteristics*

The ice cover lasted from the end of November to the middle of July, and reached its maximum thickness (2.5 m) in spring. The melting period started in May, in early July, about half of the lake surface was already free of ice. The summer stratification developed at the beginning of August and lasted approximately one month. The concentration of dissolved oxygen in upper layers of the water column dropped exceptionally below 10 mg L<sup>-1</sup>. During winter stratification, the bottom layer was characterised by a gradual decrease in oxygen concentrations. This period of oxygen depletion was finished by the mixing of the whole water column, which started in early July. The conductivity was slightly higher than 15 μS cm<sup>-1</sup> during most of the ice-cover period. The lowest values were observed in surface layers towards the end of the ice melt. pH was also strongly influenced by melting of the winter cover, which caused episodic acidification of upper layers of the water column. In June, surface values measured in laboratory dropped below 6. The lowest value (5.14) was recorded on 20 June. ANC showed a similar pattern, and reached its minimum value (−3 μeq L<sup>-1</sup>) on the same sampling date as pH. The temporal and spatial extent of episodic acidification is presented in Fig. 1.

#### *Phytoplankton species composition*

The phytoplankton of Ladové pleso was dominated by unicellular flagellates from Cryptophyceae and Chrysophyceae. The species contributing most to the total phytoplankton biomass were *Plagioselmis* (formely *Rhodomonas lacustris*, *Cryptomonas* cf. *erosa*, and *Ochromonas* spp. A comparison of phytoplankton species list from the surveys of 1990–1991 (DARGOCKÁ et al., 1997) and 2000–2001 is in Table 1. We found a total of 16 planktonic species. Some littoral or benthic species, especially diatoms, were also occasionally found in samples (e.g., *Cymbella minuta*, *Achnanthes lapidosa*, *Gomphonema montanum*, *Pinnularia borealis*). Cysts of the snow alga *Chlamydomonas* cf. *nivalis* were present in the lake water during most of the study period and originated from blooms, which form regularly on the surface of Ladové pleso winter cover. Their abundances in lake water samples were consequently highest at the end of the ice break.

## Seasonal development of phytoplankton in high mountain lake

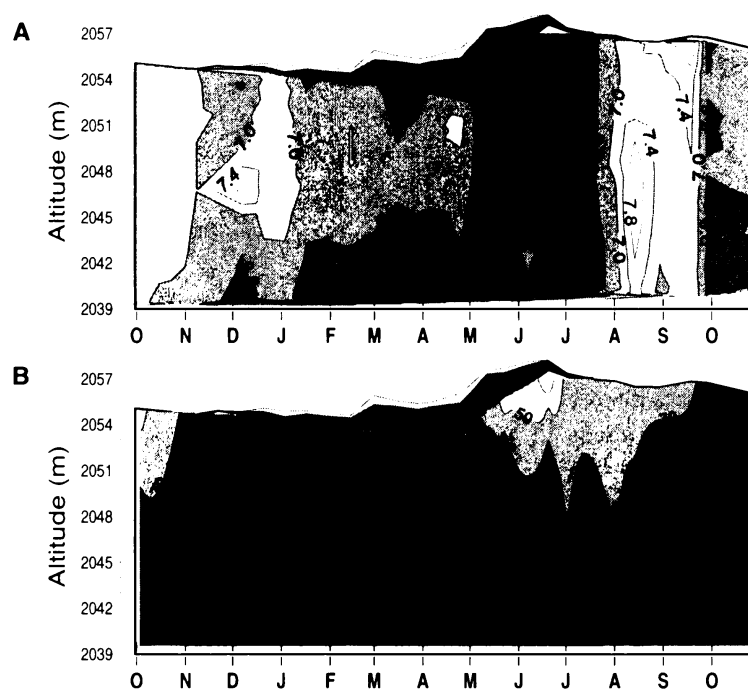


Fig. 1. Spatial and temporal distribution of pH multiprobe measurements (A), and acid neutralising capacity ( $\mu\text{eq L}^{-1}$ ) (B) in Ladové pleso in 2000–2001.

Table 1. Phytoplankton species composition of Ladové pleso in 1990–1991 (DARGOČKA et al., 1997) and 2000–2001 (this survey).

Species	1990–1991	2000–2001
Cyanobacteria:		
<i>Chroococcus dispersus</i> (Keissler) Lemmerman	+	+
<i>Pseudanabaena</i> sp.	+	+
Dinophyceae:		
<i>Gymnodinium</i> sp.	+	+
<i>Katodinium bohemicum</i> (Fott) Litvinenko	+	+
<i>Peridinium umbonatum</i> Stein	+	+
Cryptophyceae:		
<i>Cryptomonas</i> cf. <i>erosa</i> Ehrenberg	+++	+++
<i>Plagioselmis lacustris</i> (Pascher et Ruttner) Javornický	+	+++
Chrysophyceae:		
<i>Mallomonas akrokomos</i> Ruttner	+	++
<i>Ochromonas</i> sp. (large)	++	+++
<i>Ochromonas</i> sp. (small)	++	++
Bacillariophyceae:		
<i>Fragilaria</i> cf. <i>tenera</i> (W. Smith) Lange Bertalot	-	+
Chlorophyta:		
<i>Ankistrodesmus</i> sp.	+	+
<i>Koliella longiseta</i> (Vischer) Hindák	+++	+
<i>Monoraphidium</i> sp.	+	+
<i>Scenedesmus</i> sp.	-	+
<i>Tetraedron minimum</i> (A. Braun) Hansgirg	+	+
Euglenophyceae:		
<i>Trachelomonas cylindrica</i> Ehrenberg	+	-

Key: +++ dominant species; ++ important species; + present species; - species not found.

*Seasonal development and vertical distribution of phytoplankton abundances and biomass*

The range of phytoplankton biomass as biovolume in

all samples was  $0-855 \text{ mm}^3 \text{ m}^{-3}$  biovolume. Both abundances and biovolume showed a marked seasonal development with two peaks, one in autumn-early winter

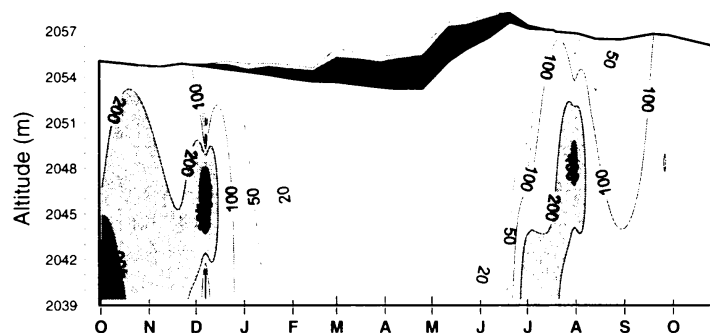


Fig. 2. Spatial and temporal distribution of phytoplankton biovolume in Ladové pleso in 2000–2001 ( $\text{mm}^3 \text{m}^{-3}$ ).

and a second just after the melting of the ice cover (July–August) (Fig. 2). The maximum recorded abundance of phytoplankton was  $\sim 7000 \text{ cells mL}^{-1}$  in December 2000. Vertical profiles of phytoplankton abundances in 2000–2001 are compared to the 1990–1991 data set (DARGOČKÁ et al., 1997) in Fig. 3. Phytoplankton biomass remained very low during most of the ice-cover period and the lowest values were reached in May. Concerning vertical distribution, phytoplankton was clearly stratified during most sampling dates. Biomass peaks were found exclusively in the deeper layers, but their position changed with the season (Fig. 2).

As regards to contribution of particular species to the total phytoplankton abundance and biomass, the most conspicuous deep water peaks were formed by *Plagioselmis lacustris*, and *Ochromonas* spp. The seasonal and depth distribution of important species (*Plagioselmis lacustris*, *Cryptomonas* cf. *erosa*, *Ochromonas* spp., and *Mallomonas akrokomos*) did not show a similar pattern indicating their different ecological requirements (Fig. 4).

#### Chlorophyll-*a* concentration and specific chlorophyll content of phytoplankton cells

The range of chlorophyll-*a* concentrations was  $0\text{--}18.6 \text{ mg mm}^{-3}$ . The general distribution pattern was similar to phytoplankton biomass (Fig. 5), but a closer examination revealed an allometric relationship between these two variables (Fig. 6):

$$\begin{aligned} \text{Chl-}a \text{ concentration } [\text{mg m}^{-3}] &= \\ &= 7.76 (\text{biovolume})^{0.65} [\text{mm}^3 \text{L}^{-1}] \end{aligned}$$

The amount of chlorophyll-*a* per unit of biovolume (specific chlorophyll-*a* content of cells) was therefore very variable ( $5.6\text{--}40.6 \mu\text{g mm}^{-3}$ , mean:  $18.7 \mu\text{g mm}^{-3}$ ). The lowest values were associated with a hypolimnetic peak of *Plagioselmis lacustris* on 30 September 2000. However, the peak of chlorophyll-*a* in December 2000, formed by the same species under thin layer of clear ice was not accompanied by a proportional increase of phytoplankton biovolume, which resulted in a fourfold increase in the specific chlorophyll content (Fig. 7). On the same date in 5 m depth, we recorded maximum value for the whole data set. Minimal as well as maximal specific chlorophyll-*a* content were thus as-

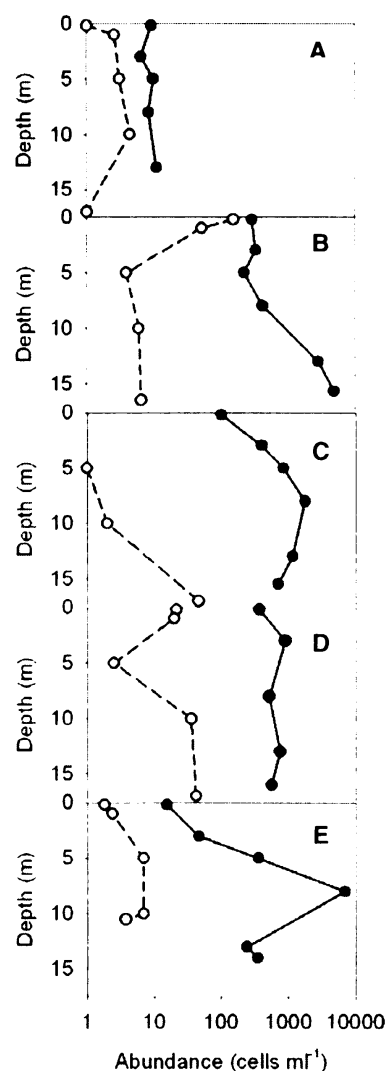


Fig. 3. Comparison of phytoplankton abundances ( $\text{n mL}^{-1}$ ) in the vertical profile of Ladové pleso during the surveys of 1990 (dotted lines, DARGOČKÁ et al., 1997), and 2000–2001 (solid lines). A – 20.II.1990, 14.II.2001; B – 24.VI.1990, 2.VII.2001; C – 27.VII.1990, 1.VIII.2001; D – 28.IX.1990, 28.IX.2001; E – 13.XII.1990, 7.XII.2000.

sociated with the predominance of *Plagioselmis lacustris*. Significantly higher values ( $P < 0.001$ ) were found on sampling dates with complete or partial ice cover.

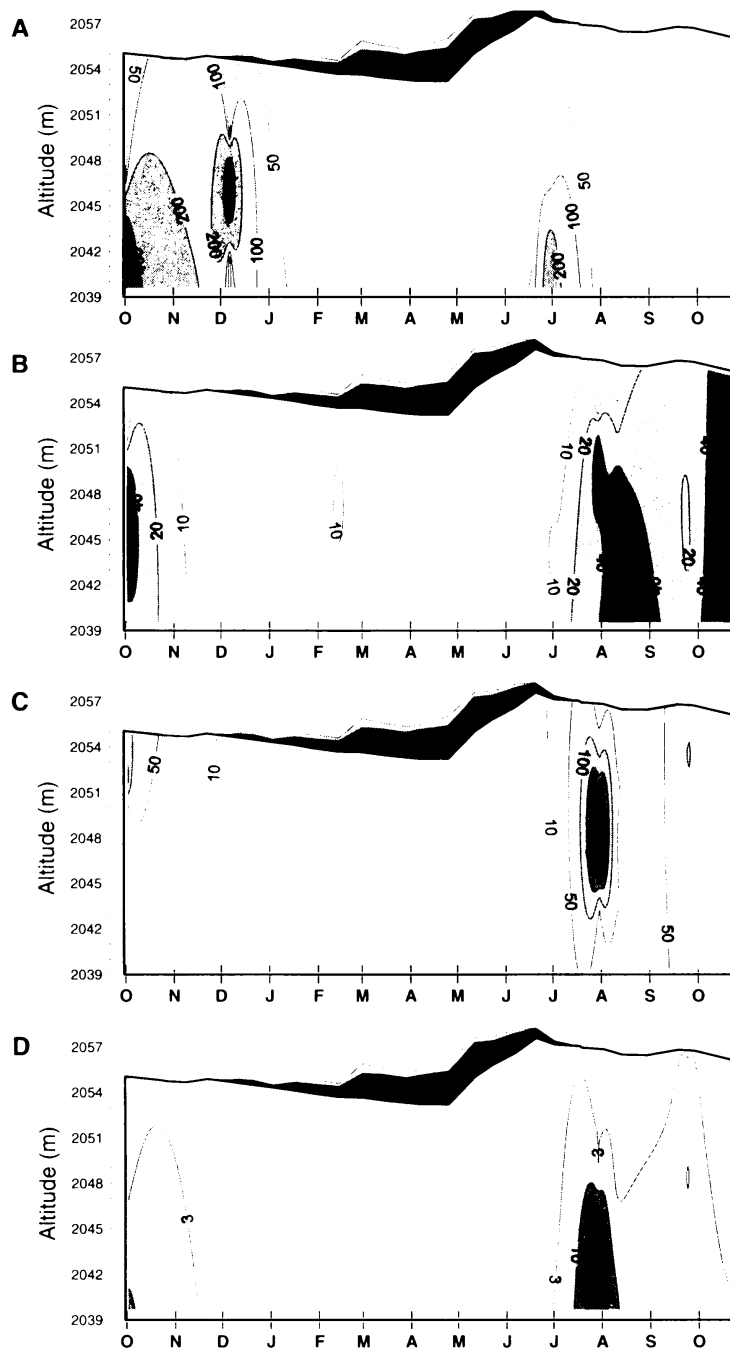


Fig. 4. Spatial and temporal distribution of biovolumes of important phytoplankton species in Ladové pleso in 2000-2001 ( $\text{mm}^3 \text{m}^{-3}$ ). A - *Plagioselmis lacustris*, B - *Cryptomonas cf. erosa*, C - *Ochromonas* spp., D - *Mallomonas akrokomos*.

When taking into account whole-lake average (volume weighted means), the specific content of chlorophyll-*a* also showed a clear seasonal trend (Fig. 7). With regards to vertical distribution, upper layers of the water column (0-3-5 m samples) were characterised by slightly higher specific content of chlorophyll-*a* ( $P < 0.05$ ) in comparison with deep layers (8-13 m - bottom samples).

#### Long-term trends

Long-term trends (1980-2004) in Ladové pleso autumn-

nal surface pH and ANC are shown in Fig. 8. Both characteristics exhibited a significant increase, indicating a response of the lake water chemistry to decreased acid deposition in the High Tatra Mts.

#### Discussion

##### *Species composition, seasonal development and vertical distribution of phytoplankton*

The phytoplankton of Ladové pleso is composed of a low number of species with the predominance of nanoplank-

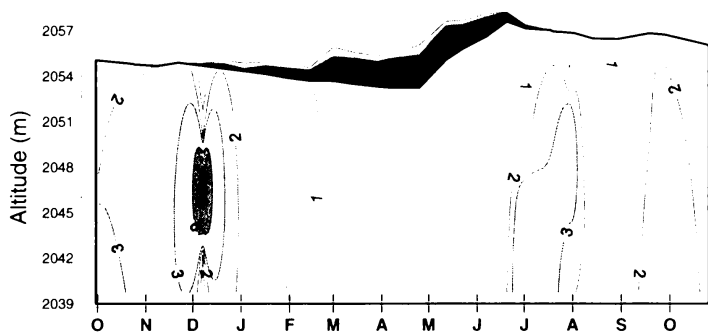


Fig. 5. Spatial and temporal distribution of chlorophyll-a concentrations in Ladové pleso in 2000–2001 ( $\text{mg m}^{-3}$ ).

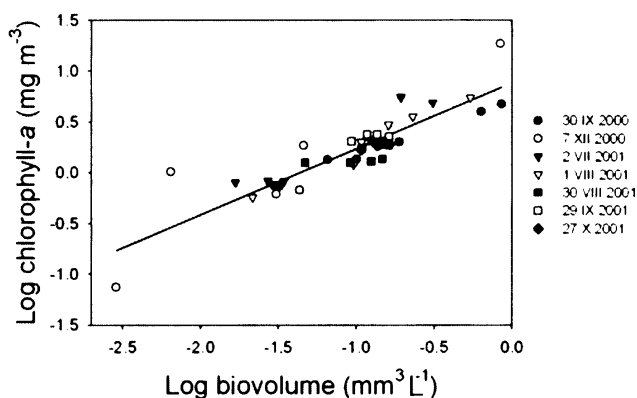


Fig. 6. Relationship between log chlorophyll-a concentrations ( $\text{mg m}^{-3}$ ) and log phytoplankton biovolume ( $\text{mm}^3 \text{L}^{-1}$ ) in Ladové pleso in 2000–2001. Solid line, linear regression;  $R^2 = 0.79$ ;  $P < 0.0001$ .

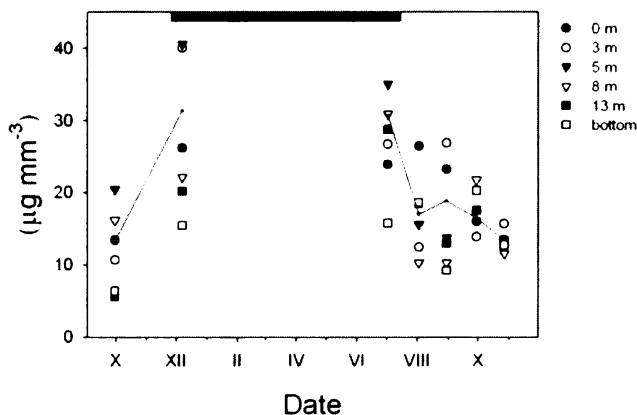


Fig. 7. Seasonal changes in chlorophyll-a concentrations per unit phytoplankton biovolume in Ladové pleso in 2000–2001 ( $\mu\text{g mm}^{-3}$ ). The line represents weighted means with respect to lake morphometry. Ice cover indicated above.

tonic flagellates, as is characteristic for oligotrophic high mountain lakes (LUKAVSKÝ, 1994; PUGNETTI & BETTINETTI, 1999). Cells of *Plagioeselmis* (formerly *Rhodomonas*) in Ladové pleso were lacking sharp hyaline tails, so they could be unambiguously determined as *Plagioeselmis lacustris* (Pascher et Ruttner) Javornický (JAVORNICKÝ, 2003). The two freshwater species of the genus *Plagioeselmis* are frequently reported in a

wide range of different habitats, and can be classified as *r*-strategists with affinities for higher pH (KLAVENESS, 1988). In Ladové pleso phytoplankton, these flagellates are found together with another cryptophyte, *Cryptomonas cf. erosa*. This association is quite common in large clear water lakes, for example in Finland (LEPISTÖ & HOLOPAINEN, 2003). The genus *Ochromonas* is known to be potentially mixotrophic, and this strategy is optimal in oligotrophic conditions (ISAKSSON, 1998). Mixotrophic species therefore often prevail in mountain lakes (e.g., PUGNETTI & BETTINETTI, 1999). *Malomonas akrokomos* has been reported from both oligotrophic and eutrophic waters, predominantly in winter (STARMACH, 1985). This species was already observed in Ladové pleso and other alpine lakes in the High Tatra Mts by JURIŠ & KOVÁČIK (1987) and by LUKAVSKÝ (1994). *Fragilaria tenera* is a widely defined species with affinities to *Fragilaria nanana* and *Fragilaria capucina* (KRAMMER & LANGE-BERTALOT, 2004). All these closely related species can be found in plankton of oligotrophic high latitude or mountain lakes all over the world including some lakes in the High Tatra Mts (e.g., JURIŠ & KOVÁČIK, 1987; HÖRNSTRÖM, 2002).

The average level of phytoplankton biomass in Ladové pleso did not exceed values established for oligotrophic conditions (LAMPERT & SOMMER, 1997). The biomass was close to zero during most of the period of thick winter cover. Even though we did not measure light intensity under the ice, studies of mountain lakes with similar thickness and structure of winter cover have shown, that no light can penetrate into the water column under such conditions (FELIP et al., 1999). A heavy snow accumulation on the ice cover, which is a regular phenomenon in the case of Ladové pleso, has an especially drastic effect on light transmittance (BOLENGA et al., 1996). The lack of light during the ice cover period is probably the main reason why the maximum of phytoplankton is associated with ice free period in the most intensively studied remote mountain lakes in Europe (CATALAN et al., 2002). A contrasting pattern with phytoplankton maximum under the ice cover of maximum thickness was observed in the high mountain lake Paione Superiore, suggesting their possible shift to heterotrophic metabolism (PUGNETTI & BETTINETTI, 1999). In Ladové pleso, such a shift did not occur, and bacteria, heterotrophic nanoflagellates

## Seasonal development of phytoplankton in high mountain lake

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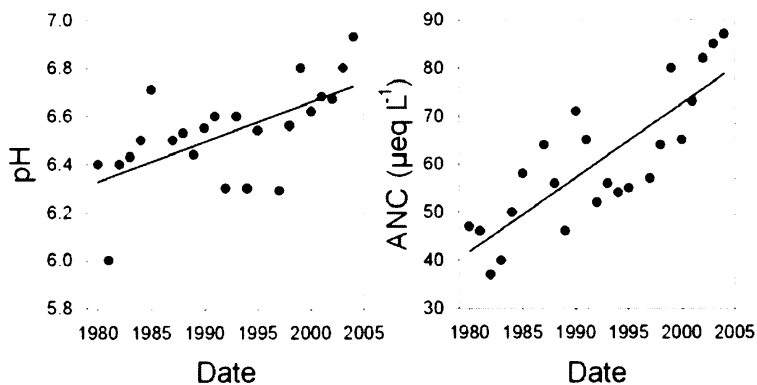


Fig. 8. Long-term trend in pH and acid neutralising capacity (ANC,  $\mu\text{eq L}^{-1}$ ) of Ladové pleso (autumnal surface samples). Solid line, linear regression;  $R^2 = 0.38$ ,  $P < 0.01$  for pH;  $R^2 = 0.68$ ,  $P < 0.0001$  for ANC.

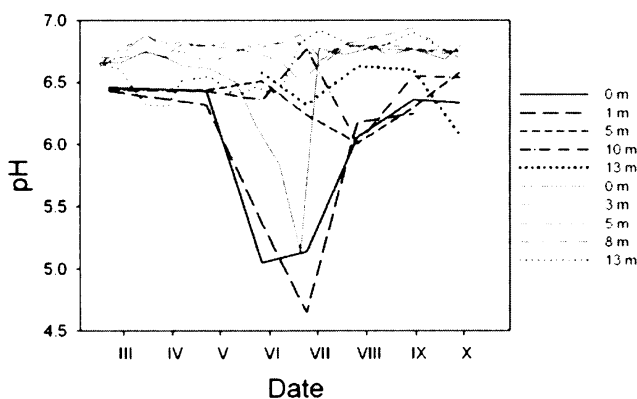


Fig. 9. Temporal and spatial extent of episodic acidification of Ladové pleso in 1990 (black lines) and 2001 (grey lines).

and ciliates formed the microbial assemblage during most of the winter cover period (NEDBALOVÁ et al., unpubl. data).

The seasonal development of phytoplankton in Ladové pleso was characterised by rapid and quite high biomass peaks occurring during short periods favourable for growth. The most important changes of environmental conditions are caused by the melting of the winter cover. Besides substantially improved light availability, nutrients and particulate organic matter accumulated in the snow and ice pack are relatively quickly released into the water column (CATALAN, 1992). This phenomenon is extremely important in Ladové pleso, as all material accumulated during the winter period in both catchment and on lake surface remain in the water column due to the seepage character of the lake. These changes resulted in a rapid restoration of phytoplankton in July. From our results, we could not infer any pattern of seasonality, but the fundamental influence of physical conditions is apparent. It is probable that the interannual variability of external forcing (especially the timing of the winter cover melting) have profound effects on seasonal development of phytoplankton in Ladové pleso (HINDER et al., 1999).

Concerning vertical distribution, high deep summer maxima, as well as one maximum of biomass under thin clear ice were found (Fig. 2). Their occurrence is a

pattern typical for many high mountain lakes (CATALAN & CAMARERO, 1990; CATALAN et al., 2002). The position of the maxima in the water column changed, suggesting active vertical migration of flagellates, which enables them to find optimal depth for growth under changing light and nutrient conditions. The light availability seems to be the crucial factor driving distribution of phytoplankton in Ladové pleso during both periods of stratification. However, the growth of phytoplankton in upper layers could also be influenced by episodic acidification.

The comparison of temporal and vertical distribution of particular species indicates differences in their ecological requirements (Fig. 4). Due to the combined effect of its growth characteristics of a  $r$ -strategist, and its obvious ability to efficiently exploit very low light intensities, *Plagioselmis lacustris* formed the most conspicuous peaks. This species is frequently recorded to increase when other populations are declining, as if a temporary niche were opening (KLAVENESS, 1988). In Ladové pleso, *Plagioselmis lacustris* was able to react very quickly to slightly increased light availability following partial ice break-up at the end of June. Its following decline could be explained by outcompetition by slowly growing large cells of *Ochromonas* spp. and *Cryptomonas* cf. *erosa*, which may be better competitors for nutrients in conditions of improved light availability during the ice-free period. However, the population of the copepode *Arctodiaptomus alpinus* could also have effect on phytoplankton quality and quantity (filtration, nutrient recycling).

#### Specific chlorophyll-*a* content

Studies on seasonal and vertical patterns of phytoplankton light adaptation were initiated by TILZER & SCHWARZ (1976). Their result showed that the highest chlorophyll-*a* to wet weight ratios (specific chlorophyll-*a* content) can be expected in plankton living under low-light conditions, either below the ice cover, or in deep layers of the lake in the summer. A physiological adaptation of cells reflecting actual underwater light climate can be thus often the cause of changes in the amount of chlorophyll per unit biovolume. However, this variable can also be influenced by species composition of phy-

toplankton, because of its dependence on cell size, life form and taxonomic composition (FELIP & CATALAN, 2000).

The range of the specific chlorophyll-*a* content of Ladové pleso phytoplankton ( $6\text{--}41\ \mu\text{g mm}^{-3}$ ) was shifted to slightly higher values in comparison with data reported in literature (TILZER & SCHWARZ, 1976; FELIP & CATALAN, 2000). Data from February to June 2001 were excluded from the analysis, because chlorophyll-*a* concentrations were close to or below the detection limit of the method used.

In the High Tatra Mts, the specific chlorophyll-*a* content was studied in detail in two lakes above timberline by FOTT et al. (1999), who found both higher values during the ice-covered periods, and immediate reaction of chlorophyll-*a* concentration to a fast change of the light conditions. In Ladové pleso, the most striking change was the up to sevenfold increase of the specific chlorophyll-*a* content in the December 2000 samples in comparison with the September 2000 samples (Fig. 7). Peaks of biomass were formed on both dates by *Plagioselmis lacustris*, indicating a physiological character of this change, and therefore a very high efficiency of light adaptation by varying the chlorophyll-*a* content of cells in this species. This is a further example that demonstrates the necessity of caution, when using the chlorophyll-*a* concentrations as a measure of phytoplankton biomass.

In contrast to significant dependence on the season (presence/absence of the ice cover), the specific chlorophyll-*a* content of Ladové pleso phytoplankton did not show an increase with sampling depth on most sampling dates, as it is typically found in deep lakes and reservoirs during periods of thermal stratification (e.g., TILZER & SCHWARZ, 1976). The absence of this pattern could be explained by different species composition of phytoplankton on vertical profiles, and by considerably lower depth of Ladové pleso. In lake Redó, differences in the taxonomic composition of phytoplankton, and therefore varying cell sizes and predominant life forms, explained most of the variability in the chlorophyll-biovolume relationship (FELIP & CATALAN, 2000).

#### *Comparison of 1990–1991 and 2000–2001 surveys and long-term trends*

The detailed survey of Ladové pleso in 1990–1991 (DARGOCKÁ et al., 1997; KNESLOVÁ et al., 1997) offers a unique opportunity to compare the seasonal development of chemical and biological characteristics of lake water to the 2000–2001 data. This comparison focused on the episodic acidification enables an alternative approach to estimate possible effects of the decrease of acid deposition during the 1990s on this non-acidified lake, than is provided by regular autumnal surveys.

Snowmelt-induced episodic acidification associated with changes in water chemistry is commonly reported from mountain streams, and has frequently profound effects on their biota (e.g., LEPORI et al., 2003). Stud-

ies reporting this phenomenon from mountain lakes are scarcer (STODDARD, 1995). Ladové pleso belongs to the category of non-acidified lakes, and its partial and episodic acidification is the results of its seepage character, as the acid water from melting snow and ice cannot leave immediately the lake basin, and it is continuously neutralised during the mixing of the water column. The comparison of the 1990–1991 (DARGOCKÁ et al., 1997) and 2000–2001 data shows that the extent of episodic acidification has diminished both in time and space, indicating a response to decreased acid deposition (Fig. 9). This trend is further confirmed by the long-term increases of autumnal surface pH and acid neutralising capacity (Fig. 8), and the data are in good agreement with general recent trends of Tatra Mts lakes chemistry (KOPÁČEK et al., 2006).

Concerning the comparison of the phytoplankton species composition in 1990–1991 and 2000–2001, we observed some important changes (Tab. 1). The most remarkable is the shift towards frequent dominance of *Plagioselmis lacustris*, which is known to prefer higher pH levels (ALMER et al., 1978). On the other hand, *Koliella longiseta*, which formed surface phytoplankton peak during severe episodic acidification in spring 1990–1991 (Fig. 3), has been found only in very low numbers in 2000–2001. This non-motile species is an important component of phytoplankton community from the near-by shallow strongly acidified Starolesnianske pleso (FOTT et al., 1999). The disappearance of planktonic diatoms from acidified lakes is among the most striking changes of affected communities (ALMER et al., 1978). Regular occurrence of *Fragilaria* cf. *tenera* in Ladové pleso in 2000–2001 can thus be viewed as another relevant change in its phytoplankton community. Although it did not reach abundances higher than  $3\ \text{cells mL}^{-1}$  in 2000–2001, finding of this pennate diatom characterised by affinities to higher pH (HÖRNSTRÖM, 2002) can also be considered as a sign of changing chemical characteristic of Ladové pleso. An increased importance of diatoms within the planktonic community in response to increasing pH was recently observed in Lago Paione Superiore in the Italian Alps (MARCHETTO et al., 2004).

The significant increase in phytoplankton abundances observed in 2000–2001 in comparison with the 1990–1991 data (Fig. 3) may be related to better bioavailability of phosphorus in current conditions of increasing pH (KOPÁČEK et al., 2001). In 1990–1991, pH of an important part of Ladové pleso volume was lower than 6 during summer months, which is the value separating non-acidified and acidified lakes (FOTT et al., 1994). In 2000–2001, pH of Ladové pleso drop below 6 for a very short period and only the surface layer was affected (Fig. 9). In the period of maximal acid deposition, acidified lakes in the High Tatra Mts were characterised by extremely low chlorophyll-*a* concentrations, whereas higher values were found both in non-acidified and strongly acidified lakes (VYHNÁLEK et al., 1994). During last decade, chlorophyll-*a* concen-

trations increased significantly in the whole lake district (KOPÁČEK et al., 2006).

The acidification-driven changes in the water quality of sensitive lakes were often accompanied by a drastic reduction of their biodiversity (ALMER et al., 1978; FOTT et al., 1994). Recently, the first signs of biological response following chemical recovery of strongly acidified mountain lakes have been reported from many regions (VRBA et al., 2003; MARCHETTO et al., 2004). Our results suggest that even lakes that were not so seriously affected by antropogenic acidification can undergo noticeable changes. However, the changes in phytoplankton species composition and abundances of *Ladové pleso* must be interpreted with caution. Planktonic communities of high mountain or high latitude lakes are known to be controlled mostly by allogenic perturbations that are characterised by high inter-annual variation (HINDER et al., 1999). Consequently, patterns of lake water recovery can also be influenced by climate change (MARCHETTO et al., 2004). Further study of *Ladové pleso* is therefore needed to confirm, whether observed change of its phytoplankton community can be unambiguously related to the decrease of acid deposition followed by the chemical recovery of lake water.

#### Acknowledgements

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## Long-term studies (1871–2000) on acidification and recovery of lakes in the Bohemian Forest (central Europe)

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### Abstract

This paper evaluates long-term changes in the atmospheric depositions of S and N compounds, lake water quality, and biodiversity at eight glacial lakes in the Bohemian Forest over the past 130 years. This time interval covers (i) the ‘background’ pre-acidification status of the lakes, (ii) a period of changes in the communities that can be partly explained by introduction of fish, (iii) a period of strong lake acidification with its adverse impacts on the communities, (iv) the lake reversal from acidity, which includes the recent status of the lakes. The lake water chemistry has followed—with a characteristic hysteresis—both the sharp increase and decline in the deposition trends of strong anions. Remarkable changes in biota have mirrored the changing water quality. Fish became extinct and most species of zooplankton (Crustacea) and benthos (Ephemeroptera and Plecoptera) retreated due to the lake water acidification. Independent of ongoing chemical reversal, microorganisms remain dominant in the recent plankton biomass as well as in controlling the pelagic food webs. The first signs of the forthcoming biological recovery have already been evidenced in some lakes, such as the population of *Ceriodaphnia quadrangula* (Cladocera) returning into the pelagial of one lake or the increase in both phytoplankton biomass and rotifer numbers in another lake.

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### 1. Introduction

Eight small glacial lakes are situated on forested slopes of the central summit of the Bohemian Forest (Böhmerwald, Šumava) along the historical border between Bavaria and Bohemia. The lakes

have been fascinating for explorers for more than 100 years. Owing to the 130-year occasional hydrobiological research and palaeolimnological studies, we are now able to document the significant changes in the lake water chemistry and the consequent changes in the plankton and benthos composition (in particular, the conspicuous reduction in crustacean and/or insect species in some lakes) as well as the extinction of fish (e.g. Veselý,

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1994; Vrba et al., 2000). Therefore, the Bohemian Forest lakes represent excellent sites for further long-term ecological research.

Like the whole region of Central Europe, the Bohemian Forest was exposed to heavy atmospheric pollution during the last century. Regional emissions of S and N compounds reached up to  $\sim 280 \text{ mmol m}^{-2} \text{ year}^{-1}$  from World War II to the 1980s, and then declined by  $\sim 80\%$  and  $\sim 35\%$ , respectively, during the 1990s (Kopáček et al., 2001, 2002). Owing to small and geologically sensitive catchments, any changes in acid deposition were reflected in the lake water composition and biota (Vrba et al., 2000; Kopáček et al., 2001). Here, we summarise all available long-term data on the acidity-derived changes in biodiversity of the Bohemian Forest lakes in the context of chemical changes, and present the recent status as a start-point of the forthcoming biological recovery.

## 2. Material and methods

### 2.1. Site description

We have studied eight glacial lakes in the Bohemian Forest (the Hercynian crystalline mountain massive, the Šumava Mts. and Bayerischer Wald;  $48^{\circ}47'–49^{\circ}11'N$ ,  $13^{\circ}07'–13^{\circ}52'E$ ), five lakes on the Czech side (Černé, Čertovo, Plešné, Prášílské, and Laka), and three on the German side (Rachelsee, Grosser Arbersee, and Kleiner Arbersee). All the lakes are of a small size (areas: 2.8–18.4 ha; volumes:  $0.04–2.88 \times 10^6 \text{ m}^3$ ; maximum depths: 3–40 m), situated in geologically sensitive catchments (bedrock of gneiss, mica-schist or granite), at altitudes between 918 and 1096 m a.s.l. Their small catchment areas (0.58–2.79  $\text{km}^2$ ) are mainly covered by Norway spruce and, more sparsely, by beech and fir (Veselý, 1994; Schaumburg, 2000).

### 2.2. Lake sampling and analyses

Samples of lake water were taken at the deepest point of each lake, primarily in September–October and occasionally in July–August. A comparative sampling of all the eight lakes was conducted

within 10 days in early September 1999. The samples for chemical analyses were immediately filtered through a 200- $\mu\text{m}$  polyamide sieve. The samples for analyses of microorganisms were fixed with either formaldehyde (for bacteria) or acid Lugol's solution (for phytoplankton). Large zooplankton were sampled by several vertical hauls with a quantitative net (200- $\mu\text{m}$  mesh size) of the Apstein type, and small zooplankton were sampled with a Van Dorn sampler from the specific depths and concentrated by a plankton net (40- $\mu\text{m}$  mesh size); both samples were preserved by formaldehyde.

The data on hydrochemistry came from the sources referred by Kopáček et al. (2001, 2002). The plankton samples were processed according to Straškrabová et al. (1999) with the exception of filamentous microorganisms (Nedoma et al., 2001). The total zooplankton (crustacea + rotifers), phytoplankton (algae + cyanobacterial filaments), and heterotrophic microbial biomass (bacteria + heterotrophic filaments) were expressed as organic carbon ( $\mu\text{g C l}^{-1}$ ; Straškrabová et al., 1999).

Benthos (mostly Ephemeroptera and Plecoptera) of the five Czech lakes were sampled 12 times between 1956 and 1995 in all seasons. The samples were collected by using metal cups or sampling nets for 10–15 min mostly by means of the 'kicking technique' according to Kershaw and Frost (1978), i.e. by digging in the stony and sandy bottoms. At the same time, the attention was also paid to submerged aquatic plants and to the surface of larger stones in the littoral zone.

### 2.3. Historical data

This paper combines the historical data on the occurrence of species and water quality of the Bohemian Forest lakes summarised by Veselý (1994), Schaumburg (2000), Vrba et al. (2000) and all further available data on zooplankton and macrozoobenthos (e.g. Ošmera, 1971; Procházková and Blažka, 1999; including unpublished data of Fott et al.). The first survey of crustacean zooplankton of all Bohemian Forest lakes was performed by Frič (1872, 1873). The recent biodiversity of crustacean zooplankton was compared to these 130-year-old observations, considered as

a representative of the pre-acidification status. Moreover, the past occurrence of cladoceran species in the lakes was independently verified by the palaeolimnological analyses of the sediment cores taken from the lakes (Pražáková and Fott, 1994; Pražáková and Fott, unpublished data). All available information concerning the fishes in the Bohemian Forest lakes, summarised by Vrba et al. (2000), was evaluated to track their original presence, historical stocking, and extinction.

The deposition trends in S and total inorganic nitrogen (TIN =  $\text{NO}_3 + \text{NH}_4$ ) in the Bohemian Forest were derived from Kopáček et al. (2001). These trends are based on estimated data (1860–1991) and measured values (since 1992; Hruška et al., 2000). The estimated deposition was based on the historical central European emission trends, the long-term Czech deposition trends, and the measured deposition of S and TIN in the Bohemian Forest. The uncertainty associated with the estimate was less than  $\pm 30\%$  (Kopáček et al., 2001).

### 3. Results and discussion

#### 3.1. Long-term changes at Černé Lake

Černé Lake, with the most detailed set of historical data on lake water quality and biodiversity, provides a characteristic example of the changing Bohemian Forest environment (Fig. 1). The changes in the rate of atmospheric deposition of acidifying pollutants were the principal driving force of all the documented changes. Under the relatively stable deposition trends in S and TIN at the beginning of the Industrial Revolution, the lake water chemistry remained apparently unaffected until the mid-1940s. The first reliable chemical analyses from 1936 showed that the epilimnetic pH was almost neutral (6.9–7.0 throughout summer), the concentrations of sulphate were low and nitrate was below the detection limit (Jírovec and Jírovcová, 1937). This implies that the forest vegetation in the catchment was still nitrogen limited. Due to the sharply increasing TIN deposition between 1950 and 1980, the nitrogen saturation of soils in the catchment became likely (already in the early 1960s) and consequently, the

lake concentrations of  $\text{NO}_3$  increased in parallel with the TIN deposition, similarly to  $\text{SO}_4$  (Fig. 1).

During the 1960s and 1970s, the massive input of acidifying compounds caused a strong acidification of the Černé Lake resulting in a drop of 2 pH units. The lake water became remarkably clear (see transparency of Secchi depths in Fig. 1). Both the deposition and the lake acidification peaked in the 1980s. During this period, the in-lake concentrations of aluminium (Al) were  $\sim 1 \text{ mg l}^{-1}$ , which was significantly above the toxic levels for fish and most zooplankton (Veselý et al., 1998b). The present decreasing trend in the Al concentrations in Černé Lake obviously reflects the decline in the concentrations of strong acid anions (Fig. 1) and is—beside a constant rise in pH—the most important symptom of the lake water reversal from the acid stress. However, the decline in the lake water concentrations of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  is not as rapid as the reduction of S and N emissions, and their present concentrations are higher than they were in the past for comparable emission rates during the increase in acidification (Kopáček et al., 2002).

The acidification-driven changes in the lake water quality were accompanied by a drastic reduction of biodiversity (Fig. 1). However, the first imbalance in the lake ecosystem was likely caused by introduction of brook trout in the 1890s and its repeated stocking (Vrba et al., 2000) and resulted in the first reduction in species richness of pelagic Crustacea. Brook trout probably reduced the number of zooplankton species (extinction of *Holopedium gibberum*, *Daphnia longispina*, *Bosmina longispina*, and *Acanthodiptomus denticornis*; Šrámek-Hušek, 1942), thereby suppressing the indigenous population of brown trout within half a century (Fig. 1). The stock of brook trout exhibited good condition and population parameters in contrast with the bad condition of a single specimen of the native brown trout caught in 1962 (Dyk, 1992). While the original species of brown trout was not observed in the lake after 1962, the brook trout (more resistant to acidification) survived under the increasing acid stress until the mid-1970s (Vrba et al., 2000). Besides the fish species, the acidification of Černé Lake obviously caused the extinction of most species of crustacean

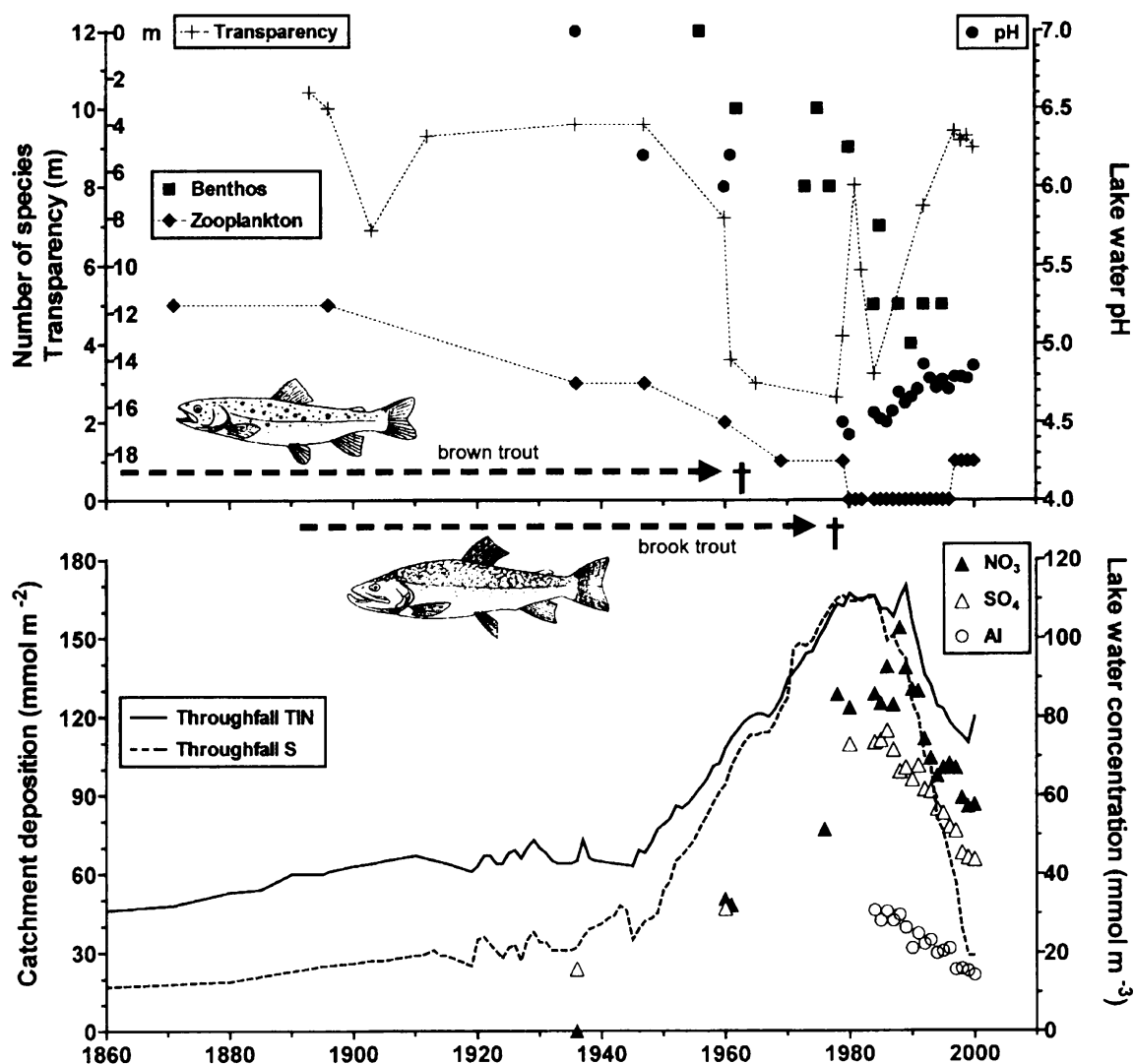


Fig. 1. Long-term limnological data on Černé Lake, 1871–2000 (from different sources): number of conspicuous zooplankton (Crustacea caught by plankton nets in the pelagial) and benthos (Ephemeroptera and Plecoptera) species (top left axis, left scale); lake water transparency as Secchi depths (top left axis, right scale), pH (top right axis), and concentrations of sulphate ( $\text{SO}_4$ ), nitrate ( $\text{NO}_3$ ), and aluminium (Al) (bottom right axis); estimated atmospheric deposition trends for sulfur (S) and total inorganic nitrogen (TIN) on the catchment (bottom left axis).

zooplankton and littoral macrozoobenthos after the 1950s/1960s (Fig. 1, Tables 2 and 3). Finally, around 1970, the only regular components of summer zooplankton were *Ceriodaphnia quadrangula*, which survived in extremely low numbers, and two rotifer species (*Microcodon clavus* and *Polyarthra remata*). The final impoverishment of zooplankton later in the 1980s was attributed to the further acidification of the lake (Fott et al., 1994). The population of *C. quadrangula* probably

survived the period of the highest acidity in the rocky littoral (though not found in the littoral samples between 1982 and 1992 either) and reappeared in the pelagial in 1997, after approximately one decade of the lake water reversal from acidification (see Fig. 1).

Similar, but not so drastic changes were also observed in the composition of benthic communities in Černé Lake. The total number of mayfly (Ephemeroptera) and stonefly (Plecoptera) species

Table 1

Historical occurrence of fish in the Bohemian Forest lakes (CN, Černé; CT, Čertovo, PL, Plešné; PR, Prášílské; RA, Rachelsee; GA, Grosser Arbersee; KA, Kleiner Arbersee; LA, Laka)

Species	CN	CT	RA	PL	KA	PR	GA	LA
Indigenous species (present before 1870):								
<i>Salmo trutta</i> L.—brown trout	E	E	A?	A?	E	A?	E	E
Stocked species (before acidification):								
<i>Salmo trutta</i> L.—brown trout			E	E		E		E
<i>Oncorhynchus mykiss</i> (Walbaum)—rainbow trout					E		E	
<i>Salvelinus fontinalis</i> (Mitchill)—brook trout	E			[E]		[E]		[E]

A, the species is absent in a lake; E, the species was present, stocked or introduced in a lake but is extinct nowadays (for details see Vrba et al., 2000). [E] means a stocking took place according to oral information, which cannot be confirmed with certainty; the lakes are fishless at present. The only documented fish, which occurred in early times in most of the lakes, was brown trout; however, three lakes seem to have been naturally fishless in the long past (A?).

dropped to one third between 1956 and 1990 (from 12 to four species; see Table 3, Fig. 1). Only the acidotolerant *Leptophlebia vespertina* (Ephemeroptera) and four acidotolerant species of stonefly were found in Černé Lake during the last sampling in 1995 (see Table 3).

### 3.2. Atmospheric acidification reduced biodiversity in all the lakes

The example of Černé Lake (Fig. 1) comprehensively describes the causes and consequences of the strong atmospheric acidification in central Europe. The value of this site for detecting environmental changes can be increased by obtaining similar data from the other Bohemian Forest lakes. Despite the less frequent historical sampling than that of Černé Lake, the available data cover all milestones of the acidification process in most Czech lakes (Vrba et al., 2000). Moreover, sampling of all the eight lakes covered not only the situation 130 years ago (Frič, 1872, 1873), but also the last two decades (Vesely et al., 1998a,b; Schaumburg, 2000; Vrba et al., 2000). The major changes in the lake water chemistry were running in parallel in seven dimictic lakes. The trends in lake water concentrations of  $\text{SO}_4$  and  $\text{NO}_3$  (e.g. Kopáček et al., 2002) as well as the palaeolimnological evidence (e.g. Arzet et al., 1986), provide us with the basis we can use to estimate the timing of individual periods of acidification with a sharp increase in the acidity during the late 1950s to the 1970s, maximum acidity (the late 1970s to

~1985), and reversal from acidity (since the late 1980s). Similarly to the other acidified regions of the Northern Hemisphere (e.g. Schindler, 1988, 1994; Stenson et al., 1993), the Bohemian Forest lakes have revealed both characteristic changes in the lake water chemistry and a significant reduction in biodiversity. The shallow Laka Lake (of the largest catchment area and the lowest retention time) probably represents a certain exception among the lakes under the study because it was acidified only temporarily (in spring), without any clear trends in the water chemistry (Vrba et al., 2000) but with some changes in biota.

Despite some uncertainty regarding the original presence of fish in three of the lakes and despite all attempts at stocking fishes during the last century, we are sure that fish became extinct in all Bohemian Forest lakes between the 1960s and 1970s (Table 1, Vrba et al., 2000). The disappearance of rainbow trout from both Kleiner Arbersee and Grosser Arbersee in the 1950s/1960s and the failure to stock this species were indeed the first indicators of ecosystem changes, which initiated the palaeolimnological research of the three lakes on the Bavarian side (e.g. Arzet et al., 1986).

Table 2 compares the present status of seven conspicuous species of Crustacea in the Bohemian Forest lakes with the situation 130 years ago (Frič, 1872, 1873). Most of the species became extinct or diminished due to the strong acidification of the lakes 20 years ago. Even at that time, two species, *Daphnia longispina* and *Cyclops abyssorum* persisted in Prášílské Lake and the latter

Table 2

Historical occurrence of the conspicuous species of crustacean zooplankton (typical littoral species not included) in the Bohemian Forest lakes (see key in Table 1)

Species	CN	CT	RA	PL	KA	PR	GA	LA
Cladocera (total number of species in the past):	4	4	1	1	2	3	4	2
<i>Bosmina longispina</i> Leydig	E	E	A	A	A	E	E	A
<i>Ceriodaphnia quadrangula</i> (O.F.M.)	S*	E	A	A	S	E	S	S
<i>Daphnia longispina</i> (O.F.M.)**	E	E	E	E	A	S	E	S
<i>Holopedium gibberum</i> Zaddach	E	E	A	A	E	A	S	A
Copepoda (total number of species in the past):	2	2	2	3	1	2	2	0
<i>Acanthodiptomus denticornis</i> (Wierz.)	E	E	A	E	E	E	E	A
<i>Cyclops abyssorum</i> Sars	E	E	E	E	A	S**	S	A
<i>Heterocope saliens</i> (Lillj.)	A	A	E	S	A	A	A	A
Crustacea (total number of surviving species):	1	0	0	1	1	2	3	2

\* In Černé lake, *C. quadrangula* was not observed in 1871 (presumably overlooked) but it was quoted from the 1890s (Frič and Vávra, 1898, under name *C. pulchella*).

\*\* According to recent molecular analyses (Petrušek, unpublished results), the *Daphnia* population from Prášílské Lake is identical with *Daphnia rosea* Sars sensu Schwenk et al. (2000).

A, the species apparently absent in a lake at any time; the remains never found in the sediment. E, the species is extinct in a lake now, but it was present 130 years ago (Frič, 1872, 1873) or the remains of Cladocera were found in the sediment. S, the surviving species have persisted the acidification of a lake.

species also in Grosser Arbersee, most likely due to the far lower Al concentrations in both lakes compared with the Al concentrations in the rest of the lakes (Fott et al., 1994; Schaumburg, 2000; Vrba et al., 2000). At present, all the species listed in Table 2, which were found by Frič, are absent in Čertovo Lake and Rachelsee, whereas only *Heterocope saliens* has survived in Plešné Lake and *Ceriodaphnia quadrangula* in Černé Lake (Table 2). The latter species has survived also in Grosser Arbersee, Kleiner Arbersee, and Laka Lake, but surprisingly, it has never been found in Prášílské Lake during the 20th century and up to the present day. In conclusion, two of the eight conspicuous species (i.e. *Bosmina longispina* and *Acanthodiptomus denticornis*) became virtually extinct in all the lakes during the last century, and two others (*Holopedium gibberum* and *Heterocope saliens*) have survived up to the present in one lake only (Table 2).

In addition to this general retreat of the crustacean zooplankton in all the lakes, we can expect a similar reduction of other invertebrate species, although it cannot be documented appropriately. We can document such a retreat of rotifers only for Plešné Lake. Frič (1872, 1873) observed a

*Conochilus* species in Grosser Arbersee and Plešné Lake that (according to the present knowledge) was most probably *Conochilus unicornis* Rousselet 1892. No *Conochilus* (an unmistakable pelagic colonial form, the live sample of which is distinguishable by eye) has been found in any Bohemian Forest lake later on. Černý (1910) found non-acidophilic species (according to Berziňš and Pejler, 1987), e.g., *Keratella quadrata* (Müll.), *K. testudo* (Ehr.), *Lecane luna* (Müll.), and *Monostyla cornuta* (Müll.) (all the names are according to the present nomenclature), which have not been observed in Plešné Lake after the acidification either.

The first information on macrozoobenthos were published already by Frič and Vávra (1898) who mentioned a mass occurrence of *Glenocorisa propinqua propinqua* (Heteroptera) in Černé and Čertovo Lake. This species was not mentioned to be present in both lakes in the late 1950s (last records by Roubal, 1957) but surviving populations was observed in Plešné and Prášílské Lake during the 1990s. However, we assume that its retreat is not directly dependent on acidification (because this species is relatively acidotolerant, Soldán et al., 1996) but more likely it is a consequence of



Table 3  
Historical occurrence of the mayfly and stonefly larvae in the five Czech lakes in the Bohemian Forest (see key in Table 1)

Species	CN	CT	PL	PR	LA
Ephemeroptera (total number of species in the 1950s):	4	3	4	4	5
<i>Siphonurus lacustris</i> (Eaton)	E	E	E	E	S
<i>Siphonurus alternatus</i> (Say)	E	A	E	E	E
<i>Ameletus inopinatus</i> Eaton	E	E	E	E	E
<i>Cloeon dipterum</i> (L.)	A	A	A	A	E
<i>Leptophlebia vespertina</i> (L.)	S	S	S	S	S
Plecoptera (total number of species in the 1950s):	8	6	10	10	9
<i>Amphinemura triangularis</i> (Ris)	S	A	S	E	S
<i>Protonemura auberti</i> Illies	E	E	E	E	E
<i>Protonemura montana</i> Kimmins + <i>P. hrabei</i> Raušer	S	S	S	E	S
<i>Nemoura cinerea</i> (Retzius)	E	E	E	E	E
<i>Nemurella picteti</i> Klapálek	A	E	E	E	E
<i>Leuctra aurita</i> Navás	S	A	S	E	S
<i>Leuctra autumnalis</i> Aubert	A	A	E	E	A
<i>Leuctra digitata</i> Kempny	S	S	S	S	S
<i>Leuctra fusca</i> (L.)	E	A	A	E	E
<i>Leuctra handlirschi</i> Kempny	A	E	E	A	A
<i>Leuctra nigra</i> (Olivier)	E	A	E	E	E
Insect larvae (total number of surviving species):	5	3	5	2	6

A, the species has been apparently absent in the lake during the last 50 years. E, the species is extinct in the lake now, but it was present in the 1950s. S, the surviving species have persisted the acidification of a lake.

significant changes in available food sources (Papáček and Soldán, 1995). This assumption can be documented by the occurrence of *G. p. propinqua* in Prášílské Lake with the cladoceran population (Kubecka et al., 2000; Kohout and Fott, 2000). Also some species with the arcto-alpine disjunction, e.g. *Molanna nigra* (Trichoptera) has survived in Prášílské Lake till the present. In general, however, benthos of the Bohemian Forest lakes still remains poorly understood.

Table 3 shows long-term changes in mayflies (Ephemeroptera) and stoneflies (Plecoptera), the only macrozoobenthos groups observed in detail in five Czech lakes since the early 1950s (e.g. Soldán et al. 1998). Total number of species has been gradually (>50%) reduced till the 1990s and the most pronounced species reduction was observed at Prášílské Lake (from 14 original to two recent species). However, the changes in the species composition did not correlate directly with the density. Within all the lakes, the mayfly *Leptophlebia vespertina* exhibits a key role. This mayfly is probably the most acidotolerant species within these groups, showing pH tolerance limits

of approximately 4.0 (Egblom and Lindell, 1983). This north European species (otherwise solitary or even rare in central Europe in general) has survived in all studied lakes; however, its population was apparently stressed by the acidification process here. This can be documented by significantly lower fecundity of females collected in 1975 and 1982. Recently, *L. vespertina* increased its population densities in all five lakes considerably, at least twice (Čertovo) or even more than 10 times (Laka).

### 3.3. Recent status of the lakes and their potential for recovery

Besides several common features of the acidified lakes studied, they showed remarkable differences in some chemical and plankton parameters (Vrba et al., 2000; Kopáček et al., 2001). On the basis of the chemistry of both tributaries and surface lake water, we have categorised the lakes as follows: (i) Rachelsee, Plešné, Černé, and Čertovo remain strongly acidified; (ii) Prášílské and Kleiner Arbersee are moderately acidified but still with

a depleted carbonate buffering system; whereas (iii) Grosser Arbersee and Laka have low bicarbonate alkalinity (Vrba et al., 2000). Accordingly, the less acidified lakes (with lower Al concentrations) show higher numbers (2–3) of surviving species (Table 2) and a higher biodiversity of rotifers and Crustacea (Table 4).

In consequence of different loading with acidifying agents and nutrients (Vrba et al., 2000), the lakes represent a fascinating set of unique freshwater bodies, which differ in particular aspects from those found at acidified lakes elsewhere (cf. Stenson et al., 1993). Both plankton biomass and pelagic food webs became dominated by microorganisms because of the reduction of biodiversity and thus of absence of the higher trophic levels. There is observed a characteristic and extremely high heterotrophic microbial biomass with an extreme proportion of filaments, which is usually as high as phytoplankton biomass (Fig. 2). The only exception represents the plankton of Plešné Lake, where phytoplankton accounts for ~80% of the total biomass due to the higher inflow concentration of phosphorus compared with the other lakes (Vrba et al., 2000; cf. Fig. 2). The summer zooplankton biomass mostly accounted for <1% of the total biomass (Fig. 2). Tables 4 and 5 summarise most invertebrate (except for insects) and phytoplankton taxa, respectively, recently found in the Bohemian Forest lakes.

The recent phytoplankton composition of seven dimictic lakes is surprisingly similar (two filamentous Cyanobacteria and 20–23 algae commonly found in the preserved samples), whereas a lower number of algal taxa (13) has been found in Laka Lake (Table 5). However, the actual number of algal species in the lakes might be higher if sampled repeatedly (cf. presence of *Cryptomonas* spp. in Čertovo and Plešné Lake, Table 5). Schaumburg (2000) reported as many as 39 and 63 phytoplankton species present in Grosser Arbersee and Kleiner Arbersee, respectively, (but just 22 items in Rachelsee) during longer observation periods. Although the species composition is similar, the phytoplankton biomass in Plešné Lake is usually dominated by non-motile species (*Monoraphidium dybowskii* and Cyanobacteria), whereas phytoflagellates dominate the phytoplankton bio-

mass in all other lakes studied (cf. Table 5; Nedbalová and Vrtiška, 2000). All recently present species at these lakes were observed in Černé Lake as early as in 1936 (B. Fott, unpublished); the only species of his list which was not found at present is *Cyclotella* sp. (Vrba et al., 2000). Thus, the comparison of the present status with the old records suggests that many phytoplankton species of acid-sensitive oligotrophic lakes were able to survive when the lakes became acidic (Fott et al., 1994).

On the other hand, owing to lack of detailed phytoplankton data in the past, we can hardly prove a phytoplankton recovery in these lakes, except for an almost 50% increase in the chlorophyll *a* concentration in Plešné Lake between 1994 and 1998 (Vrba, unpublished data). While the total phosphorus concentrations remained unchanged during this period, the increase in phytoplankton biomass was likely a consequence of less phosphorus immobilisation by Al (Kopáček et al., 2000) due to the decreasing trend in the Al concentration in the lake (up to now ~50% reduction compared with the maximum values ~15 years ago; Veselý et al., 1998b). Therefore, this ongoing Al decrease seems to be the key factor of any possible biological recovery of the lake ecosystems studied. First, the current Al concentrations are less impoverishing the phosphorus availability for phytoplankton (Bittl et al., 2001; Vrba et al., unpublished) and second, any toxic effects of Al on the aquatic biota decreased significantly during the chemical reversal of the lakes.

Unlike the recovery of Černé Lake, which is indicated by the increase in the cladoceran population (*C. quadrangula*), the beginning of zooplankton recovery in Plešné Lake seems to be manifested by the increase in pelagic rotifers. While their mean numbers were almost negligible (30–180 ind. m<sup>-3</sup>) in summer 1990–1992, the numbers increased by 2–3 orders of magnitude (11 000–37 000 ind. m<sup>-3</sup>) in summer 1997–1999. Because the acidotolerant species of rotifers (cf. Table 4; Berziniš and Pejler, 1987) are generally dominant in the plankton, the increase in their abundance apparently resulted from the increase in

Table 4

Recent list of all rotifer and crustacean taxa (common open-water species in bold) in the Bohemian Forest lakes, 1997–2000 (see key in Table 1)

Species	CN	CT	RA	PL	KA <sup>*</sup>	PR	GA <sup>*</sup>	LA <sup>*</sup>
Rotatoria (only pelagic samples):								
<i>Bdelloidea</i> g. sp.	x	x	x	x	x	x	–	–
<b><i>Brachionus sericus</i> Rous.</b> **	(x)	–	–	x	–	–	–	–
<b><i>Collotheca pelagica</i> (Rous.)</b> **	x	x	(x)	x	–	x	–	–
<i>Colurella</i> sp.	(x)	–	–	–	–	–	–	–
<i>Erignatha clastopis</i> (Gosse)	–	–	–	x	–	x	–	–
<i>Euchlanis dilatata</i> (Ehr.)	–	–	–	–	(x)	–	(x)	(x)
<b><i>Keratella serrulata</i> (Ehr.)</b> **	x	x	x	xx	x	x	(x)	(x)
<b><i>Keratella ticinensis</i> (Call.)</b> **	–	–	–	–	–	–	–	x
<i>Lecane ligona</i> (Dunl.)	–	(x)	x	(x)	–	(x)	–	–
<i>Lecane mira</i> (Murr.)	–	–	–	–	(x)	–	–	–
<i>Lecane stichaea</i> Harr.	x	x	–	x	x	x	(x)	x
<i>Lepadella acuminata</i> (Ehr.)	(x)	(x)	–	x	(x)	–	–	–
<b><i>Microcodon clavus</i> Ehr.</b> ***	xx	xx	x	x	–	x	–	x
<i>Monommata</i> sp.	–	–	–	–	–	(x)	–	–
<i>Monostyla lunaris</i> (Ehr.)	x	x	x	x	x	x	x	(x)
<i>Monostyla pyriformis</i> (Dad.)	–	–	–	–	–	x	–	–
<b><i>Polyarthra major</i> Burckh.</b> **	–	–	–	–	x	–	x	xx
<b><i>Polyarthra remata</i> Skor.</b> **	xx	xx	–	–	xx	xx	x	xx
<b><i>Synchaeta oblonga</i> Ehr.</b> **	xx	x	–	xx	x	xx	(x)	–
<b><i>Synchaeta pectinata</i> Ehr.</b> **	–	–	–	–	–	–	–	x
<i>Trichocerca</i> spp.	(x)	(x)	(x)	(x)	–	x	–	(x)
<i>Trichotria tetractis</i> (Ehr.)	–	–	–	–	–	x	–	–
Cladocera (both pelagic and littoral samples):								
<i>Acantholeberis curvirostris</i> (O.F.M.)	x	x	x	–	x	x	x	x
<i>Acroperus harpae</i> Baird	x	x	x	x	x	x	x	x
<i>Alona affinis</i> (Leydig)	(x)	x	x	x	x	x	(x)	x
<i>Alona guttata</i> Sars	x	x	–	x	x	x	–	(x)
<i>Alona quadrangularis</i> (O.F.M.)	–	–	–	–	x	–	–	x
<i>Alonella excisa</i> (Fisch.)	x	x	(x)	x	x	x	x	x
<i>Alonella nana</i> (Baird)	–	–	–	–	(x)	–	–	–
<i>Alonopsis elongata</i> (Sars)	x	x	–	–	x	(x)	x	x
<b><i>Ceriodaphnia quadrangula</i> (O.F.M.)</b> ***	xx	–	–	–	xx	–	x	x
<i>Chydorus sphaericus</i> (O.F.M.)***	–	–	–	–	x	–	–	–
<b><i>Daphnia longispina</i> (O.F.M.)</b> **	–	–	–	–	–	xx	–	x
<i>Euryercus lamellatus</i> O.F.M.	–	–	–	–	x	–	x	x
<i>Graptoleberis testudinaria</i> (Fisch.)	–	–	–	–	–	–	–	(x)
<b><i>Holopedium gibberum</i> Zaddach</b> **	–	–	–	–	–	–	x	–
<i>Iliocryptus sordius</i> (Liévin)	(x)	–	–	–	–	–	–	–
<i>Peracantha truncata</i> (Müller)	–	–	–	–	–	–	x	x
<i>Polyphemus pediculus</i> L.	–	–	–	–	x	x	x	x
<i>Sida crystallina</i> (O.F.M.)	–	–	–	–	x	–	x	–
<i>Simocephalus vetulus</i> (O.F.M.)	–	–	–	–	–	–	–	x
Copepoda (both pelagic and littoral samples):								
<b><i>Acanthocyclops vernalis</i> (Fisch.)</b> ***	–	xx	–	xx	x	–	–	x
<b><i>Cyclops abyssorum</i> Sars</b> **	–	–	–	–	–	xx	xx	–
<i>Diacyclops nanus</i> (Sars)	x	x	(x)	x	–	x	x	–
<i>Eucyclops serrulatus</i> (Fisch.)	x	–	–	x	(x)	(x)	(x)	(x)
<b><i>Heterocope saliens</i> (Lillj.)</b> ***	–	–	–	xx	–	–	–	–
<i>Macrocyclus fuscus</i> (Jur.)	x	x	(x)	–	x	x	x	x
<i>Paracyclops fimbriatus</i> (Fisch.)	–	–	–	(x)	–	–	–	–
Total number of present taxa:	23	20	13	21	26	26	22	27

\* We sampled the lake only in September 1999.

\*\* A typical pelagic species.

\*\*\* A littoral-pelagic (tychoplanktic) species.

x, present species; (x), only 1–2 specimens in some samples; xx, important component of zooplankton biomass; –, absent species.

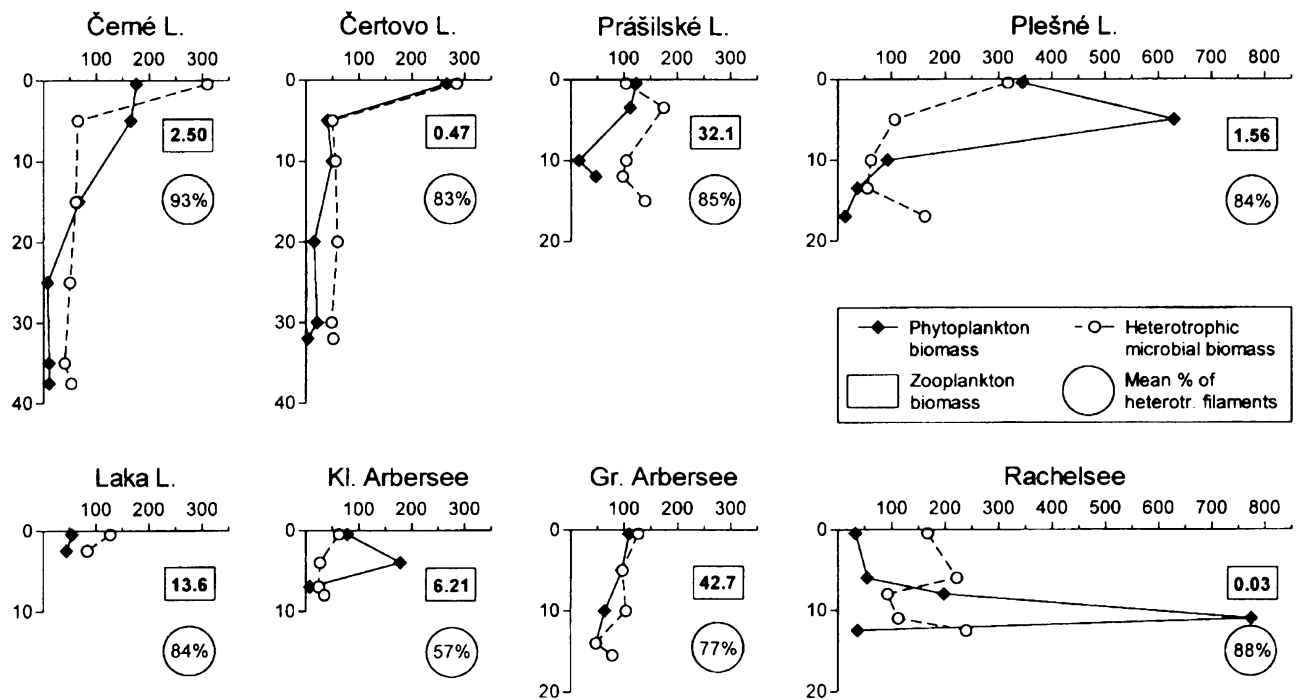


Fig. 2. A comparison of plankton biomass (in  $\mu\text{g C l}^{-1}$ ) in the Bohemian Forest lakes in September 1999: volume weighted means of zooplankton biomass (bold numbers, in rectangles) and vertical profiles of phytoplankton and heterotrophic microbial biomass (average proportion of filaments in per cent of the heterotrophic microbial biomass in each lake, in circles).

food resources in Plešné Lake rather than from a direct effect of the chemical reversal.

As far as some groups of aquatic insect are concerned, we have not yet noticed any remarkable recovery trends in the original fauna. The present samples exhibit the same total numbers species or higher by 1–2 species of macrozoobentos compared with those in the early 1990s (cf. Fig. 1). However, the total number of specimens and their densities are evidently increasing in the littoral zone of all the lakes studied. Besides the 10-fold increase in mayfly density (*L. vespertina*), the recent regular occurrence of planktonic larvae of *Chaoborus obscuripes* van der Wulp (Diptera) is typical for the shallow Laka Lake with a large biomass of submerged vegetation. This invertebrate predator likely controls the zooplankton community. Unlike in the other lakes with crustacean zooplankton (cf. Table 4 and Fig. 2), rotifers are dominant in zooplankton biomass in Laka Lake, whereas both *D. longispina* and *C. quadrangula* occur at low numbers. Such a dominance of invertebrate predators and rotifers in the absence

of fish were described as the first period of plankton recovery of the acidified Swedish lakes treated by liming. No further recolonisation of those lakes by dense cladoceran populations (e.g. *D. longispina*, *H. gibberum*, and *C. quadrangula*) followed unless the introduced fish eliminated the invertebrate predators (Stenson et al., 1993).

As far as we know, no return of fish has been documented in any Bohemian Forest lake as yet. It is typical that the decrease in the lake water concentration of  $\text{SO}_4$  drags behind the drop in atmospheric S depositions (Fig. 1) and that the consequent hysteresis in the chemical reversal of lakes from acidification further delays any biological recovery (Kopáček et al., 2002). The recent survey of all the lakes presents the first comprehensive background data for a more detailed study and evaluation of the process of biological recovery of the lakes. The set of long-term data reviewed in this paper shows that the Bohemian Forest lakes represent a unique opportunity to study the natural biological recovery processes in lake ecosystems damaged from an extremely strong acid stress.

Table 5

Recent list of common phytoplankton taxa in the Bohemian Forest lakes, survey of September 1999 (see key in Table 1)

Species	CN	CT	RA	PL	KA	PR	GA	LA
Cyanobacteria:								
<i>Limnothrix</i> sp.	x	x	x	xx	x	x	x	x
<i>Pseudanabaena</i> sp.	x	x	x	xx	x	x	x	x
Dinophyceae:								
<i>Gymnodinium uberrimum</i> (Allman) Kofoid et Sweezy	xx	xx	xx	–	xx	xx	xx	–
<i>Gymnodinium</i> sp.	x	x	x	x	x	x	x	x
<i>Katodinium bohemicum</i> (Fott) Litvinenko	x	x	x	x	x	x	x	x
<i>Katodinium planum</i> (Fott) Loeblich III	x	–	x	x	–	–	–	–
<i>Peridinium umbonatum</i> Stein	xx	xx	xx	xx	xx	xx	xx	xx
Cryptophyceae:								
<i>Cryptomonas erosa</i> Ehrenberg	x	x	x	x	x	xx	x	x
<i>Cryptomonas gracilis</i> Skuja	–	x*	–	x*	x	x	x	–
<i>Cryptomonas marssonii</i> Skuja	–	x*	x	x	xx	x	xx	–
<i>Cryptomonas reflexa</i> Skuja	–	–	–	–	xx	–	xx	xx
Chrysophyceae:								
<i>Bitrichia ollula</i> (Fott) Bourrelly	x	x	x	x	x	x	x	x
<i>Dinobryon</i> spp.	xx	xx	xx	xx	x	xx	x	xx
<i>Mallomonas</i> sp.	x	x	–	x	x	x	x	–
<i>Ochromonas</i> sp. (large)	x	x	x	x	x	x	x	x
<i>Ochromonas</i> sp. (small)	–	x	–	x	–	x	–	–
<i>Spiniferomonas</i> sp.	x	x	x	x	x	x	xx	x
<i>Synura echinulata</i> Korschikov	x	x	x	x	xx	x	x	x
Xanthophyceae:								
<i>Isthmochloron trispinatum</i> (W. et G.S. West) Skuja	x	x	x	x	x	x	x	–
Chlorophyta:								
<i>Arthrodesmus incus</i> (Bréb.) Hass.	–	–	–	–	x	–	–	–
<i>Carteria multifilis</i> (Fres.) Dill + <i>C. radiosa</i> Korschikov	x	x	xx	x	x	x	x	–
<i>Chlamydomonas</i> sp.	x	x	x	x	x	x	x	x
<i>Chlorogonium fusiforme</i> Matwienko	x	x	x	x	x	x	x	–
<i>Chloromonas angustissima</i> (Ettl) Gerl. et Ettl	x	x	x	x	x	x	x	x
<i>Koliella corcontica</i> Hind.	x	x	x	x	x	x	x	x
<i>Monoraphidium dybowskii</i> (Wolosz.) Hind. et Kom.-Legn.	x	x	x	xx	x	x	x	–
<i>Tetraedron minimum</i> (A. Br.) Hansg.	x	x	x	–	x	–	x	–
Phytoplankton (total number of common taxa):	22	24	22	23	25	23	24	15

\* Absent *Cryptomonas* species in samples from the survey of September 1999, but commonly present in the lake phytoplankton during 1997–1998 (Nedbalová and Vrtiška, 2000).

x, present species; xx, important component of total phytoplankton biomass; –, absent species.

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## A key role of aluminium in phosphorus availability, food web structure, and plankton dynamics in strongly acidified lakes

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**Abstract:** We studied extracellular acid phosphatase activity (AcPA) of planktonic microorganisms, aluminium (Al) speciation, and phosphorus (P) cycling in three atmospherically acidified (pH of 4.5–5.1) mountain forest lakes: Čertovo jezero (CT), Prášílské jezero (PR), and Plešné jezero (PL) in the Bohemian Forest (Šumava, Böhmerwald). Microorganisms dominated pelagic food webs of the lakes and crustacean zooplankton were important only in PR, with the lowest Al concentrations ( $193 \mu\text{g L}^{-1}$ ) due to 3–4 times lower terrestrial input. The lakes differed substantially in Al speciation, i.e., in the proportion of ionic and particulate forms, with the highest proportion of ionic Al in the most acid CT (pH = 4.5). The P concentration in the inlet of PL (mean:  $22.9 \mu\text{g L}^{-1}$ ) was about five times higher than in CT and PR ( $3.9$  and  $5.1 \mu\text{g L}^{-1}$ , respectively). Average total biomass of planktonic microorganisms in PL ( $593 \mu\text{g C L}^{-1}$ ) was, however, only ~2-times higher than in CT and PR ( $235$  and  $272 \mu\text{g C L}^{-1}$ , respectively). Enormous AcPA (means:  $2.17$ – $6.82 \mu\text{mol L}^{-1} \text{h}^{-1}$ ) and high planktonic C : P ratios suggested severe P limitation of the plankton in all lakes. Comparing 1998 and 2003 seasons, we observed changes in water composition (pH and Al speciation) leading to a significant increase in phytoplankton biomass in the lakes. The increase in the seston C : P ratio during the same time, however, indicates a progressive P deficiency of the lakes. The terrestrial Al inputs, together with in-lake processes controlling the formation of particulate Al, reduced P availability for planktonic microorganisms and were responsible for the differences in AcPA. At pH < 5, moreover, ionic Al forms caused inhibition of extracellular phosphatases. We postulate that both particulate and ionic Al forms affect P availability (i.e., inhibition of extracellular phosphatases and inactivation of P), specifically shape the plankton composition in the lakes and affect plankton recovery from the acid stress.

**Key words:** Acidification, recovery, carbon biomass, plankton stoichiometry, bacterioplankton, phytoplankton, zooplankton, extracellular phosphatases.

### Introduction

Atmospheric acidification of geologically sensitive lake districts of the Northern Hemisphere has been one of the major challenges in limnology of the second half of the 20<sup>th</sup> century (e.g., SCHINDLER, 1988; SCHINDLER et al., 1985). Many mechanisms and processes of lake acidification and reversal from acidity have been suggested and experimentally tested during the last three decades. In particular, numerous negative effects of aluminium (Al) on different organisms and acidified lake ecosystems have been reported (e.g., JANSSON, 1981; SCHINDLER, 1988; HAVAS & ROSSELAND, 1995). Not all questions, however, have yet been answered fully or satisfactorily. For instance, BRAKKE et al. (1994) point out that ‘we have little information to address

the important question of phosphorus (P) cycling’. No evidence that the internal P cycle is disrupted by acidification has been provided (e.g., OLSSON & PETERS-SON, 1993; SCHINDLER, 1994), with the exception of experiments by NALEWAJKO & PAUL (1985), who observed significant depressions of photosynthesis and P availability in Al treated waters in the pH range of 5–7.

Small glacial lakes in the Bohemian Forest (Šumava, Böhmerwald) had been strongly acidified due to sulphur and nitrogen deposition before the 1990s and have been recovering from both acidity and nitrogen saturation afterwards, due to extensive reduction in emission rates of atmospheric pollutants in Central Europe (e.g., KOPÁČEK et al., 2002; VESELÝ et al., 1998). The lakes represent unique ecosystems, with con-

spicuous dominance of microorganisms in their plankton at present (VRBA et al., 2003a, b). As a consequence of anthropogenic acidification of the lakes, fish became extinct in all the lakes during the 20<sup>th</sup> century, and pelagic crustacean zooplankton disappeared from most of them. Pelagic populations of *Daphnia longispina* (O.F. Müller, 1776) and *Cyclops abyssorum* Sars, 1863 have survived only in one lake (Prášilské jezero: FOTT et al., 1994; VRBA et al., 2003a).

The lakes have revealed all signs of severe P limitation, e.g., very high C : P and N : P molar ratios in seston and exceptionally high activities of extracellular phosphatases (VRBA et al., 1996; HEJZLAR et al., 1998). KOPÁČEK et al. (2000a) published evidence for an abiotic mechanism controlling P mobility: In-lake pH and alkalinity is increased compared to tributaries due to biological processes (e.g., assimilation of  $\text{NO}_3^-$ , decomposition, and dissimilatory reduction of  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ) which affect the Al speciation. The ionic Al species supplied from terrestrial sources hydrolyse at higher pH in the lakes and form Al oxyhydroxides ( $\text{Al}_{\text{part}}$ ), strongly binding orthophosphate ( $\text{P}_i$ ). Unlike ferric oxyhydroxides,  $\text{Al}_{\text{part}}$  complexes are not sensitive to redox changes and are stable under anoxic conditions. Hence, such  $\text{P}_i$  binding to  $\text{Al}_{\text{part}}$  is a powerful mechanism of P immobilisation in the acidified lakes (KOPÁČEK et al., 2000a; ULRICH & PÖTHIG, 2000).

JANSSON (1981) observed that high Al concentrations had an inhibitory effect on extracellular phosphatases at low pH, but they stimulated production of extracellular phosphatases by the plankton of acidified lakes (JANSSON et al., 1986; JOSEPH et al., 1995). We believed that this was also the case in the Bohemian Forest lakes where total Al concentrations peaked at  $\sim 1000 \mu\text{g L}^{-1}$  (Čertovo and Plešné lakes) and  $\sim 500 \mu\text{g L}^{-1}$  (Prášilské Lake) in the late 1980s (FOTT et al., 1994; VESELÝ et al., 1998). BITTL et al. (2001), however, showed that the inhibiting effect of Al could not fully explain the extreme activities of phosphatases. Further analysis of the data suggested that the above-mentioned mechanism of P immobilisation by  $\text{Al}_{\text{part}}$  might be the complementary cause of the extreme activities of phosphatases.

STERNER & ELSER (2002) have recently proposed a fundamental role of chemical stoichiometry in ecology. One can deduce that, under excess of nitrogen, the distinct P stoichiometry (C : P ratio) of autotrophic and heterotrophic microorganisms or animals may cause serious constraints of the food web structure in the acidified lakes.

The present study proposes the existence of complex interactions between Al and P in three strongly acidified lakes. We suggest strong and complex effects of Al on P metabolism in the lakes. Moreover, we hypothesise that the changes in Al speciation may transmit, in particular through the plankton stoichiometry, impacts of other environmental factors, like pH or alkalinity, to the plankton.

## Material and methods

### Water sampling and analyses

We sampled three small glacial lakes in the Bohemian Forest ( $48^\circ 47' 49'' \text{N}$ ,  $13^\circ 12' 13'' \text{E}$ ): Prášilské jezero (Prášilské Lake) in 1998, Čertovo jezero (Čertovo Lake) in 1998 and 2003, and Plešné jezero (Plešné Lake) in 1998, 2001, and 2003. The lakes are small, with surface areas of 4.2–10.3 ha, maximum depths of 16–36 m, and water residence times of 0.5, 2 and 0.8 years, respectively. The lakes are situated at elevations of 1028–1090 m a.s.l. in steep catchments, forested almost exclusively by Norway spruce (for details, see NEDBALOVÁ et al., 2006). Samples of lake water were taken monthly at the deepest points of the lakes during the ice-free period (late April to October) from 0.5 m depth. The samples were filtered immediately through a 200- $\mu\text{m}$  polyamide sieve (except for samples for chlorophyll, bacteria, and phytoplankton). Large zooplankton were sampled by several vertical hauls with a quantitative net (200- $\mu\text{m}$  mesh size) of the Apstein type, and small zooplankton were sampled with a Van Dorn sampler from the epilimnion and concentrated by a plankton net (40- $\mu\text{m}$  mesh size; STRÁŠKRABOVÁ et al., 1999).

Tributaries were sampled in 3–4-week intervals near their inlets to the lakes and the discharge was estimated by means of a bucket and stopwatch. Samples were filtered immediately through a 40- $\mu\text{m}$  polyamide sieve to remove coarse particles. Each inlet sample was analysed separately and the element fluxes for each lake were obtained by linking discharge data with the corresponding concentration data by the method of period-weighted mean (LIKENS et al., 1977). For Prášilské Lake, only seasonal data from 1998 were used; for Čertovo and Plešné lakes, the annual average inputs were measured in 1998–2003 and 2000–2003 periods, respectively.

In the laboratory, samples were filtered with 0.4- $\mu\text{m}$  glass-fibre filters (MN-5, Macherey Nagel) for the analyses of dissolved P and Al. Dissolved organic carbon (DOC) and particulate carbon ( $\text{C}_{\text{part}}$ ) were analysed with a LiquiTOC analyser (Foss/Heraeus) for the filtrate, and by combustion of the glass-fibre filter for the retained particulate organic matter, respectively. Acid neutralising capacity (ANC, Gran titration), pH, soluble reactive P (SRP, molybdate method), total P ( $\text{P}_T$ ) and dissolved P ( $\text{P}_D$ ) were analysed within 24 hours of sampling.  $\text{P}_T$  and  $\text{P}_D$  were determined by perchloric acid digestion. Particulate P ( $\text{P}_{\text{part}}$ ) was the difference between  $\text{P}_T$  and  $\text{P}_D$ . Fractionation of aluminium, i.e., total reactive Al ( $\text{Al}_T$ ), dissolved Al ( $\text{Al}_D$ ), and organically bound Al ( $\text{Al}_{\text{org}}$ ), were analysed in non-filtered samples, filtered samples, and cation exchange treated samples after their filtration, respectively. Ionic Al ( $\text{Al}_i$ ) was obtained as the difference between  $\text{Al}_D$  and  $\text{Al}_{\text{org}}$ . Particulate Al ( $\text{Al}_{\text{part}}$ ) was the difference between  $\text{Al}_T$  and  $\text{Al}_D$ . More details on analytical methods are given in KOPÁČEK et al. (2004, 2006a).

### Plankton analyses

All plankton samples (bacteria, protozoa, phyto- and zooplankton) were sampled, preserved, and processed according to STRÁŠKRABOVÁ et al. (1999) with the exception of filamentous microorganisms (NEDOMA et al., 2001).

Bacterial samples were preserved in 2% formaldehyde. Bacterioplankton were counted and sized after DAPI staining on black Poretics filters using an epifluorescence microscope (Olympus BX-60 or AX-70) and an image analysis system (Lucia D 3.52 or G/F 4.11, Laboratory Imaging,

www.lim.cz). We quantified separately unicellular bacteria and used the line intercept method for quantification of the total length of bacterial filaments (NEDOMA et al., 2001). Finally, both items were converted to carbon biomass and added to calculate heterotrophic biomass (HB) as described elsewhere (VRBA et al., 2003b).

Phytoplankton samples (~1 L) were preserved by acid Lugol's solution and pre-sedimented (5:1, except for Plešné Lake). Algal species were counted and measured in Utermöhl's sedimentation chambers on an inverted microscope (Nikon Diaphot). Individual cell volumes of the main species were approximated according to STRAŠKRABOVÁ et al. (1999) to calculate total algal biovolume; total biovolume of filamentous cyanobacteria was estimated in sedimentation chambers using the line intercept method (NEDOMA et al., 2001). Total phytoplankton biomass (PB) was expressed as organic carbon using the conversion factor 0.2 mg C mm<sup>-3</sup> (STRAŠKRABOVÁ et al., 1999) applied to the total phytoplankton biovolume (cyanobacteria + algae). Concentration of chlorophyll-*a* (Chl-*a*) was determined spectrophotometrically on Whatman GF/C filters after acetone extraction (LORENZEN, 1967); values were not corrected for phaeopigments.

Concentrated samples of zooplankton were preserved in 4% formaldehyde and processed as follows: Abundance of zooplankton was expressed as individuals per litre; size (length) of individual species was measured to estimate their biovolume (rotifers, nauplii) or dry weight (other crustaceans); total zooplankton biomass (ZB) was expressed as organic carbon using the following conversion factors: 0.5 mg C mg<sup>-1</sup> of crustacean dry weight and 0.05 mg C mm<sup>-3</sup> of rotifers and/or nauplii biovolume (STRAŠKRABOVÁ et al., 1999).

#### Phosphatase assays

Two distinct fluorogenic substrates were used for detection of extracellular phosphatase activity. Hydrolysis of 4-methylumbelliferyl phosphate (MUFPP, Sigma) was measured fluorometrically in lakewater samples. Saturation kinetics of bulk acid phosphatase activity (AcPA) was measured in a set of duplicate 4.0 ml subsamples supplemented with 0.5 ml of acetate buffer (*in situ* pH, 1 mmol L<sup>-1</sup> final concentration) and 0.5 ml of MUFPP solution (0.002–20 μmol L<sup>-1</sup> final concentration). Samples were mixed and incubated at *in situ* temperature for 6 to 15 min (for details, see BITTL et al., 2001). Fluorescence was read on a Spekol 11 photometer with a fluorometric device (Zeiss).

Hydrolysis of ELF<sup>®</sup>97 phosphate (ELFP, Molecular Probes) by individual microbial cells was detected according to NEDOMA et al. (2003b). One-mL subsamples of lake water were supplemented with ELFP (20 μmol L<sup>-1</sup> final concentration), mixed, and incubated at 25 °C for 30 min. Then each sample was immediately filtered through 0.8-μm Poretics filter over mild vacuum (< 20 kPa). Filters were inspected in an epifluorescence microscope (Olympus AX-70) and quantified according to NEDOMA et al. (2003b).

#### Statistical analyses

Saturation kinetic data on AcPA were analysed using non-linear regression (Prism 4.0, www.graphpad.com). Data were plotted with the Michaelis-Menten equation,  $v = V_{\max} \times [S]/(K_M + [S])$ ; where  $v$  is velocity of hydrolysis,  $V_{\max}$  is maximum velocity,  $K_M$  is Michaelis constant, and  $[S]$  is substrate concentration.

Table 1. Alphabetical list of abbreviations and symbols used throughout the text.

AcPA	acid phosphatase activity
Al <sub>D</sub>	dissolved aluminium
Al <sub>i</sub>	ionic aluminium
Al <sub>org</sub>	organically bound aluminium
Al <sub>part</sub>	particulate aluminium
Al <sub>T</sub>	total reactive aluminium
ANC	acid neutralising capacity (alkalinity)
Chl- <i>a</i>	Chlorophyll- <i>a</i> concentration
C <sub>part</sub>	particulate organic carbon
DOC	dissolved organic carbon
ELFP	ELF <sup>®</sup> 97 phosphate
HB	heterotrophic microbial biomass (bacteria plus filaments)
K <sub>M</sub>	Michaelis (half-saturation) constant
MUFPP	4-methylumbelliferyl phosphate
N <sub>part</sub>	particulate nitrogen
PB	phytoplankton biomass
P <sub>D</sub>	dissolved phosphorus
P <sub>i</sub>	orthophosphate
P <sub>org</sub>	(dissolved) organic phosphorus
P <sub>part</sub>	particulate phosphorus
P <sub>T</sub>	total phosphorus
SRP	soluble reactive phosphorus
T <sub>t</sub>	turnover time (of MUFPP)
V <sub>max</sub>	maximum velocity (potential acid phosphatase activity)
ZB	zooplankton biomass

Nonparametric tests (Mann Whitney or Kruskal-Wallis with Dunn's multiple comparison test, Prism 4.0) were applied for comparison of seasonal data sets.

All abbreviations and symbols of chemical, biological and enzymatic parameters frequently used throughout the text are summarised in Table 1.

## Results

### Chemistry

Low pH (means of 4.51–5.11), negative ANC (means of -35 to -2 μeq L<sup>-1</sup>), and permanent P depletion (SRP of <1 μg L<sup>-1</sup>; mean molar C<sub>part</sub>:P<sub>part</sub> of 616–1043) in the epilimnion were common features of all lakes studied (Tab. 2). In contrast, the lakes differed in some chemical and plankton parameters, such as P<sub>T</sub> (means of 4.5–13.2 μg L<sup>-1</sup>), Al<sub>T</sub> (means of 193–565 μg L<sup>-1</sup>), bulk extracellular AcPA (mean V<sub>max</sub> of 2.17–6.82 μmol L<sup>-1</sup> h<sup>-1</sup>), and plankton biomass and composition (Tab. 2, Fig. 1). As shown for Plešné Lake, most of the parameters studied have been changing consistently with the epilimnetic pH during lake recovery from acidity (Fig. 2). The most remarkable change was the increase in the seston C<sub>part</sub>:P<sub>part</sub> ratios both in Plešné and Čertovo lakes during the study period (Tab. 2).

The differences in the lakewater composition among the lakes were due to distinct chemistry of their tributaries. Lake water pH and ANC were substantially higher than in tributaries (Tabs 2, 3). The difference in P<sub>D</sub> inlet concentrations into the lakes (Tab. 3) led to the lowest P<sub>D</sub> load for Čertovo Lake, slightly higher

Table 2. Selected characteristics of surface lake water and epilimnetic plankton of three acidified lakes in the Bohemian Forest.

Parameter	Čertovo Lake		Prášilské Lake		Plešné Lake	
	1998 ( <i>n</i> = 8) Apr. 27–Oct. 12	2003 ( <i>n</i> = 8) Apr. 17–Oct. 15	1998 ( <i>n</i> = 7) May 25–Oct. 12	1998 ( <i>n</i> = 8) Apr. 28–Oct. 14	2001 ( <i>n</i> = 6) May 2–Oct. 1	2003 ( <i>n</i> = 7) May 5–Oct. 13
pH	4.51 ± 0.03	4.60 ± 0.10	4.90 ± 0.11	4.83 ± 0.07	5.02 ± 0.13	5.11 ± 0.17
ANC (μeq L <sup>-1</sup> )	-35 ± 7	-26 ± 12	-12 ± 5	-13 ± 4	-4 ± 4	-2 ± 4
DOC (mg L <sup>-1</sup> )	2.22 ± 0.50	2.57 ± 0.58	3.88 ± 0.89	2.36 ± 0.79	3.39 ± 0.70	3.46 ± 1.01
C <sub>part</sub> (mg L <sup>-1</sup> )	0.74 ± 0.22	0.98 ± 0.54	0.75 ± 0.40	2.28 ± 0.79	3.13 ± 0.61	3.83 ± 0.96
C <sub>part</sub> :P <sub>part</sub> (molar)	665 ± 207	817 ± 279	616 ± 205	769 ± 238	871 ± 173	1043 ± 575
N <sub>part</sub> :P <sub>part</sub> (molar) <sup>a</sup>	59 ± 26	72 ± 29	62 ± 62	78 ± 22	77 ± 11	67 ± 37
P <sub>D</sub> (μg L <sup>-1</sup> )	1.6 ± 0.2	1.5 ± 0.8	2.9 ± 0.8	1.3 ± 0.5	2.4 ± 0.3	2.5 ± 0.8
SRP (μg L <sup>-1</sup> ) <sup>b</sup>	<1	<1	<1	<1	<1	<1
P <sub>part</sub> (μg L <sup>-1</sup> )	3.0 ± 1.0	3.4 ± 2.0	3.6 ± 2.3	7.6 ± 1.0	9.3 ± 0.5	10.7 ± 3.1
P <sub>T</sub> (μg L <sup>-1</sup> )	4.5 ± 0.6	4.9 ± 2.2	6.4 ± 1.8	8.9 ± 1.2	11.7 ± 0.6	13.2 ± 2.4
Al <sub>D</sub> (μg L <sup>-1</sup> )	537 ± 39	365 ± 80	145 ± 47	373 ± 107	283 ± 103	215 ± 152
Al <sub>org</sub> (μg L <sup>-1</sup> )	28 ± 20	48 ± 24	68 ± 28	36 ± 27	99 ± 28	88 ± 35
Al <sub>i</sub> (μg L <sup>-1</sup> )	509 ± 29	318 ± 61	77 ± 29	337 ± 92	184 ± 94	127 ± 121
Al <sub>part</sub> (μg L <sup>-1</sup> )	28 ± 34	54 ± 36	48 ± 25	144 ± 46	210 ± 74	257 ± 87
Al <sub>T</sub> (μg L <sup>-1</sup> )	565 ± 24	420 ± 49	193 ± 27	517 ± 75	492 ± 33	473 ± 82
Chl- <i>a</i> (μg L <sup>-1</sup> )	2.65 ± 0.91	4.81 ± 3.36	4.32 ± 1.89	8.76 ± 6.47	23.1 ± 14.2	17.9 ± 2.7
PB (μg C L <sup>-1</sup> ) <sup>c</sup>	98(42%)	ND	188(69%)	466(79%)	744(84%)	ND
HB (μg C L <sup>-1</sup> ) <sup>c</sup>	137(58%)	ND	84(31%)	127(1)	146(16%)	ND
PB + HB (μg C L <sup>-1</sup> )	235	ND	272	593	890	ND
AcPA (μmol L <sup>-1</sup> h <sup>-1</sup> )	2.20 ± 1.02	6.13 ± 5.78	2.17 ± 1.06	5.39 ± 2.41	6.80 ± 2.61	6.82 ± 1.97

Explanations: Mean ± standard deviation; *n* – number of samples; ND – not determined; <sup>a</sup> N<sub>part</sub> – particulate nitrogen (for methods see KOPACEK et al., 2004); <sup>b</sup> in most cases, SRP below detection limit; <sup>c</sup> in parentheses as % of the total microbial biomass (PB + HB = 100%).

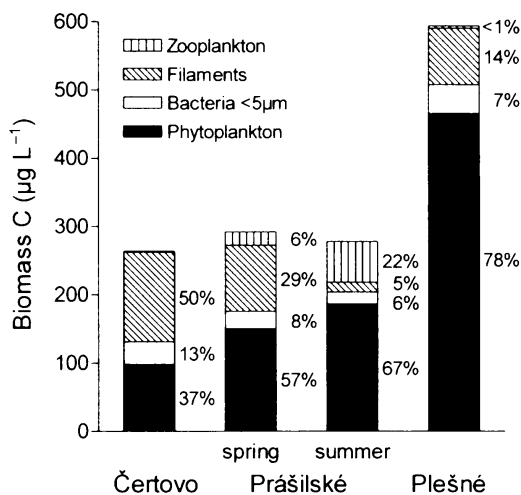


Fig. 1. Comparison of plankton biomass in the epilimnion of three Bohemian Forest lakes. Total biomass (seasonal mean) and its composition (% of total biomass) was relatively stable in Čertovo and Plešné lakes (both with negligible zooplankton biomass) throughout the season (May–September 1998). In contrast, it changed markedly in Prášilské Lake as the consequence of zooplankton development (spring = May–early July, summer = August – September; for details, see Fig. 1 in VRBA et al., 2003b).

for Prášilské Lake but several times higher for Plešné Lake. Another difference among the lakes was in Al<sub>D</sub> in-

let concentrations, with the lowest values for Prášilské Lake.

The studied lakes clearly differed in the subsequent fate of the incoming Al<sub>i</sub>. While Al<sub>T</sub> concentrations in the lakes were relatively stable, Al speciation changed seasonally, following changes in the lakewater pH. The lowest pH, and accordingly the highest Al<sub>i</sub> concentration, occurred in the lakes during spring snowmelt. The maximum Al<sub>i</sub> concentrations after the 1998 spring overturn reached ~120, ~520 and ~550 μg L<sup>-1</sup> in the epilimnion of Prášilské, Plešné and Čertovo lakes, respectively. While high Al<sub>i</sub> concentrations remained roughly stable in Čertovo Lake due to its stable pH throughout the 1998 season, the seasonal increase in lakewater pH (compared to tributaries, Tab. 3) caused the massive formation of Al<sub>part</sub> in the Plešné epilimnion (Tab. 2). Little formation of Al<sub>part</sub> was observed in Prášilské Lake, due to almost one-order-of-magnitude lower Al<sub>i</sub> concentrations (Tab. 2).

Small differences between P concentrations in tributaries and lake water were observed in Prášilské and Čertovo lakes, with the low formation of Al<sub>part</sub> (Tabs 2, 3). In contrast, mean epilimnetic P<sub>D</sub> concentrations were ~10–20-times lower in Plešné Lake than in its tributaries, and SRP concentrations were <1 μg L<sup>-1</sup> throughout the year (Tab. 2).

Average Al<sub>T</sub> concentrations in the Plešné epil-

## Aluminium affects food webs in acidified lakes

Table 3. Selected chemical characteristics of inlet water of the Bohemian Forest lakes.

Parameter	Čertovo Lake	Prášílské Lake	Plešné Lake
pH	4.25 ± 0.03	4.69 ± 0.19	4.39 ± 0.03
ANC ( $\mu\text{eq L}^{-1}$ )	-55 ± 4	-23 ± 12	-48 ± 1
DOC ( $\text{mg L}^{-1}$ )	5.5 ± 1.8	6.21 ± 2.3	8.3 ± 0.8
$P_D$ ( $\mu\text{g L}^{-1}$ )	2.8 ± 1.0	5.1 ± 1.0	21.4 ± 0.8
SRP ( $\mu\text{g L}^{-1}$ ) <sup>a</sup>	0.6 ± 0.3 (21%)	0.9 ± 0.9 (18%)	17.3 ± 1.1 (81%)
$P_{\text{part}}$ ( $\mu\text{g L}^{-1}$ )	1.1 ± 0.8	0.8 ± 0.7	1.4 ± 0.9
$P_T$ ( $\mu\text{g L}^{-1}$ )	3.9 ± 1.5	5.9 ± 1.6	22.9 ± 0.6
$Al_D$ ( $\mu\text{g L}^{-1}$ )	608 ± 42	194 ± 69	742 ± 19
$Al_{\text{org}}$ ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>	160 ± 41 (26%)	129 ± 38 (67%)	284 ± 11 (38%)
$Al_i$ ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>	448 ± 60 (74%)	65 ± 34 (33%)	458 ± 20 (62%)
$Al_{\text{part}}$ ( $\mu\text{g L}^{-1}$ )	9 ± 5	10 ± 13	19 ± 8
$Al_T$ ( $\mu\text{g L}^{-1}$ )	617 ± 44	204 ± 69	761 ± 16

Explanations: Prášílské Lake = seasonal mean ± standard deviation (SD) for 7 samplings (May 25 – October 12) in 1998; Čertovo Lake = mean ± SD of annual averages for the 1998–2003 period; Plešné Lake = mean ± SD of annual averages for the 2000–2003 period; <sup>a</sup> in parentheses as % of  $P_D$ ; <sup>b</sup> in parentheses as % of  $Al_D$ .

innion did not change significantly between 1998 and 2003. Contrary, a significant drop in  $Al_i$  and increase in  $Al_{\text{part}}$  concentrations occurred there during this period (Figs 2E–G) due to changes in pH (Fig. 2A). These changes were accompanied by a significant increase in epilimnetic  $P_T$  concentrations in Plešné Lake (Fig. 2B), despite the stable level and low inter-annual  $P_T$  variability in the tributaries (Tab. 3). In Čertovo Lake, we documented a significant drop in  $Al_T$  concentrations (followed by a significant decrease in  $Al_i$  concentrations) and increase in the variability of  $P_T$  concentrations between 1998 and 2003 (Tab. 2).

### Plankton

Zooplankton were negligible in terms of biomass both in Čertovo and Plešné lakes. Contrary, ZB in Prášílské Lake accounted for <10% in spring but >20% of the total biomass in the late summer (Fig. 1). Both protozoa (data not shown; for the 1998 season, see MACEK, 2002) and rotifers were usually scarce, accounting for a negligible proportion of the planktonic biomass in all lakes. Proportions of mean carbon biomass of both dominant plankton groups, i.e., phytoplankton (PB) and heterotrophic bacteria (HB), as well as their composition differed markedly among the lakes (Fig. 1). Mean Chl-*a* values were roughly proportional to mean PB (Tab. 2) but their trends differed. Total plankton biomass (the sum of PB and HB) in Plešné Lake was two-times higher than in the other lakes (Tab. 2). We also observed a significant increase in Chl-*a* and both absolute and relative increase in PB in Plešné Lake between 1998 and 2001 (Fig. 2C–D). Also in Čertovo Lake, a similar, but insignificant ( $P > 0.5$ ), trend in Chl-*a* was evident between 1998 and 2003 (Tab. 2).

PB always accounted for >50% of total biomass in Prášílské Lake and averaged ~80% in Plešné Lake. In contrast, HB always exceeded PB in Čertovo Lake (Fig. 1). The same phytoplankton species (mostly flagellates, cf. NEDBALOVÁ et al., 2006) generally occurred in all three lakes, but their dominance dif-

fered. In Čertovo Lake, two dinoflagellates, *Gymnodinium uberrimum* (Allman) Kofoid et Sweezy and *Peridinium umbonatum* Stein, dominated and *Dinobryon* spp. (Chrysophyceae) formed a summer epilimnetic maximum (for details, see VRBA et al., 2003b). *G. uberrimum* dominated the phytoplankton of Prášílské Lake during the whole 1998 season. The phytoplankton of Plešné Lake differed markedly from those of the other lakes. The species structure of its phytoplankton was singular with a predominance of non-motile forms: *Monoraphidium dybowskii* (Woloszynska) Hindák et Komárková-Legnerová (Chlorophyceae) accounted for ~50–80% of PB, accompanied by two filamentous cyanobacteria (*Pseudanabaena* sp. and *Limnnothrix* sp.) and *P. umbonatum*.

Filamentous microorganisms dominated the bacterioplankton of all three lakes. They were extremely long (usually >100  $\mu\text{m}$ ) and belonged mostly to Eubacteria as described elsewhere (VRBA et al., 2003b). The filaments contributed on average to ~70–80% of HB in the epilimnion of all lakes except for the late summer period in Prášílské Lake (Fig. 1).

### Extracellular phosphatases

Concomitantly with the highest  $C_{\text{part}}:P_{\text{part}}$  and  $N_{\text{part}}:P_{\text{part}}$  ratios, the highest AcPA were found in Plešné Lake (Tab. 2). Generally for all lakes in 1998,  $V_{\text{max}}$  values were in the range of 0.7–9.9  $\mu\text{mol L}^{-1} \text{h}^{-1}$  and the apparent Michaelis constant ( $K_M$ ) ranged from 1.0–5.8  $\mu\text{mol L}^{-1}$ . The turnover time ( $T_t = K_M/V_{\text{max}}$ ) of MUFP, usually shorter than one hour (range of 0.2–3.0 h), suggested a high planktonic need for P in all lakes.

Compared to 1998, the AcPA values increased in Plešné and Čertovo lakes during the study (Tab. 2). While the maximum values of 2001 and 2003 did not differ much from those of 1998 in Plešné Lake (Fig. 2), the maximum AcPA in Čertovo Lake in 2003 (16.8  $\mu\text{mol L}^{-1} \text{h}^{-1}$ ) increased more than four times compared to 1998 and twice (May–June 2003) even exceeded those of Plešné Lake.

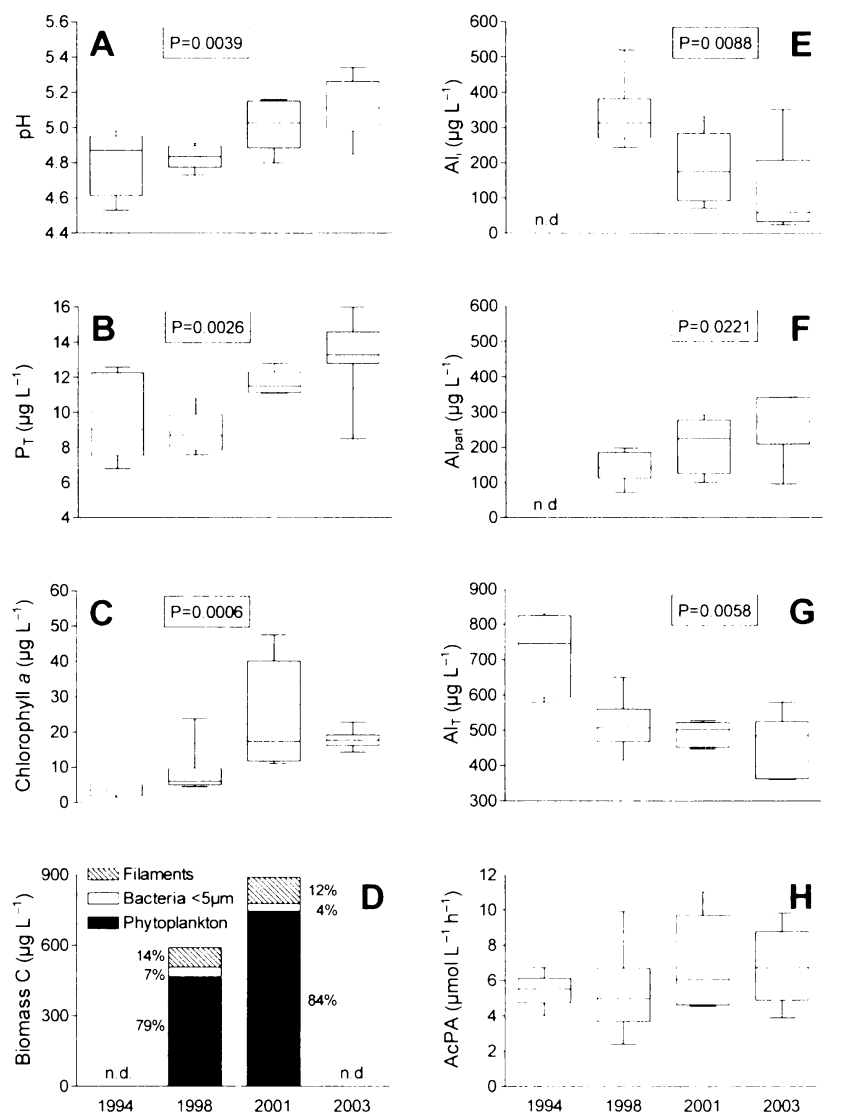


Fig. 2. A comparison of trends in the selected parameters in the epilimnion of Plešné Lake during four seasons of the last decade (the 1994 data come from HEJZLAR et al., 1998). A–C, E–H: median, 25% and 75% quartiles (box), minimum and maximum (bars; framed are significant P values, Kruskal-Wallis test), note the same absolute scaling for Al forms (E–G); D - total plankton biomass and its composition (%; as in Fig. 1); n.d. - not determined.

Direct microscopic detection of extracellular phosphatases with ELFP (during 2003) showed that AcPA was not evenly distributed, neither in phytoplankton nor in bacterioplankton of Plešné and Čertovo lakes (cf. NEDOMA & VRBA, 2006; ŠTROJŠOVÁ & VRBA, 2006). Phosphatase sites were usually detectable on heterotrophic filaments, some bacteria, both filamentous cyanobacteria, and certain algal species, in particular *M. dybowskii*, *Koliella corcontica* Hindák, *Chlorogonium fusiforme* Matwienko (all Chlorophyceae), *Chlamydomonas* sp. (Chlamydomophyceae), *Spiniferomonas abei* Takahashi, *Mallomonas* sp. (Chrysophyceae), *P. umbonatum* and *G. uberrimum*. On the other hand, many bacteria and other algae, like *Dinobryon* spp. or *Cryptomonas* spp., did not exhibit any AcPA at all.

## Discussion

This study is a comprehensive synthesis of data sets that have mostly been published elsewhere (Tab. 4). Regular data on lake water chemistry of the three Bohemian Forest lakes have shown the overall behaviour of dimictic lakes, but with some specific features due to their strong acidification. Full description of seasonal dynamics of each lake, however, goes far beyond this study, focused on the role of Al in P availability and plankton dynamics. Globally, the Bohemian Forest lakes represent a rather extreme situation, with Al levels much higher than found in most acidified lakes worldwide. Therefore, the lakes offer a unique opportunity for studying the complex effects of Al on the

Table 4. List of references providing additional information on the lakes under study.

Year	Čertovo Lake	Prášílské Lake	Plešné Lake
1998	KOPÁČEK et al. (2000b) <sup>a</sup> ; MACEK (2002) <sup>b</sup> ; VRBA et al. (2003b) <sup>c</sup>	KOPÁČEK et al. (1999) <sup>a</sup> ; MACEK (2002) <sup>b</sup> ; VRBA et al. (2003b) <sup>c</sup>	KOPÁČEK et al. (2000a) <sup>a</sup> ; MACEK (2002) <sup>b</sup> ; VRBA et al. (2003b) <sup>c</sup>
2001	KOPÁČEK et al. (2006a) <sup>a</sup>		KOPÁČEK et al. (2004) <sup>a</sup>
2003	KOPÁČEK et al. (2006a) <sup>a</sup> ; NEDBALOVÁ et al. (2006) <sup>d</sup> ; NEDOMA & VRBA (2006) <sup>c</sup>	NEDBALOVÁ et al. (2006) <sup>d</sup>	KOPÁČEK et al. (2006b) <sup>a</sup> ; NEDBALOVÁ et al. (2006) <sup>d</sup> ; ŠTROJSOVÁ & VRBA (2006) <sup>c</sup>

Explanations: <sup>a</sup> seasonal (annual) stratification of lakewater chemistry; <sup>b</sup> seasonal stratification of ciliates; <sup>c</sup> seasonal plankton development; <sup>d</sup> September inter-lake comparison; <sup>e</sup> cell- or species-specific AcPA (ELFP assay).

plankton and its potential for recovery from acid stress. When evaluated separately, the AcPA data were too puzzled, without any clear correlation, neither with the seasonal development nor with the vertical distribution of plankton biomass (HB or PB) and/or Al speciation in the water column. Thus, we took advantage of this natural 'experimental lake area' and compared the average seasonal data from each lake on both inter-lake and inter-annual bases.

The average  $C_{\text{part}}:P_{\text{part}}$  (616 1043) and  $N_{\text{part}}:P_{\text{part}}$  (59 78) molar ratios in the epilimnetic seston of the studied lakes were substantially higher than the average particulate C:P (306) and N:P (24) molar ratios of temperate lakes studied by HECKY et al. (1993), indicating much stronger P deficiency of the plankton in the Bohemian Forest lakes. In accordance with the extreme AcPA and  $T_t < 1$  h, the highest  $C_{\text{part}}:P_{\text{part}}$  and  $N_{\text{part}}:P_{\text{part}}$  ratios indicated the highest P limitation in Plešné Lake (Tab. 2), despite of the highest inlet P concentration among the lakes (Tab. 3). Surprisingly, the seston  $C_{\text{part}}:P_{\text{part}}$  ratios even increased in Plešné and Čertovo lakes between 1998 and 2003. This increase most probably reflected the recovery of phytoplankton in these lakes, i.e., an increase in PB with less homeostatic stoichiometry compared to HB (cf. STERNER & ELSER, 2002). The present AcPA exceeds most of the published activities of extracellular phosphatases reviewed by BITTL et al. (2001) and clearly indicates a persisting microbial struggle for P regeneration in the studied lakes.

#### Aluminium effects

We hypothesise that Al may govern the P availability in the acidified lakes, as well as the food web structure through the consumers' and resources' stoichiometry (STERNER & ELSER, 2002). There are at least the following three major effects of Al on the plankton structure and dynamics in these ecosystems: (i) direct toxicity of Al for aquatic biota, (ii) inhibition of extracellular phosphatases by Al<sub>i</sub>, and (iii) inactivation of P by Al<sub>part</sub>. While this element is the most abundant metal in the lithosphere with a potential impact on biochemical evolution, its biogeochemistry in natural waters has been overlooked for a long time (EXLEY, 2003).

Toxicity of aqueous Al species (e.g., DRISCOLL, 1985; HÖRNSTRÖM et al., 1984; HAVAS & ROSELAND, 1995) played an important role in the formation of the current food web structure of the Bohemian Forest lakes. The Al toxicity was the most likely cause of fish and zooplankton extinction during the acidification progress. This effect, however, differed among the lakes and crustacean species had survived in the less toxic water of Prášílské Lake (FOTT et al., 1994) with the lowest terrestrial Al inputs (Tab. 3). On the other hand, this lower Al input has clearly affected the less severe plankton stoichiometry (i.e., low seston C:P) in this lake compared to the others (Tab. 2, this study; NEDBALOVÁ et al., 2006), which might have been critical for the survival of, in particular, *Daphnia longispina* (cf. STERNER & ELSER, 2002).

Several authors have already suggested the negative effects of Al<sub>i</sub> on AcPA (JANSSON, 1981; JANSSON et al., 1986; JOSEPH et al., 1995) or on the entire P metabolism of an alga (RAI et al., 1998). In laboratory experiments with artificial lake water, BITTL et al. (2001) observed a synergistic effect of pH (between 4.0–5.2) and Al (0–1000 µg L<sup>-1</sup>) on AcPA of the plankton >2 µm (on filters) from Plešné Lake. They found a significant reduction of AcPA in the presence of Al<sub>i</sub> compared to the control without Al and suggested a direct inhibitory effect (competitive or non-competitive) of Al<sub>i</sub> on extracellular phosphatases. Thus, such an inhibitory effect of Al<sub>i</sub> on AcPA depended on both Al<sub>i</sub> concentration and pH, and should be even more pronounced at the lower natural substrate concentrations in the lake water (BITTL et al., 2001). If the competitive effect of Al<sub>i</sub> controlled AcPA, a drop in Al<sub>i</sub> should be accompanied by a decrease in AcPA due to reduced P deficiency. In other words, a release from the Al<sub>i</sub> inhibitory effect should reduce microbial overproduction of the extracellular phosphatases (JANSSON, 1981). However, such a general correlation was not observed in the lakes under study, where the conversion of Al<sub>i</sub> to Al<sub>part</sub> was likely pH-controlled at relatively stable Al<sub>T</sub> concentrations.

On the other hand, natural P immobilisation by Al<sub>part</sub> has been recently recognised as a common and important mechanism in any water body with elevated Al input and a pH gradient between its inlet and outlet (KOPÁČEK et al., 2004). The controlling effects of Al

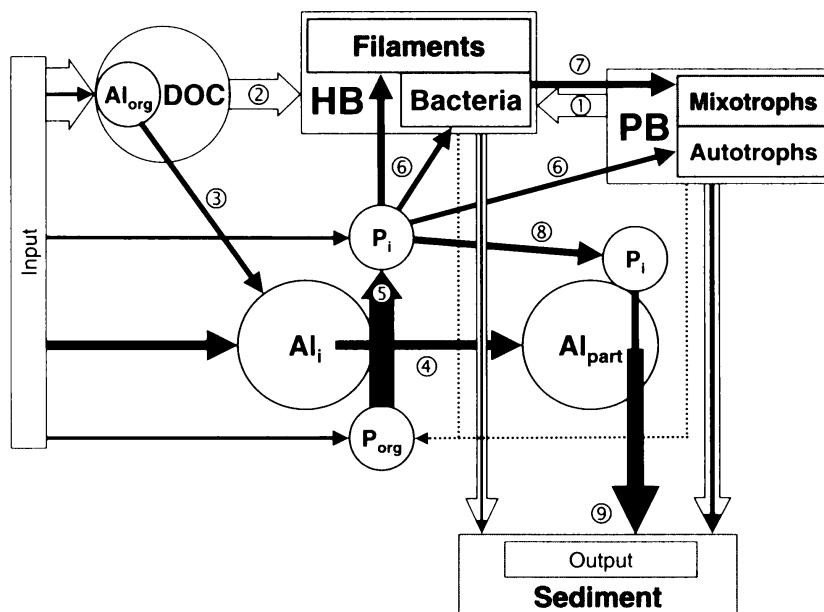


Fig. 3. A chart flow model of major fluxes and relationships between carbon, phosphorus and aluminium forms in the pelagic zone (epilimnion) of acidified lakes (for abbreviations, see Tab. 1). Black, grey and white arrows from left indicate major inputs of P, Al and DOC, respectively, from terrestrial and atmospheric sources. The arrows' thickness and blocks' size indicate the relative importance of particular fluxes and chemical forms or microbial biomass, respectively. Their actual proportions, however, differ among the lake studied (see Tabs 2 and 3, Fig. 1, and the text for details). The major processes involved are numbered as follows: 1 – Photosynthesis supplies autochthonous DOC; 2 – Summer photodegradation of allochthonous DOC liberates additional substrate for bacterioplankton (HB); 3 – The same mechanism liberates  $Al_i$  from  $Al_{org}$ ; 4 – pH (in-lake ANC generation) controls further hydrolysis of  $Al_i$  to  $Al_{part}$ ; 5 – Enzymatic hydrolysis of  $P_{org}$  by extracellular phosphatases represents an overall mechanism of  $P_i$  regeneration in the P-deficient lakes that is impaired as well by  $Al_i$  at pH < 5, however, most of AcPA actually is largely species-specific and localised on the surfaces of microbial cells; 6 – Bacterioplankton are superior to phytoplankton (PB) in  $P_i$  uptake at its low concentrations; 7 – Phagotrophic mixotrophs take advantage of bacterivory in P acquisition; 8 –  $P_i$  binding to fresh  $Al_{part}$  represents an important mechanism of abiotic in-lake P immobilisation; 9 – Sedimentation of  $Al_{part}$ -P complexes represents a significant P loss from the water column (besides an outlet output). While the lake sediments represent the major sink of P, both filaments and mixotrophs may represent other (seasonal) P sinks in the plankton biomass.

were superior in Plešné Lake; both  $Al_i$  and  $Al_{part}$  were important in governing P availability (and thus AcPA) due to high input and variable in-lake speciation of Al (Tabs 2, 3). The AcPA levels, however, reflected both the reduced P availability (more phosphatase is needed to gain P at elevated Al concentrations) and the amount of microorganisms. Consequently, the highest AcPA in Plešné Lake resulted also from the highest abundance of phytoplankton due to the highest P and SRP inputs into this lake (cf. PB and total plankton biomass in Fig. 1).

In contrast to Plešné Lake, heterotrophic microorganisms dominated the plankton and were the major phosphatase producers in Čertovo Lake, with low seasonal and spatial variation in the lake chemistry (conservative Al speciation) and small seasonal plankton dynamics. Apart of decreasing  $Al_T$  concentrations, the P immobilisation by  $Al_{part}$  has become more powerful during a lake recovery (due to a more pronounced pH gradient) and apparently enhanced AcPA (cf. 1998 and 2003 in Tab. 2).

There was neither any significant relationship between bulk AcPA to plankton biomass, nor any correlation between biomass-specific AcPA with either PB or HB, in any of the lakes. The qualitative microscopic

detection (using ELFP) showed obvious differences in species-specific AcPA, which may explain such a weak relationship. Only part of the present microbial populations expressed extracellular phosphatases, as suggested by two 2003 seasonal studies on (i) group-specific AcPA in the bacterioplankton of Čertovo Lake (NEDOMA & VRBA, 2006), and (ii) cell-specific AcPA in the *M. dybowskii* population of Plešné Lake (ŠTROJSOVÁ & VRBA, 2006). These results showed that, besides the green alga *M. dybowskii* in Plešné Lake, certain distinct bacterial morphotypes usually were the major producers of extracellular phosphatases, while only some phytoplankton species were active but not always those dominant ones. RENGEFORS et al. (2001) and ŠTROJSOVÁ et al. (2003) reported similar observations in P-deficient phytoplankton of an oligotrophic lake and a eutrophic reservoir, respectively.

The formation of  $Al_{part}$  strongly affected both AcPA and seston stoichiometry, suggesting that this process controlled availability, regeneration and cycling of P in the acidified lakes. When acidification peaked in the Bohemian Forest lakes (pH ~ 4.5 and  $Al_T$  ~ 1 mg L<sup>-1</sup>, VESELÝ et al., 1998), planktonic microorganisms likely released more extracellular phosphatases to overcome an inhibitory effect of  $Al_i$ . Circumstances prob-



ably have become even more complex during the recent reversal of the lake chemistry from acidity (Fig. 3). At  $\text{pH} > 5$ , the in-lake concentration of  $\text{Al}_i$ , supplied by more acidic inlets and by photochemical liberation of  $\text{Al}_{\text{org}}$  (KOPÁČEK et al., 2005), decreases due to  $\text{Al}_i$  hydrolysis, thus increasing the pool of  $\text{Al}_{\text{part}}$  that binds  $\text{P}_i$  released by the phosphatases. We suppose that the P metabolism of plankton microorganisms is most severely stressed at  $\text{pH} \sim 5$  due to the simultaneous effects of both  $\text{Al}_i$  and  $\text{Al}_{\text{part}}$ , occurring at the 4.5–5.2 pH range. Such critical pH gradients are actually common between inputs and outputs of all the lakes studied.

#### *Phosphorus cycling and food web structure in the pelagic zone*

We suggest that the plankton (i.e., total biomass, its allocation in HB and PB, presence of zooplankton, species diversity, stoichiometry, and food web structure) are influenced by the interplay of Al effects and P availability. Both primary and bacterial production increased along a trophic gradient of the lakes: Čertovo–Prášilské–Plešné (NEDOMA et al., 2003a). On seasonal average (1998), phytoplankton exudation accounted for 18%, 17%, and 11% of total primary production, respectively, and could hardly cover the carbon demand of bacterioplankton in any lake (NEDOMA et al., 2003a). Unlike most freshwater lakes, filamentous microorganisms dominate bacterioplankton of the Bohemian Forest lakes. The abundance of unicellular bacteria (range:  $0.5\text{--}1.2 \times 10^6$  cells  $\text{mL}^{-1}$ , see VRBA et al., 1996, 2003b; HEJZLAR et al., 1998), however, matches common values of oligotrophic to mesotrophic lakes. Epilimnetic bacteria, present in high biomass, utilise also photodegraded allochthonous DOC (KOPÁČEK et al., 2003; PORCAL et al., 2004). Both low P supply (or availability) and allochthonous DOC input can alternate or interplay in the bottom-up control of the plankton dynamics in the lakes (VRBA et al., 2003b). On the other hand, any top-down control of the pelagic community by insect top-predators is unlikely; it cannot explain the very low ZB neither in Čertovo nor in Plešné lakes (cf. NEDBALOVÁ et al., 2006).

Generally, the less  $\text{P}_T$  in the studied lake, the higher was the proportion of HB to the plankton biomass. In Čertovo and Prášilské lakes with lower  $\text{P}_T$  inputs, phototrophic and mixotrophic flagellates dominate PB, likely taking advantages from their motility and/or phagotrophy (VRBA et al., 2003b). Contrary, the higher  $\text{P}_T$  and/or SRP input to Plešné Lake likely favours some non-motile algae (*Monoraphidium dybowskii*, *Koliella corcontica*) and filamentous cyanobacteria, which slowly sediment from the euphotic layer during the summer stagnation period. Hence they form conspicuous hypolimnetic maxima of both Chl-*a* and PB (NEDBALOVÁ & VRTIŠKA, 2000) and even AcPA (HEJZLAR et al., 1998). The sedimenting PB represents the dominant (biotic) P loss from the water column. This mechanism is of a great importance in Plešné Lake,

representing a net sink of all nutrients and removing  $\sim 50\%$  of TP and DOC (KOPÁČEK et al., 2004, 2006b).

The dominance of *Monoraphidium dybowskii* and cyanobacteria in the acidified lake is not usual. Another cyanobacterium, *Merismopedia tenuissima* Lemmermann, accompanied by *Monoraphidium griffithii* (Berkeley) Komárková-Legnerová and *M. dybowskii* were found in some Swedish lakes with pH 6.0–6.5 (ROŠÉN, 1981; BLOMQUIST et al., 1993). *M. dybowskii* was also reported from acid lakes ( $\text{pH} < 5.3$ ) with an especially rich nutrient supply (HÖRNSTRÖM et al., 1984). Its importance in acid waters was explained by the fact that its growth was not affected by high concentrations of toxic Al (HÖRNSTRÖM et al., 1995). All these features, in particular the high terrestrial inputs of both Al and P, fully applied to Plešné Lake as well. On the other hand, the dominance of *Pseudanabaena* sp. and *Limnothrix* sp. has so far never been recorded in acidified lakes.

The high proportion of HB (typically  $> 40\%$ ) in the studied lakes resulted in significant amounts of P retained in the bacterioplankton and, thus, more favourable C:P stoichiometry (Tab. 2). Some mixotrophic flagellates, in particular *Dinobryon* spp., most likely covered their P demand by phagotrophy on bacterioplankton as suggested by JANSSON et al. (1996). This alternative strategy of 'eating their competitors' (THINGSTAD et al., 1996) was successful in the epilimnion of Čertovo and Prášilské lakes during the 1998 season when the *Dinobryon* grazing on single-celled bacteria drove the bacterioplankton to the cell elongation and characteristic filament formation (VRBA et al., 2003b). In the absence of zooplankton, these filaments dominating HB represented an important sink of P for other pelagic microorganisms in the Bohemian Forest lakes. Consequently the defence strategy of bacterioplankton provided another benefit in their competition for P.

#### *Plankton recovery*

The rapid decrease in  $\text{Al}_T$  concentrations (by  $\sim 50\%$  during the last two decades, (VESELÝ et al., 1998) is the most significant change in lakewater chemistry of the Bohemian Forest lakes during their reversal from acidity. If Al is the major factor stressing the lake ecosystems, we should observe certain biological improvements after such a decline in Al concentration. It is worth mentioning in this context that Al concentrations in the Bohemian Forest lakes peaked at higher values than in other acidified lake districts (e.g., JANSSON et al., 1986) and the recent  $\text{Al}_T$  concentrations are still very high (Tab. 2). Hence, P cycling remains limited in the lakes. Nevertheless, this study suggests a phytoplankton recovery in Plešné Lake (Figs 2C, D). The changes in lakewater chemistry (higher pH and reduced  $\text{Al}_i$  toxicity) and increasing food resources (PB) were accompanied by a significant increase (by 2–3 orders of magnitude during the 1990s)

in the numbers of pelagic rotifers (NEDBALOVÁ et al., 2006).

Overall, our results appear to be of particular interest in the debate on possible oligotrophication during acidification. OLSSON & PETERSSON (1993) critically reviewed relevant hypotheses and concluded that 'acidification, generally, did not result in significantly decreased P concentrations or phytoplankton production'. If the term oligotrophication concerns the  $P_T$  content or the total plankton biomass in the lake, then we could hardly prove any oligotrophication (at pH between 5.5 and 4.5). On the other hand, if the term is restricted to PB or Chl-*a*, some 'oligotrophication by acidification' or 'eutrophication by recovery' was observed similarly to some cases reported by OLSSON & PETERSSON (1993). Unlike the cases reviewed by SCHINDLER (1994), our data suggest that the internal P cycle of the lakes can be disrupted by acidification because Al affects P availability and utilisation by the plankton. The 2001-2003 results from Plešné Lake have revealed a significant increase in  $P_T$  concentrations, likely caused by higher PB (Fig. 2); however, the impaired resources' stoichiometry may prevent a recovery of cladoceran zooplankton. We argue that many former acidification studies have neglected or overlooked the role of bacterioplankton at all. We have to emphasise in this context that neither the decline in the number of phytoplankton species nor the appearance of benthic algal mats (SCHINDLER, 1994) were observed in the lakes under study. On the other hand, the heterotrophic filaments in plankton are characteristic for the acidified Bohemian Forest lakes (VRBA et al., 2003a, b).

To conclude, the present study demonstrates the key role of aluminium in overall phosphorus availability, food web structure, plankton dynamics, and recent recovery of chronically acidified lakes of the Bohemian Forest. The inter-lake comparison suggests that the Al-driven processes seriously affect stoichiometry of resources and consumers, as well as functional diversity of the acidified ecosystems.

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## Massive occurrence of heterotrophic filaments in acidified lakes: seasonal dynamics and composition

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### Abstract

We documented permanent presence of heterotrophic filaments in three acidified lakes (pH < 5) in the Bohemian Forest. Due to acidification, crustacean zooplankton were absent in all but one lake. In terms of carbon flow, microorganisms were thus almost exclusive players in the pelagic food webs. Variety of extremely long (> 100 µm) heterotrophic filaments occurred in the lakes. The filaments usually accounted for > 50% of total heterotrophic microbial biomass in the pelagic zone (medians of the total biomass: 82–108 µg C l<sup>-1</sup>), except for anoxic bottom layers and for episodic appearance of *Daphnia longispina* in one lake. Seasonal filament formation was mainly induced and maintained by grazing of mixotrophic flagellates (*Dinobryon* spp.) on small unicellular bacteria in the absence of cladoceran filtration. The filaments were less active than unicellular suspended bacteria. Fluorescence in situ hybridisation revealed that usually < 50% of total bacteria were targeted with oligonucleotide probes for Eubacteria.

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**Keywords:** Bacterioplankton; Phylogenetic identification; Heterotrophic filament; Bacterial activity; *Dinobryon* spp.; *Daphnia longispina*

### 1. Introduction

Recent limnological literature has listed an increasing number of freshwater environments where filamentous microorganisms comprise a significant part of pelagic microbial biomass. Forces, like protistan grazing inducing the development and importance of grazing-resistant bacteria, have been recognised in planktonic systems (see [1,2] for review). The size distribution and composition of planktonic bacteria, and particularly the appearance of filaments has come into the focus of aquatic microbial ecology in the context of studies of predator–prey interactions [3,4]. The temporary occurrence of the filaments has been observed in most aquatic ecosystems, in particular as the consequence of an increased grazing pressure of bacteriv-

orous protists and/or absence of filter-feeding cladocera [5–9]. It has been shown in the laboratory [3,10–12] as well as in field experiments [13–15] that the filamentous morphotypes are adaptations of some microbes to enhanced protistan grazing, but there are probably numerous other reasons for bacteria to elongate far beyond their typical sizes [16].

However, it is under debate whether such a protistan grazing pressure selects rather for phenotypical adaptations or genotypical shifts in the bacterioplankton. Many grazing-induced morphological changes observed in the bacterioplankton were found to be accompanied by changes in taxonomic community structure and bacterial activity (see [2] for review). The grazing-resistant filaments were affiliated to certain taxonomic groups, such as the alpha- and beta-subclasses of Proteobacteria or the *Cytophaga-Flavobacterium* cluster [11,13–17]. On the other hand, according to our knowledge, no substantial information has yet been published on partitioning of microbial activity between small unicellular bacteria and filaments.

Only a few papers have reported on the permanent oc-

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currence of the filaments in freshwater ecosystems, such as a hypertrophic lake [18], a high mountain lake [16,17], or anthropogenically acidified lakes [19]. In all the cases, negligible cladoceran filtration was found in parallel.

Eight small acidified lakes in the Bohemian Forest (Central Europe) are rich in filamentous bacteria and represent very unusual ecosystems. They were exposed to strong atmospheric deposition of sulphur and nitrogen compounds in the second half of the last century and, consequently, fish became extinct and zooplankton diminished in most of the lakes (e.g. [20]). Consequently, microorganisms have dominated pelagic food webs and plankton biomass of the lakes. The filaments have formed a conspicuous and characteristic component of the plankton [19], though their quantification was rather difficult due to methodological obstacles. Only recently a suitable tool has emerged by adopting the line intercept method for appropriate quantification of such very long filaments [21]. Despite a certain reversal of the lake water chemistry from acidification during the last ~15 years, biological recovery of the plankton in most of the Bohemian Forest lakes seems to be significantly delayed [20].

Currently, the Bohemian Forest lakes offer a unique set of natural aquatic environments with highly specific dynamics of the bacterioplankton in the context of various predator–prey interactions. This study reports on the massive occurrence of filaments and their dynamics in relation to either presence or absence of both cladoceran filtration and protistan grazing. Also possible impacts of other environmental factors are discussed. We present here the first data on phylogenetic bacterioplankton composition in the Bohemian Forest lakes and on partition of bacterial activity between different size fractions.

## 2. Materials and methods

### 2.1. Study sites and sampling

We studied three small lakes of glacial origin in the Bohemian Forest (Šumava Mts., Böhmerwald): Čertovo, Plešné, and Prášilské (dimictic; areas: 10.3, 7.5, and 4.2 ha; volumes:  $1.85 \times 10^6$ ,  $0.62 \times 10^6$ , and  $0.35 \times 10^6$  m<sup>3</sup>; maximum depths: 36, 18, and 15 m; water residence times: 1.6, 0.7, and 0.5 year, respectively). All three lakes are situated in catchments with bedrock composition sensitive to acid deposition, at altitudes between 1028 and 1090 m a.s.l. Their small catchments are predominantly covered with Norway spruce forest. Detailed descriptions of the lakes and maps of their catchments were published elsewhere [22–24].

Samples of lake water were taken from two to five depths at the deepest point of each lake in 1997 (three samplings), 1998 (7–10), and 1999 (4–6). We regularly analysed samples of surface (0.5 m) and bottom (~0.5 m above the sediment) layers and three further depths (2,

~5, and 8 or 10 m) for lake water chemistry and plankton biomass. Large zooplankton were sampled by several vertical hauls with a quantitative net (200- $\mu$ m mesh size) of the Apstein type. Temperature and dissolved oxygen were measured with the DataSonde 4 (Hydrolab, USA) at 0.5-m intervals.

### 2.2. Chemical and plankton analyses

In the laboratory, samples were filtered through 0.4- $\mu$ m glass-fibre filters (MN-5, Macherey Nagel) for the analysis of dissolved organic carbon (DOC; LiquiTOC analyser, Foss/Heraeus). Acid neutralising capacity (Gran titration), pH, and total phosphorus (TP) were analysed within 24 h of sampling. TP was determined by perchloric acid digestion [25]; 20–30 ml of sample were evaporated to obtain a detection limit of about 0.5  $\mu$ g P l<sup>-1</sup>.

We determined concentration of chlorophyll *a* (Chl*a*) spectrophotometrically on Whatman GF/C filters after acetone extraction [26]; values were not corrected for phaeopigments. Phytoplankton samples (~1 l) were preserved by acid Lugol's solution and pre-sedimented (5 $\times$ ). *Dinobryon* species were counted and measured in Utermöhl's sedimentation chambers on an inverted microscope (Nikon Diaphot, phase contrast, objective 60 $\times$ ). Individual cell volumes of algae were approximated and their total biovolume was converted to organic carbon (biomass) using a factor of 0.2 mg C mm<sup>-3</sup> [27].

Concentrated samples of crustacean zooplankton were preserved by formaldehyde (final concentration, 4%). Abundance of *Daphnia longispina* was expressed as individuals per litre. Size (length) of the individuals was measured to estimate their dry weight, which was converted to carbon biomass [27].

### 2.3. Microbial abundance and biomass

Bacterial samples were preserved in formaldehyde (final concentration, 2%). Unicellular bacterioplankton (cell length < 5  $\mu$ m) were counted and sized after DAPI staining on black Poretics filters using an epifluorescence microscope (Olympus BX-60) and the PC-based image analysis system Lucia D 3.52 (Laboratory Imaging, www.lim.cz). Bacterial biomass ( $B_B$ ) was calculated from cell dimensions according to [9] using the Norland's [28] formula. However, very long filamentous microorganisms commonly dominated bacterioplankton of the lakes. Thus the line intercept method was used to quantify the total length of all heterotrophic filaments [21], which was converted to carbon biomass using the following formula:  $B_F = (1/4)\pi \times D_F^2 \times L_F \times f_C$ , where  $B_F$  is the biomass of heterotrophic filaments,  $L_F$  is the total length of filaments per litre of a sample,  $D_F$  is an average diameter of filaments, and  $f_C$  is a conversion factor. Two classes of the filaments were recognised and measured independently: with  $D_F = 0.3$  and 0.7  $\mu$ m and the respective  $f_C = 14.9$

and  $51.4 \text{ fg C } \mu\text{m}^{-1}$ . The conversion factors are based on the widely accepted allometric relationship between bacterial cell volume and carbon content [28] and an assumption that each filament may be satisfactorily approximated with a chain of  $2\text{-}\mu\text{m}$  bacterial rods of the particular  $D_F$ . This reflects a linear increase in carbon content for both the length and volume of a filament of given  $D_F$ . The total heterotrophic microbial biomass (HB) was the sum of  $B_F$  and  $B_B$ .

#### 2.4. Microbial activity

Two microbial activity parameters were measured in Prášilské Lake during the 1999 summer season to assess their share in two size fractions ( $0.2\text{--}2 \mu\text{m}$  and  $>2 \mu\text{m}$ ) of the bacterioplankton. Both glucose uptake [29] and bacterial production (thymidine uptake according to [30]) were measured at in situ temperature using a similar setup: for each substrate concentration, two sets of triplicate samples plus duplicate blanks (prefixed with 2% formaldehyde) were processed in parallel. After incubation (see below), these parallel sets were filtered through membrane filters (Poretics; pore size:  $2 \mu\text{m}$  or  $0.2 \mu\text{m}$ ) and further processed as described below.

For glucose uptake, 5-ml samples were supplemented with six different concentrations of [ $\text{U-}^{14}\text{C}$ ]glucose (final concentrations,  $5\text{--}200 \text{ nmol l}^{-1}$ ; Amersham), incubated for 120 min, and then fixed with formaldehyde. For bacterial production, 5-ml samples were incubated for 60 min with [methyl- $^3\text{H}$ ]thymidine (final concentration,  $20 \text{ nmol l}^{-1}$ ; Amersham) and then preserved with formaldehyde. Thymidine incorporation was saturated at thymidine concentrations  $>10 \text{ nmol l}^{-1}$ . Filters were extracted (for 1 min) 10 times using 1 ml of ice-cold 5% trichloroacetic acid.

The substrate incorporation rate in the  $0.2\text{--}2\text{-}\mu\text{m}$  fraction was calculated by subtraction of the  $0.2\text{-}\mu\text{m}$  from the  $2\text{-}\mu\text{m}$  incorporation rates. Because up to 40% of thin filaments could pass through the  $2\text{-}\mu\text{m}$  filter [21], a complete separation of the filaments was unlikely. Parameters of saturation kinetics were calculated with non-linear regression using the Michaelis Menten equation (Prism 3.0, www.graphpad.com).

#### 2.5. Fluorescence in situ hybridisation (FISH) with oligonucleotide probes

In all lakes, we tentatively analysed bacterioplankton composition in three surface and two bottom samples in 1997 and other four to six analyses of samples from the two layers in 1998. The analysis was carried out by in situ hybridisation with fluorescent oligonucleotide rRNA-targeted probes on membrane filters [17]. Subsamples for FISH were prefixed with alkaline Lugol's solution followed by formaldehyde fixation and decolourised by the addition of sodium thiosulphate. Twenty ml of a sample were filtered onto a membrane filter (Poretics; diameter,  $47 \mu\text{m}$ ; pore size,  $0.2 \mu\text{m}$ ). The filter was rinsed with distilled water and stored frozen until further processing (within 1–6 months). FISH of filter sections with specific Cy3-labelled oligonucleotide probes (for Eubacteria, *Cytophaga-Flavobacterium* cluster, alpha-, beta-, and gamma-Proteobacteria; Interactiva, Ulm, Germany) and DAPI staining were used to estimate percentages of specifically labelled bacteria in the epifluorescence microscope (see [14] for more details).

### 3. Results

#### 3.1. Seasonal development in the lakes

Common features of the studied lakes were low pH (means: 4.52–4.94, Table 1) and permanent phosphorus depletion ( $<1 \mu\text{g l}^{-1}$  of dissolved reactive P throughout the year in all lakes [22–24]). Čertovo Lake was the most acidic one with the lowest DOC concentrations whereas the opposite was true for Prášilské Lake (see medians in Table 1). In all lakes, the minimum pH values but elevated DOC concentrations were observed in spring after snow-melt and during lake mixing (data not shown). Higher pH values ( $\text{pH} > 5$ , cf. its ranges in Table 1) were typical for the lake water above the sediments, which usually became anoxic during both summer and winter stagnation periods. Except for one sampling (late April in 1998), dissolved oxygen was always significantly depleted ( $<0.5 \text{ mg l}^{-1}$ ) above the bottom in all the lakes (data not shown).

Table 1  
Selected chemical and microbiological parameters in the Bohemian Forest lakes (1997–1999)

	pH	DOC ( $\text{mg l}^{-1}$ )	TP ( $\mu\text{g l}^{-1}$ )	Chla ( $\mu\text{g l}^{-1}$ )	Total HB ( $\mu\text{g C l}^{-1}$ )	Bacteria ( $\mu\text{g C l}^{-1}$ )	Filaments ( $\mu\text{g C l}^{-1}$ )
Čertovo Lake	4.52 (4.4–5.9)	2.46 (1.5–11.2)	4.2 (2.3–30)	2.66 (1.3–5.6)	82.4 (8.5–325)	17.9 (3.7–325)	51.7 (0–279)
Plešně Lake	4.83 (4.6–5.9)	3.22 (1.4–11.7)	8.9 (5.3–61)	11.1 (4.3–43.5)	108 (13.7–430)	32.3 (6.8–206)	62.6 (0–311)
Prášilské Lake	4.94 (4.7–5.7)	3.77 (2.9–6.4)	5.9 (3.4–58)	4.64 (0.35–9.7)	82.2 (14.5–195)	22.5 (2.2–70.1)	42.4 (0–167)

Median (minimum–maximum) values of pH, concentrations of dissolved organic carbon (DOC), total phosphorus (TP), chlorophyll *a* (Chla), heterotrophic microbial biomass (HB), biomass of small ( $<5 \mu\text{m}$ ) unicellular bacteria, and biomass of heterotrophic filaments along the water column.

The lakes also differed in TP concentrations (Table 1). Generally, the values were several times lower at the surface (median TP: 4.5, 5.5, and 8.9  $\mu\text{g l}^{-1}$ ) than above the bottom (median TP: 17.1, 22.9, and 16.9  $\mu\text{g l}^{-1}$ ) in Čertovo, Prášilské, and Plešné lakes, respectively. The median TP concentrations were roughly proportional to the median of phytoplankton biomass in each lake (Chla in Table 1). Minima of Chla concentrations (0.35 and 0.99  $\mu\text{g l}^{-1}$ ) were observed in Prášilské Lake, whenever the pop-

ulation of *D. longispina* peaked (22 July 1997 and 27 July 1998, respectively; cf. Fig. 1). We did not observe such a dense *Daphnia* population in 1999 and its biomass in Prášilské Lake remained extremely low (Fig. 1). The bacterioplankton development showed completely different patterns without any clear relationships to TP, Chla or DOC concentrations ( $P > 0.05$ ). Medians of total HB were very similar in Čertovo and Prášilské lakes but by  $\sim 30\%$  higher in Plešné Lake (Table 1, Figs. 1–3).

### Prášilské Lake

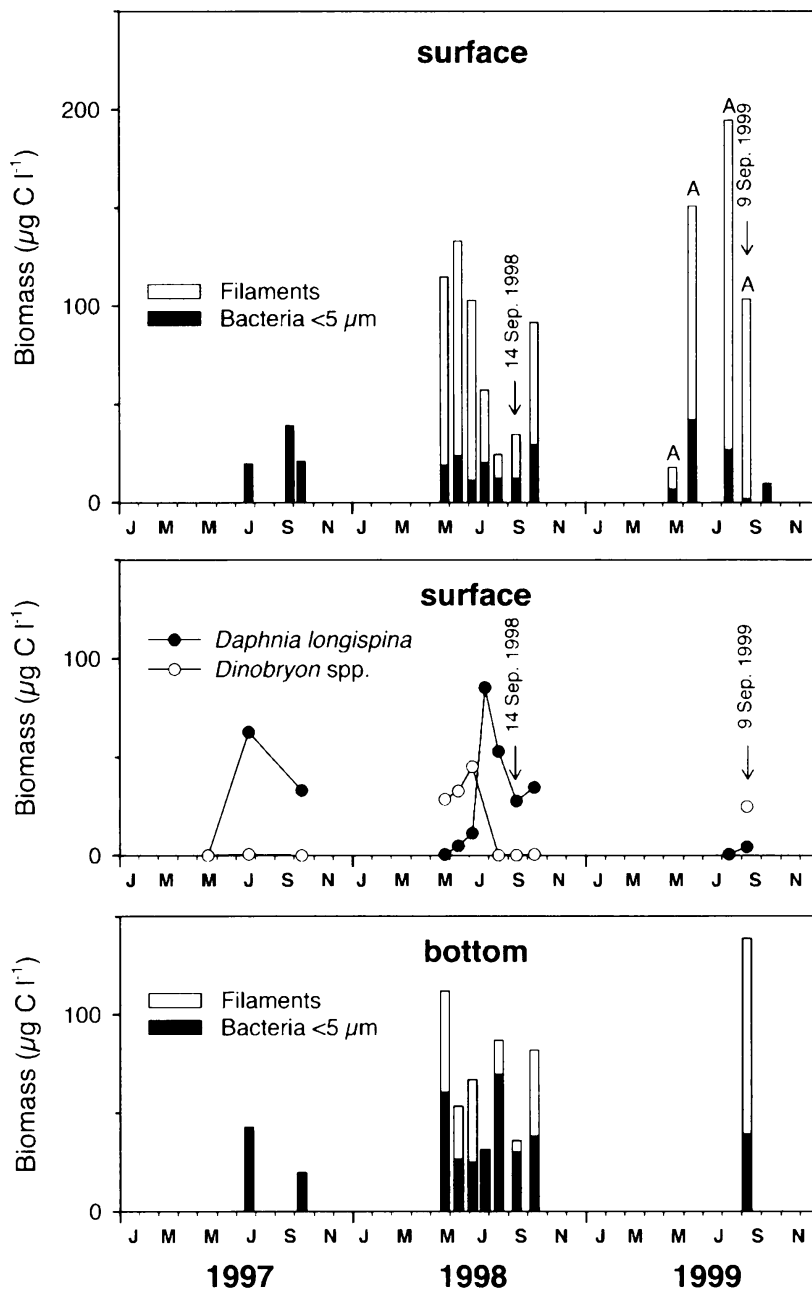


Fig. 1. Prášilské Lake (1997–1999). Seasonal development of HB at the surface and bottom layers expressed as the proportion of small (< 5  $\mu\text{m}$ ) unicellular bacteria and heterotrophic filaments in the total HB. Arrows with dates refer to the vertical profiles exhibited in Fig. 5; letters A indicate the samples assayed for size fractionation of microbial activity (see Fig. 6). Seasonal trends in the biomass of two prominent bacterial predators are given in the middle.



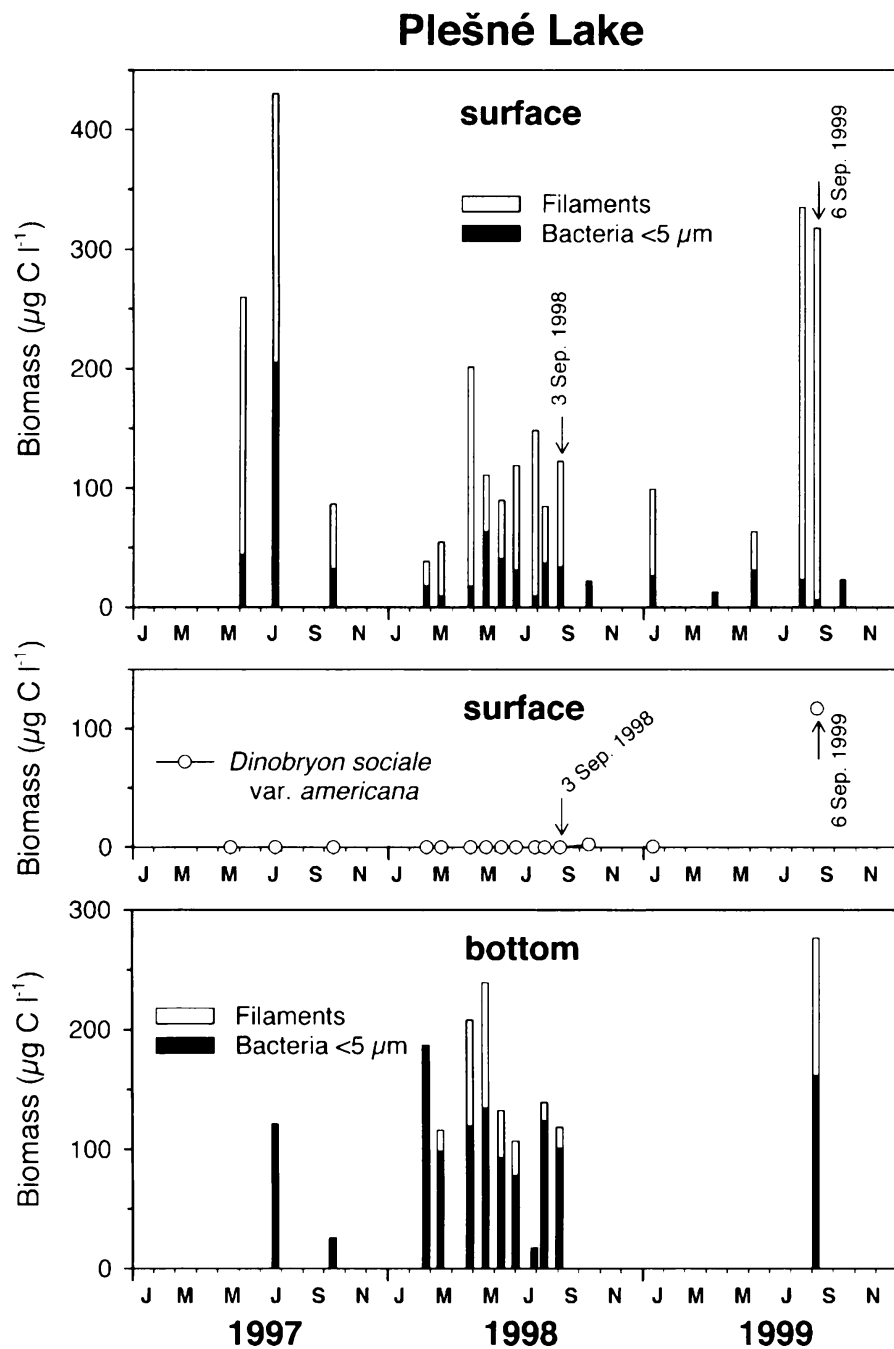


Fig. 2. Plešné Lake (1997–1999). Seasonal development of HB at the surface and bottom layers with the proportion of small (<5 µm) unicellular bacteria and heterotrophic filaments in the total HB. Arrows with dates refer to the vertical profiles exhibited in Fig. 5. Seasonal trends in the biomass of the prominent bacterial predator are given in the middle.

Central panels in Figs. 1–3 also show seasonal dynamics of dominant bacterivores, which were commonly the mixotrophic *Dinobryon* spp. in both oligotrophic lakes (Čertovo and Prášilské). *Dinobryon pediforme* was often the most abundant (median: 1016 cells ml<sup>-1</sup>, maximum: 6084 cells ml<sup>-1</sup>) phytoplankton species as well as the most abundant bacterivore in the epilimnion of Čertovo Lake; however, not dominant in biomass. *Dinobryon sociale* var. *americana* occurred in Plešné Lake but usually at

low abundance and negligible biomass, except for the maximum (4864 cells ml<sup>-1</sup>) at the surface layer in 1999 (Fig. 2). Both *Dinobryon* species were also the most abundant (median: 1382 cells ml<sup>-1</sup>, maximum: 4760 cells ml<sup>-1</sup>) in the epilimnion of Prášilské Lake until the large *Daphnia* population appeared. The top-down control of their growth by *D. longispina* was obvious both in 1997 and 1998 seasons (Fig. 1).

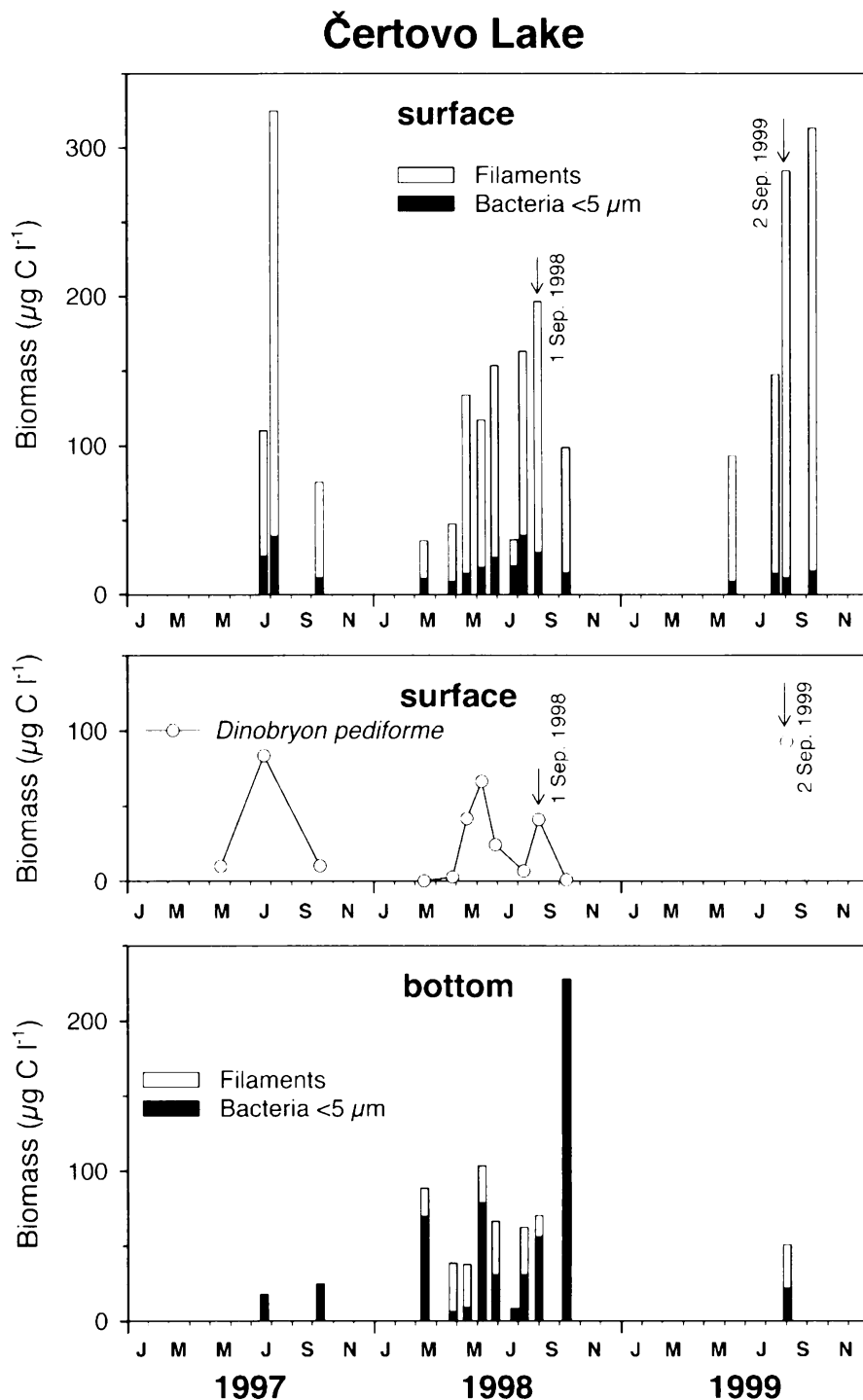


Fig. 3. Čertovo Lake (1997–1999). Seasonal development of HB at the surface and bottom layers with the proportion of small ( $< 5 \mu\text{m}$ ) unicellular bacteria and heterotrophic filaments in the total HB. Arrows with dates refer to the vertical profiles exhibited in Fig. 5. Seasonal trends in the biomass of the prominent bacterial predator are given in the middle.

### 3.2. Seasonal dynamics of heterotrophic filaments

Long ( $> 100 \mu\text{m}$ ) heterotrophic filaments were common and very characteristic attributes of lake bacterioplankton (Fig. 4). Despite the distinct seasonal variability (Figs. 1–3), median proportions of the filaments in the total HB were 52% in Prášílské Lake, 58% in Plešné Lake, and 63%

in Čertovo Lake. The high summer abundance of *D. longispina* in Prášílské Lake led to conspicuous reduction (1998) or total absence (1997) of the filaments (Fig. 1). The highest median biomass of small ( $< 5 \mu\text{m}$ ) unicellular bacteria was in Plešné Lake (Fig. 2), whereas the lowest one was in Čertovo Lake (Fig. 3). In Prášílské Lake, the range of biomass was much narrower with no extreme

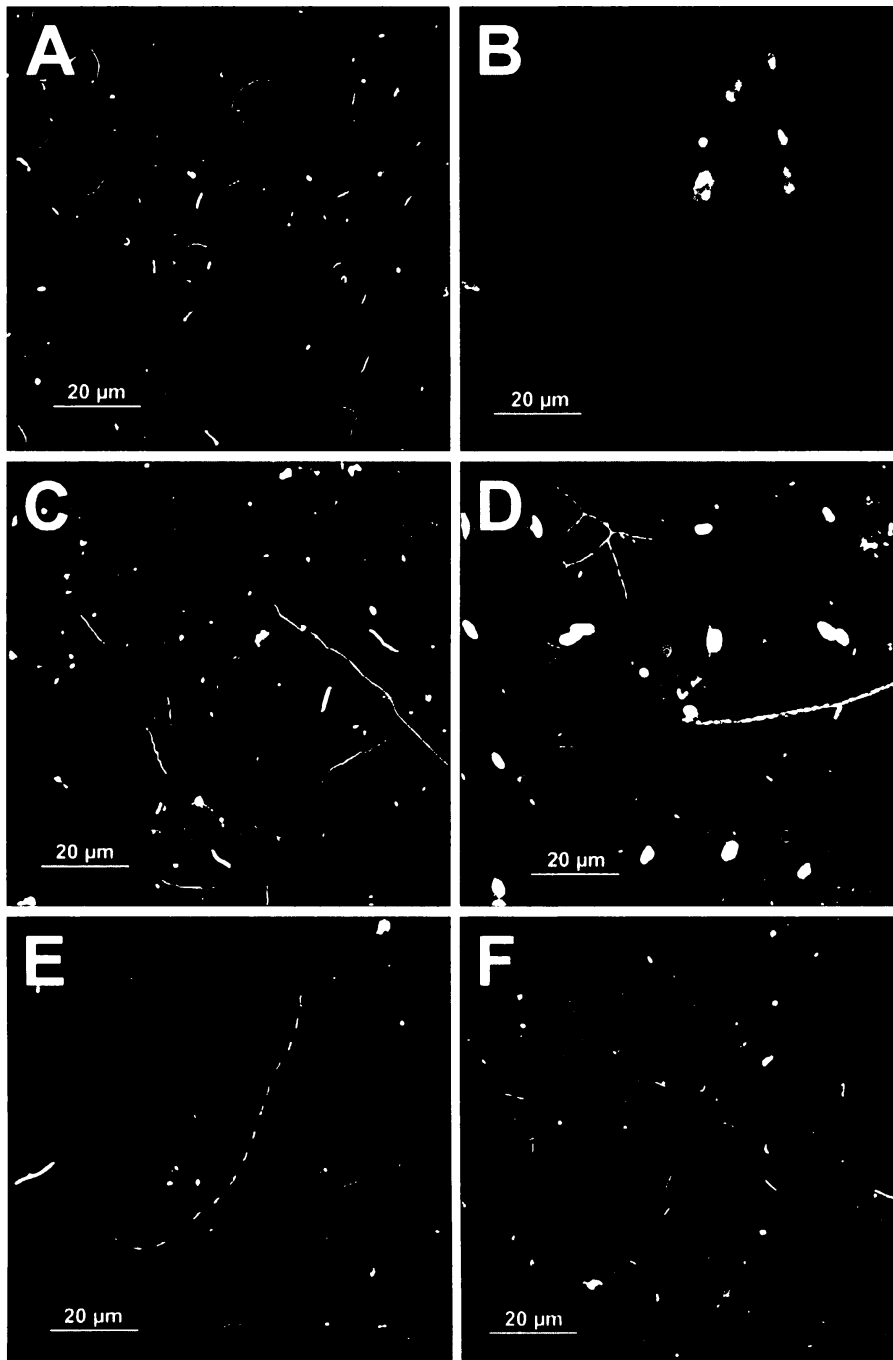


Fig. 4. Typical morphotypes of microplankton of the acidified lakes in the Bohemian Forest. Images of DAPI-stained microorganisms in the epifluorescence microscope (Olympus BX 70) were taken with a monochromatic digital integrating camera DVC-1300 (DVC, Austin, TX, USA) and processed with the image analysis software Lucia G/F 4.2 (Laboratory Imaging, Prague, Czech Republic) [21]. A–E: Water column samples; F: anoxic bottom sample. All images are at a magnification of  $\times 400$ . Besides various morphotypes of the heterotrophic microplankton (see text for more details), common phytoplankton species are shown, like colonial mixotrophs *D. pediforme* (B), green algae *Monoraphidium dybowskii* (D, bright oval cells), and an autotrophic filament of cyanobacterium *Limnothrix* sp. (D, the thick object from centre to right). The latter filaments, however, are not included in the filaments' biomass under study.

values of the biomass recorded (Table 1). Over the whole period studied (1997–1999,  $n = 11$ ), cladoceran biomass showed a significant negative correlation to biomass of filaments (Spearman  $r = -0.770$ ,  $P = 0.0056$ ) but not to biomass of unicellular bacteria (Spearman  $r = 0.105$ ,  $P = 0.76$ ).

Depth profiles of total HB were rather uniform

throughout the water columns in all lakes. Fig. 5 shows typical examples of vertical distribution of unicellular bacteria and filaments. Remarkably higher biomass of the filaments was usually observed in the epilimnion compared to the deeper layers, while under anoxic conditions above the sediments, the filaments were occasionally missing (cf.

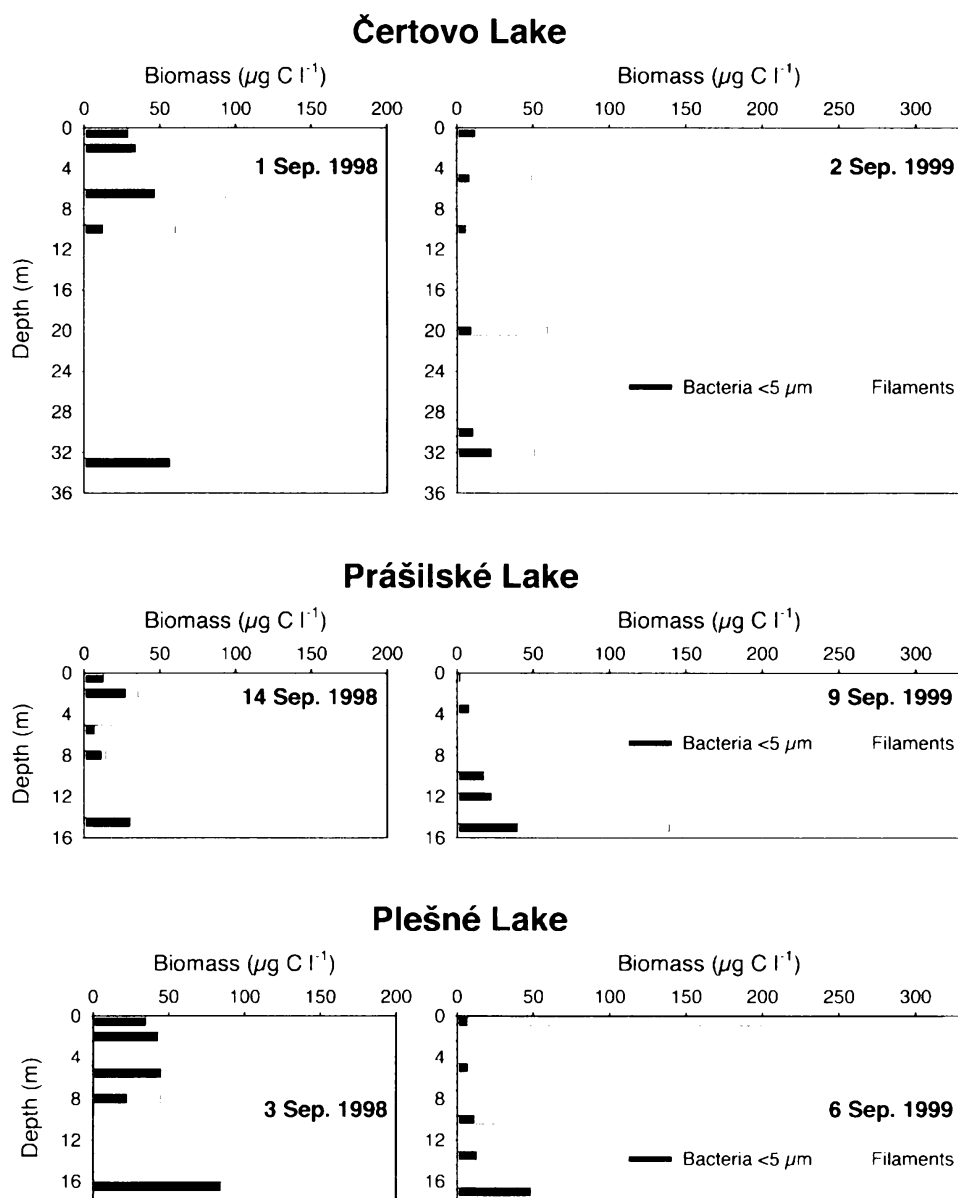


Fig. 5. Selected vertical distributions of HB in all three lakes in 1998 (left) and 1999 (right). Note the same scale factor of all plots.

Figs. 1–3). Pelagic unicellular bacteria were significantly less abundant (medians:  $5.1 \times 10^5$ ,  $6.2 \times 10^5$ , and  $9.1 \times 10^5$  cells  $\text{ml}^{-1}$  in Čertovo, Prášílské, and Plešné lakes) than above the bottom (medians:  $1.3 \times 10^6$ ,  $1.4 \times 10^6$ , and  $1.6 \times 10^6$  cells  $\text{ml}^{-1}$ , respectively), where they commonly accounted for majority of HB (Figs. 1–3). In Plešné Lake, unicellular bacteria were larger (median of mean cell volume:  $0.172 \mu\text{m}^3$ ) at the bottom (Fig. 4F) than at the surface layer ( $0.139 \mu\text{m}^3$ ), but they were of similar size in both layers in Čertovo and Prášílské lakes.

Seasonally, total HB increased several times in all lakes – from low values after ice-out, followed by rising filament biomass in late spring (May–June), to a summer maximum of HB (cf. surface values in Figs. 1–3). Exceptions were the 1997 and 1998 summer minima of HB in Prášílské

Lake, where the cladocera severely reduced the filaments. In contrast to the epilimnions, HB did not show any clear seasonal trends in the anoxic bottom layers of the lakes (Fig. 1–3).

### 3.3. Filament morphology and phylogenetic composition

Threadlike filaments, poorly DAPI-stained (Fig. 4B), were common especially in the oligotrophic Čertovo Lake. Other common morphotypes of all lakes were *Caulobacter*-like bacteria with long flagella and bright DAPI fluorescence of cells (Fig. 4A,C). In certain periods, particularly in spring, a small proportion of the heterotrophic filaments was branched resembling aquatic micromycetes (Fig. 4D).

Table 2  
Bacterioplankton composition in three Bohemian Forest lakes (1997–1998)

		EUB (% DAPI)	ALF (% DAPI)	BET (% DAPI)	GAM (% DAPI)	CF (% DAPI)
Čertovo Lake surface ( $n=9$ )	Bacteria	41 (22–56)	8.3 (0–14)	3.7 (0–19)	0.0 (7.3)	1.5 (0–3.6)
	Filaments	39 (0–80)	0.0 (0–15)	0.0 (4.1)	0.0 (42)	0.0 (19)
Čertovo Lake bottom ( $n=8$ )	Bacteria	23 (0–34)	4.1 (0–9.5)	4.0 (0–13)	0.0 (0–0.7)	0.6 (0–1.2)
	Filaments	32 (0–79)	1.1 (0–13)	0.0	0.0	0.0
Plešné Lake surface ( $n=9$ )	Bacteria	45 (17–59)	11 (0–33)	1.5 (0–27)	0.0 (5.3)	1.4 (0–5.0)
	Filaments	27 (0–60)	0.0 (0–50)	0.0 (5.6)	0.0	0.0
Plešné Lake bottom ( $n=8$ )	Bacteria	30 (25–37)	3.7 (0–13)	15 (5.6–27)	0.0 (5.4)	0.0 (4.9)
	Filaments	26 (19–63)	0.0 (0–4.0)	0.0	0.0	0.0
Prášilské Lake surface ( $n=7$ )	Bacteria	47 (17–61)	2.8 (0–27)	17 (0.7–26)	0.0 (1.0)	0.0 (0–4.3)
	Filaments	56 (0–96)	0.0	0.0	0.0	0.0 (3.7)
Prášilské Lake bottom ( $n=6$ )	Bacteria	27 (22–53)	0.8 (0–2.4)	11 (1.0–23)	0.0 (0.7)	0.0 (0–9.2)
	Filaments	18 (0–98)	0.0	0.0 (50)	0.0	0.0

Proportions of Eubacteria (EUB), alpha-Proteobacteria (ALF), beta-Proteobacteria (BET), gamma-Proteobacteria (GAM), and *Cytophaga-Flavobacterium* (CF) cluster in the total DAPI-stained unicellular bacteria and in the total DAPI-stained heterotrophic filaments (median, range of values in parentheses).

Table 2 shows bacterioplankton composition (assessed by FISH) in 1997 and 1998. In all the lakes, the general probe for Eubacteria hybridised with some 40–60% of the total bacteria or the total filaments (both DAPI stained) at the surface layer but usually <30% at the bottom (Table 2). The branched filaments were never targeted by any probe.

Further FISH analyses showed almost no hybridisation with the specific probes for gamma-Proteobacteria and the *Cytophaga-Flavobacterium* cluster; positive results were rather exceptional and in all but one case (filaments in Čertovo Lake, October 1997) did not exceed 10% of the total DAPI-stained cells (Table 2). More significant results in some samples gave FISH of the unicellular bacteria with two other specific probes, i.e. for alpha- or beta-Proteobacteria. The former accounted for >5% (median) of the total DAPI-stained bacteria in Čertovo and Plešné lakes (surface samples), while the latter accounted for >10% in Plešné Lake (bottom) and in Prášilské Lake (surface and bottom samples). FISH of the filaments with both probes was generally very low in most samples (Table 2).

### 3.4. Size fractionation of microbial activity

In general, bacterial production and maximum capacity of glucose uptake, measured at the surface of Prášilské Lake in 1999, showed similar seasonal trends (Fig. 6), roughly following the trend in the total HB (cf. columns A in Fig. 1). The total incorporation of both thymidine and glucose increased by one order of magnitude from May to August, and then it dropped in September (Fig. 6). However, the size fractionation of either substrate showed contrast patterns. From May to August, about 70% of thymidine but only ~40% of glucose incorporated into a smaller fraction (0.2–2  $\mu\text{m}$ ) and the rest of either substrate incorporated into a larger fraction (>2  $\mu\text{m}$ ). In contrast, there was a totally opposite trend in partitioning

of both substrates in September: while 71% and 37% in the larger fraction, 29% and 63% of thymidine and glucose, respectively, were found in the smaller fraction.

The biomass of unicellular bacteria formed ~40% of HB in May but accounted for as little as 2% in September (Fig. 1). Assuming the unicellular bacteria and filaments were localised in the fractions 0.2–2  $\mu\text{m}$  and >2  $\mu\text{m}$ , respectively, we calculated biomass-specific incorporation rates of either substrate for the small bacteria and for the filaments. The former always showed higher specific activity than the latter (Table 3). Despite two-order-of-magnitude higher specific incorporation rates of glucose compared to those of thymidine, the ratio of bacterial-to-filament incorporation was surprisingly similar for both

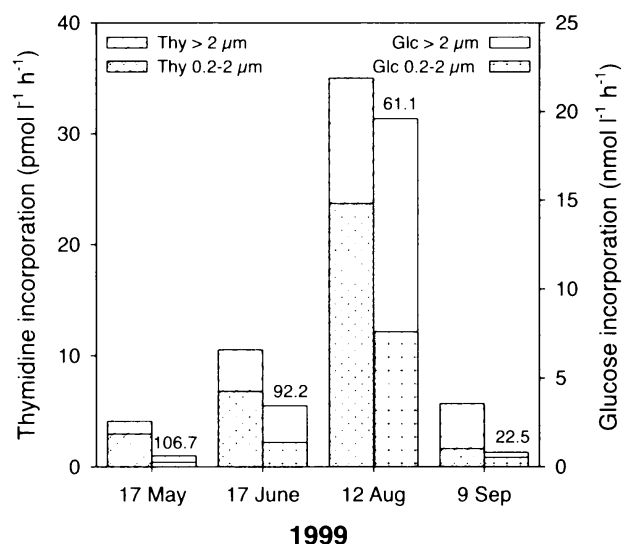


Fig. 6. Microbial activity at the surface of Prášilské Lake in 1999. Partition of both thymidine and glucose incorporation into two fractions of the bacterioplankton (for the corresponding biomass of both small unicellular bacteria and heterotrophic filaments also see the columns labelled A in Fig. 1). The half-saturation constant ( $K_s$ , the value above each column) is given for the corresponding total glucose incorporation rate.

Table 3  
Biomass-specific incorporation rates of thymidine and glucose into the two plankton fractions of Prášílské Lake in 1999

Size fraction:	Thymidine (pmol $\mu\text{g}^{-1} \text{h}^{-1}$ )			Glucose (pmol $\mu\text{g}^{-1} \text{h}^{-1}$ )		
	Bacteria (0.2–2 $\mu\text{m}$ )	Filaments (> 2 $\mu\text{m}$ )	Ratio bacteria:filaments	Bacteria (0.2–2 $\mu\text{m}$ )	Filaments (> 2 $\mu\text{m}$ )	Ratio bacteria:filaments
17 May	0.405	0.389	1.0	35.2	33.6	1.0
17 June	0.160	0.097	1.6	32.3	19.0	1.7
12 August	0.865	0.209	4.1	277.6	71.8	3.9
9 September	0.736	0.056	13.1	237.7	2.8	84.3

The incorporation rates into the fraction 0.2–2  $\mu\text{m}$  are expressed per biomass of unicellular bacteria and the incorporation rates into the fraction > 2  $\mu\text{m}$  are expressed per biomass of filaments.

substrates, with increasing trends throughout the season (Table 3).

The half-saturation constant ( $K_s$ ) of the total glucose uptake progressively decreased throughout the studied period (Fig. 6). At each sampling date, however, the values of  $K_s$  were similar in both fractions.

#### 4. Discussion

Our 3-year study documented the permanent presence of the heterotrophic filaments in all lakes under study, except for the 1997 season in Prášílské Lake. Common occurrence of the filaments has been observed in all eight lakes in the Bohemian Forest, regardless of that are acidified strongly or moderately at present [19,20]. Such a permanent [16–18] or temporary [5–8,13,14] appearance of the filaments has been reported from many freshwaters. However, as far as we know, this study is the first attempt at estimation of the filaments' biomass and its seasonal dynamics in the pelagic zone; it usually accounted for > 50% of HB (Fig. 5). Furthermore, HB was usually as high as phytoplankton biomass in all Bohemian Forest lakes, except for the plankton of Plešné Lake, where phytoplankton accounted for ~80% of the total biomass (e.g. [20], J. Vrba and L. Nedbalová, unpublished).

##### 4.1. Bottom-up and top-down control of bacterioplankton dynamics in the acidified lakes

Our results clearly show the remarkably large proportion of HB in the plankton biomass of the acidified lakes in the Bohemian Forest and yield the following question: Why is the proportion of HB so high and mostly composed of filaments?

Generally in freshwater ecosystems, different sources of autochthonous and allochthonous DOC fuel the major part of bacterial production. In the lakes studied, both sources likely affected growth and seasonal dynamics of the bacterioplankton to a distinct extent. Overall, the epilimnetic total HB usually showed similar seasonal developments with characteristic summer peaks (Figs. 1–3) and also the median values of total HB were similar in the lakes (Table 1). However, detailed analysis showed differ-

ent HB dynamics in each lake with distinct importance of bottom-up factors, e.g. supply of P or DOC and microbial competition for P, and top-down factors, i.e. the presence or absence of grazers.

A trophic status of the lakes increases in the following order: Čertovo < Prášílské < Plešné. Table 1 shows a general increase along the gradient in both TP and Chl *a* concentrations in the lakes. Seasonal data on Chl *a* concentrations, phytoplankton biomass (L. Nedbalová, unpublished), and primary production (J. Nedoma, unpublished) clearly indicate an increasing role of the autochthonous organic carbon supply along the trophic gradient. The above parameters differ by a factor of 3–5 between Čertovo and Plešné lakes. Seasonal pattern of the epilimnetic bacterioplankton, in particular the increases in the filaments' biomass in late spring (Figs. 1–3) suggested that there were likely additional substrate sources besides primary production. Despite the above difference in the phytoplankton of Čertovo and Plešné lakes, the epilimnetic values of HB were almost identical (medians: 126 and 115  $\mu\text{g l}^{-1}$ , respectively; cf. Figs. 2 and 3, Fig. 5).

The allochthonous DOC input into the studied lakes supplied an additional substrate for the bacterioplankton and obviously favoured bacterial growth [31]. Overall, DOC concentrations in the tributaries were about 2–10 times higher compared to those at the surface of the particular lake and the inflow water contained more humic matter than the lake water (see [22–24] for details). While the inflow DOC concentrations were usually similar to the maximum values in the lakes, the minimum DOC concentrations (see the DOC range for each lake in Table 1) occurred in the surface lake water during summer stratification periods. We hypothesised that sunlight caused photochemical degradation of the allochthonous DOC at the surface layer, which was followed by bacterial utilisation of low molecular compounds. The hypothesis was verified both in laboratory [31] and lake experiments (P. Porcal and J. Kopáček, unpublished). Thus an enhanced bacterial growth in response to photochemical transformation of DOC [32] played very likely an important role in the epilimnion of the lakes under study. This conclusion is supported by the vertical distributions of HB with frequent remarkable epilimnetic maxima of the filaments (Fig. 5).

In summer, also higher epilimnetic temperatures supported microbial activity and growth.

Although the P input into Plešné Lake ( $270 \mu\text{g m}^{-2} \text{ year}^{-1}$ ) is several times higher compared to Čertovo and Prášilské ( $54$  and  $64 \mu\text{g m}^{-2} \text{ year}^{-1}$ , respectively; [33] and J. Kopáček, unpublished), concentrations of dissolved reactive P in all the lakes are always below the detection limit ( $< 1 \mu\text{g l}^{-1}$ ), indicating strong P limitation of all lake ecosystems. In the acidified lakes, the actual P availability for the plankton is further controlled by an interplay of pH and aluminium. A slight P inactivation by aluminium was likely in Čertovo and Prášilské lakes but it was enormous in the water column and sediments of Plešné Lake [23]. Furthermore, an urgent need of P for the microplankton in all the lakes was indicated by extremely high activities ( $0.39$ – $9.93 \mu\text{mol l}^{-1} \text{ h}^{-1}$ ) of extracellular phosphatases [34] and high molar C:P ratios (on average  $> 500$  [22–24]).

While the phytoplankton biomass roughly corresponded to a different TP concentration in each lake, the total HB values were similar (Table 1). Consequently, the proportion of total HB within the entire plankton biomass showed a clear trend, which was inverse to that of the lake trophic status. In other words, the lower the P supply, the higher was the share of HB in the plankton. The bacterioplankton were apparently more efficient in P acquisition than the phytoplankton under the very P-limited conditions. A competition for P is generally an important aspect of algal-bacterial relationships with bacteria considered to be the superior competitors to algae [35].

In P-limited systems, algae stressed by the lack of P respond by enhanced extracellular release of DOC, thus in fact stimulating their competitors for P uptake ('the paradox of phytoplankton-bacteria interactions' [36]). This mechanism may favour bacteria in competition for resources, e.g. in oligotrophic lakes with low DOC and P-limited phytoplankton [37]. Also in all three acidified lakes under study, the phytoplankton experienced likewise severe P limitation due to impairing the P availability by aluminium [23,34].

From a 'top-down point of view', the formation of grazing-resistant filaments is generally considered to be the consequence of a grazing pressure by protists (e.g. [2]). Overall in freshwaters, a heavy grazing of heterotrophic flagellates upon small bacteria results in the filament formation [7,13–15,18]. In the acidified lakes under study, however, abundance of both heterotrophic flagellates ( $\sim 10^2 \text{ cells ml}^{-1}$ ) and ciliates (means:  $< 1 \text{ cells ml}^{-1}$  [38]) were too low to exert a significant grazing pressure. On the other hand, summer abundance of the mixotrophic flagellates, particularly two *Dinobryon* species in the epilimnion of Prášilské and Čertovo lakes, clearly exceeded limited abundance of the heterotrophic flagellates and direct measurements during some *Dinobryon* blooms suggested the major role of these mixotrophs in bacterial consumption [19,39]. Preliminary estimates in both lakes

suggested that grazing of *Dinobryon* spp. usually accounted for  $> 90\%$  (maximum:  $98\%$  in August 1998, Čertovo Lake) of the total bacterivory, whereas heterotrophic flagellates for only  $< 10\%$  (K. Šimek, unpublished).

In some lakes studied, *Dinobryon* spp. appeared to be the principal grazers daily cropping up to  $\sim 50\%$  of standing stock of the unicellular bacterioplankton. For instance in Čertovo Lake in 1998, the highest ingestion rate was found on 29 June ( $9 \text{ bacteria } \text{Dinobryon}^{-1} \text{ h}^{-1}$ ), when this mixotroph turned over as much as  $\sim 260\%$  of bacterial production, and then its grazing pressure gradually decreased throughout the season [39]. Such a strong grazing obviously drove the bacterioplankton (i) to a rapid turnover of small unicellular bacteria and (ii) to the shift of size distribution towards longer cells and to filament formation. Later, the parallel drop in *Dinobryon* biomass (Fig. 3) and its grazing pressure [39] apparently enabled a slight recovery of the unicellular bacterial biomass in August 1998 (Fig. 3).

The mixotrophy was often observed especially among chrysophytes (see [40] for review) under P limitation. Abundant *Dinobryon* likely took the advantage of mixotrophic growth covering its P demand by phagotrophy on the single-celled bacterioplankton. The mixotrophic *Dinobryon* spp. occurred in the epilimnion of both oligotrophic lakes, Čertovo and Prášilské (Figs. 1 and 3). However, as yet in the mesotrophic Plešné Lake, autotrophic phytoplankton species [20,41] were usually dominant over *D. sociale* (cf. Fig. 2) due to the elevated P supply.

In recent limnology it has been widely accepted that the presence of cladocera prevents bacterial filament formation (e.g. [1]). Prášilské Lake clearly exemplifies an evidence of such a direct impact of *D. longispina* on the bacterioplankton (Fig. 1). A large population of *Daphnia* always caused entire (1997) or marked (1998) reduction of the filament biomass, whereas its small population (1999) was inefficient in preventing the development of filament biomass as high as in the other lakes (cf. Figs. 2, 3 and 5).

To summarise, the low P supply and the allochthonous DOC supply can alternate or interplay in the bottom-up control of the plankton dynamics in the studied acidified lakes in the Bohemian Forest. The overall P shortage seems to favour the growth of bacterioplankton over phytoplankton. Moreover, photochemical degradation of allochthonous DOC supplies an additional advantage for the bacterial growth, in particular under the less P input and higher transparency (Čertovo Lake). Such conditions also favour the massive occurrence of the filaments. Their formation is at least partly induced and maintained by protistan grazing on the bacterioplankton. On the other hand, the effective cladoceran filtration (Prášilské Lake) episodically reduces the protistan grazers as well as the filaments. The example of Plešné Lake (1997–1998), however, suggests that some filaments may develop even in the absence of the principal protistan grazers. In addition, other environmental conditions, rather than the impact

of protistan grazing alone, may control the occurrence of filaments, as well (e.g. [11]). Indeed higher pH and/or oxygen depletions at the bottom might cause the low occurrence or absence of the filaments.

#### 4.2. Bacterioplankton composition, morphology, and activity

Tentative analyses of the bacterioplankton composition in the acidified lakes under study showed a generally lower FISH detection limit than in an oligotrophic high mountain lake, Gossenköllesee, where the detection with the probe for Eubacteria averaged 55% (range: 26–82%) of all DAPI-stained objects and 87% (68–100%) of filamentous cells [16,17]. Our study revealed rather lower proportions in unicellular bacteria and altogether hybridisation of the filaments by the probe for Eubacteria was far lower in the Bohemian Forest lakes than in Gossenköllesee [16].

Overall, the proportions of the bacterioplankton, which were detected by the specific probes for alpha- and beta-Proteobacteria in the acidified lakes, matched the ranges commonly reported for both subclasses. It holds also for the very low detection of gamma-Proteobacteria resembling data from most other lakes [13–17]. The detected proportions of alpha- and beta-Proteobacteria slightly differed among the lakes under study as well as between the surface and bottom layers in one lake. We consistently found higher FISH detection limits at the surface than at the bottom layers in all lakes. The only exception was the bottom layer in Plešné Lake, where members of beta-Proteobacteria were more commonly detected than at the surface (Table 2).

Various filamentous morphotypes occurred in the bacterioplankton of the lakes (Fig. 4). Compared to available literature data, so low detection of the filaments by any specific probe is rather exceptional but it was typical for this study. In most freshwater environments, the majority of various filamentous morphotypes were targeted by probes for alpha- or beta-Proteobacteria, or they were affiliated to the *Cytophaga-Flavobacterium* cluster [13–17]. For instance in Gossenköllesee, Pernthaler et al. [16] observed that the filaments affiliated to the *Cytophaga-Flavobacterium* cluster constituted seasonally even the largest pool of bacterioplankton biomass. They also observed an autumnal peak of threadlike and rod-shaped Archaea in the lake [16]. It was possible that the presence of similar filamentous microorganisms in the Bohemian Forest lakes could explain a low proportion of hybridised filaments with the probe for Eubacteria and their negligible detection with the specific probes; however, we did not use the probe for Archaea in this study.

Also, some branched filaments reminded rather fungal mycelia (Fig. 4D). Most likely, those filaments were aquatic micromycetes or streamborne fungal spora, which came from the forested catchments. Their occurrence was documented in many streams of the Bohemian Forest [42].

Although fungi are known to be typical of acidic habitats [43], the branched filaments occurred neither regularly nor frequently in our lake samples; they were more common in early spring. Also, even if present, they were too seldom for separate quantifying with the line intercept method [21]. Therefore, they could hardly account for a significant portion of the total length of the heterotrophic filaments or the total HB in the lakes under study.

Another explanation of the overall low hybridisation efficiency for the filaments in this study might be a matter of their slower growth and lower activity, implying also less ribosomes per cell [17], approaching thus the detection limit of the FISH method. Very low biomass-specific activity of the filaments, in particular their thymidine uptake rates (Table 3) actually indicated such a possibility. Our seasonal study on the bacterioplankton activity in Prášílské Lake showed an increasing trend in growth rate of unicellular bacteria (Table 3), most likely responding to the *Dimobryon* grazing (cf. Fig. 1), whereas the maximum growth of the filaments was observed already in May, i.e. prior to building up their high biomass.

#### 4.3. Conclusions

The virtually permanent and massive occurrence of bacterial filaments classifies both Plešné and Čertovo lakes among rather rare and unique freshwater environments (e.g. [16,18]) mostly without cladoceran zooplankton, where majority of P is likely sequestered in microbial biomass. It seems quite likely that, in the absence of cladoceran filtration, the filaments may gradually withdraw most of the available P throughout a season. From a 'bottom-up point of view', they may represent bacterial populations adapted to the specific conditions (low pH and/or higher DOC supply) and simply a sink of P for microbial competitors in the lakes. Typically, most of the heterotrophic filaments were affiliated to Eubacteria but they were apparently less active and slower growing than unicellular bacteria.

Our study has yielded a basic description of the major morphological and genotypic characteristics of the bacterioplankton, its seasonal dynamics and changes in the selected activity parameters in anthropogenically heavily acidified lakes in the Bohemian Forest. However, as yet a couple of questions, namely those related to the taxonomic affiliation of the abundant filaments and other environmental factors allowing their massive development in the acidified lakes, are left largely unsolved and thus in need of future studies.

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## 4. SUMMARY AND CONCLUSIONS

This thesis deals chiefly with various aspects of plankton ecology in mountain lakes recovering from anthropogenic acidification. A brief summary of attached publications and general conclusions with an emphasis on phytoplankton assemblages follow.

### **Biological recovery of the Bohemian Forest lakes from acidification (NEDBALOVÁ & al. 2006a)**

Based on data collected in autumn 2003, this study of eight Bohemian Forest lakes focused in detail on an evaluation of the current progress in their chemical and biological recovery from acidification. In comparison with a 1999 survey, four lakes (Kleiner Arbersee, Prášílské Lake, Grosser Arbersee and Laka) have recovered their carbonate buffering system. However, the other four lakes (Černé Lake, Čertovo Lake, Rachelsee and Plešné Lake) still remain chronically acidified. The composition and amount of volume-weighted plankton biomass reflected differences in the acidification status and nutrient loading to particular lakes. While bacterioplankton was the main component in acidified lakes except for Plešné Lake, phytoplankton and zooplankton co-dominated the plankton biomass in the recovering lakes. A dominance of phytoplankton was characteristic for mesotrophic Plešné Lake, because of higher phosphorus input to this lake. Overall, the species composition and amount of phytoplankton biomass reflected differences in acidification status, nutrient availability and zooplankton status among the lakes. For example, grazing pressure of filtering zooplankton seems to be the cause for the high proportion of the large dinoflagellate *Gymnodinium uberrimum* in the phytoplankton biomass of some lakes. Concerning newly observed signs of biological recovery, we documented an increase in zooplankton biomass in the recovering lakes and a two-order-of-magnitude increase in rotifer abundance in acidified Plešné Lake. Moreover, this survey is the first evidence of the return of some macrozoobenthos species that were extinct during acidification (in particular from the Ephemeroptera and Plecoptera).

### **Phytoplankton of a high mountain lake (L'adové pleso, the Tatra Mountains, Slovakia): seasonal development and first indications of a response to decreased acid deposition (NEDBALOVÁ & al. 2006b)**

In this one-year study, we examined the seasonal and vertical distribution of phytoplankton in a non-acidified high mountain lake (L'adové Lake, High Tatra Mts) with an emphasis on the effect of the currently less-pronounced episodic acidification of surface layers during melting periods. Unicellular flagellates, namely from the Cryptophyceae and Chrysophyceae

dominated the phytoplankton, and different seasonal and depth distributions of important species (*Plagioselmis lacustris*, *Cryptomonas* cf. *erosa*, *Ochromonas* spp. and *Mallomonas akrokomos*) indicated differences in their ecological requirements. Total phytoplankton biomass did not exceed values typical for oligotrophic lakes, and showed a marked seasonal development with two high peaks in the deeper layers: one in December under thin clear ice and one just after the improvement of growth conditions due to melting of the winter cover. The specific content of chlorophyll-*a* showed clear patterns in both seasonal and vertical distribution. Concerning long-term trends in the lake water chemistry from 1980–2004, a significant increase in surface pH and acid neutralising capacity was observed, and the extent of episodic acidification has diminished both in time and space. We suppose that the shift in species composition, as well as the increase in phytoplankton abundances in comparison with a 1990–1991 study can be considered as the first sign of a biological response to decreased acid deposition.

#### **Long term studies (1871–2000) on acidification and recovery of lakes in the Bohemian Forest (Central Europe) (VRBA & al. 2003a)**

In this paper, we documented long-term changes in acid deposition, lake water chemistry and biodiversity of eight Bohemian Forest lakes. Due to a 130-year research tradition, we were able to cover the pre-acidification period, the period of strong acidification as well as the period of reversal from acidity, especially in the case of the most extensively studied Černé Lake. During the 1960s and 1970s, heavy acid deposition caused a drop of 2 pH units in this lake. The shift in lake water chemistry was accompanied by a drastic reduction in biodiversity, which included the extinction of most species of crustacean zooplankton, littoral macrozoobenthos (Ephemeroptera, Plecoptera) and fish. Acidification resulted in reduced biodiversity in all lakes, and pelagic food webs became dominated by microorganisms. Concerning phytoplankton, the 1999 survey documented the presence of 20–23 species in the seven dimictic lakes. Due to scarce historical records, we could not evaluate acidification-driven changes in phytoplankton species composition and biomass in detail. However, a comparison of the 1999 survey with older data suggested that many phytoplankton species were able to survive when the lakes became acidic. As regards signs of potential biological recovery from acidification, we observed an almost 50 % increase in chlorophyll-*a* concentrations in Plešné Lake between 1994 and 1998, which was likely a consequence of reduced phosphorus immobilisation by aluminium. Moreover, the population of *Ceriodaphnia*

*quadrangula* returned to the pelagial of Černé Lake and the number of rotifers started to increase in Plešné Lake.

**A key role of aluminium in phosphorus availability, food web structure, and plankton dynamics in strongly acidified lakes (VRBA & al. 2006)**

This study focused on the evaluation of aluminium as a key factor driving plankton structure in four acidified lakes in the Bohemian Forest. The highest proportion of ionic aluminium was found in the most acid Čertovo Lake; on the other hand, the highest phosphorus input was characteristic for Plešné Lake. Phytoplankton in all lakes was apparently phosphorus limited, which was demonstrated both by elevated carbon to phosphorus ratios and the extremely high activity of acid extracellular phosphatases. The changes in lake water chemistry (pH and aluminium speciation) between 1998 and 2003 resulted in a significant increase in phytoplankton biomass. We suggested that both ionic and particulate aluminium may govern phosphorus availability namely through direct toxicity, inhibition of extracellular phosphatase activity and phosphorus inactivation. In lakes with lower concentrations of phosphorus, flagellates probably dominate the phytoplankton biomass due to their capacity for active migration. Moreover, mixotrophic species (e.g. frequent *Dinobryon* spp.) can partially satisfy their phosphorus demand by grazing on unicellular bacteria. Non-motile species (e.g. *Monoraphidium dybowskii*) likely take advantage of the higher phosphorus input to Plešné Lake. The dominance of this species in Plešné Lake can be further supported by its tolerance to high levels of toxic aluminium species. Hence, a unique set of specific food webs dominated by microorganisms have developed in the pelagic zone of particular lakes as the result of differences in aluminium and phosphorus input and acidification status.

**Massive occurrence of heterotrophic filaments in acidified lakes: seasonal dynamics and composition (VRBA & al. 2003b)**

We documented the massive presence of extremely long (> 100 µm) heterotrophic filaments in the plankton of acidified lakes in the Bohemian Forest. Our aim was to evaluate possible reasons for their seasonal development patterns and their high proportion in the plankton biomass in particular lakes. The main factors influencing filament dynamics were identified as both grazing of mixotrophic flagellates from the genus *Dinobryon* (Chrysophyceae) on unicellular bacteria and filtration of *Daphnia longispina*, which occasionally reduced protistan grazers as well as the filaments. In both oligotrophic lakes (Čertovo and Prášilské), *Dinobryon pediforme* was often the most abundant phytoplankton species, taking advantage

of mixotrophic growth in conditions of severe phosphorus limitation. On the other hand, *Dinobryon sociale* var. *americana* occurred in mesotrophic Plešné Lake usually in low abundance, and autotrophic phytoplankton species dominated in this lake due to the elevated nutrient supply. The plankton composition and dynamics in the lakes clearly showed the influence of both protistan grazing and the top-down control of cladoceran grazing on shaping the structure of pelagic assemblages.

Overall, we tried to determine key factors and mechanisms influencing actual species composition and structure of plankton biomass in acidified lakes under study. Through the effects of direct toxicity, phosphorus immobilisation and inhibition of extracellular phosphatases, aluminium was shown to shape the specific structure of pelagic food webs in particular Bohemian Forest lakes. The amount of total plankton biomass was apparently the result of a complex interplay between pH and phosphorus and aluminium concentrations in tributaries and lake water. In addition, protistan grazing by *Dinobryon* spp. was identified as the reason for an unusually high proportion of heterotrophic filaments in the plankton biomass. Concerning phytoplankton, higher nutrient input seems to favour the occurrence of non-motile forms. On the other hand, flagellates often with the ability of mixotrophic nutrition dominate in the lakes with lower nutrient input, as it is typical for oligotrophic mountain lakes elsewhere.

Furthermore, the results of an extensive limnological survey have demonstrated in detail that the beginning of biological recovery of the lakes from acidification has been significantly delayed after the chemical reversal. However, a significant progress was recorded in comparison with previous studies. Due to the harsh growth conditions in the lakes already during the pre-acidification period, the response of phytoplankton communities was manifested chiefly through increases in biomass as a result of reduced phosphorus immobilisation by aluminium in conditions of higher pH. However, we have documented a shift in phytoplankton species composition in a lake characterised by diminishing extent of episodic acidification. Most likely, further reversal in lake water chemistry in both lake districts will be reflected in changes in biomass and relative proportion of species due to the varying importance of bottom-up and top-down control. In general, we suppose that namely the ecosystem resistance may prevent full biological recovery of the lakes.

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