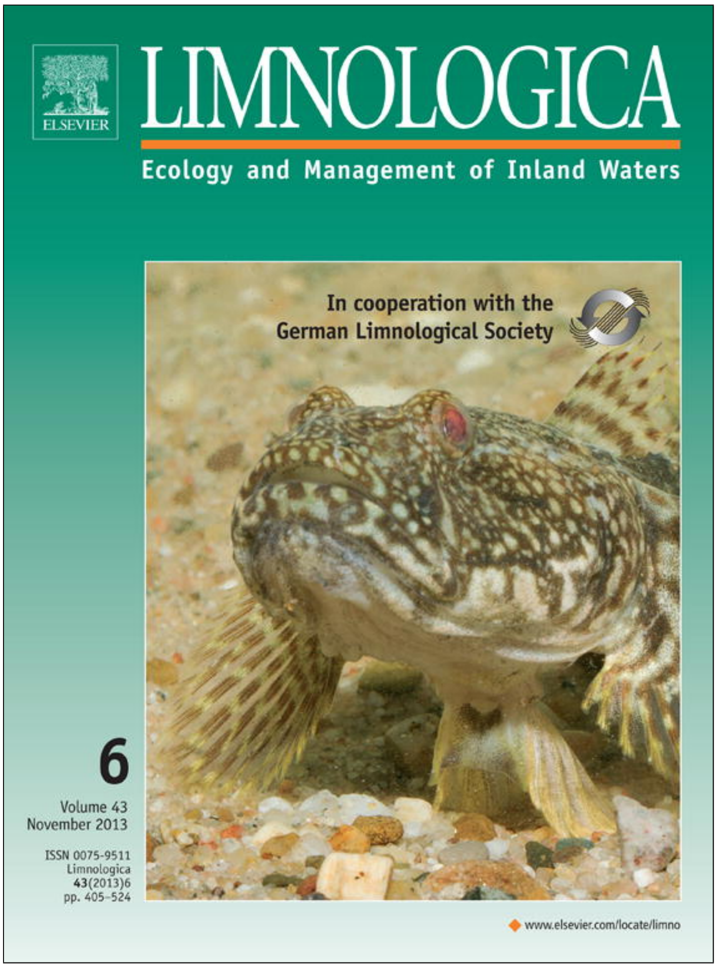
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The unique environment of the most acidified permanently meromictic lake in the Czech Republic

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ABSTRACT

Changes in the water properties and biological characteristics of the highly acidic Hromnice Lake (Western Bohemia) were investigated. This 110-year-old lake, formed as a consequence of the mining of pyritic shales, is permanently meromictic. Two chemoclines separate an extremely acidic (pH ~ 2.6) mixolimnion from a metal-rich anoxic monimolimnion. The absence of spring mixolimnetic turnover due to ice melting and very slow heat propagation through the chemocline with a 6-month delay were observed. Extreme mixolimnetic oxygen maxima (up to 31 mg l^{-1}) in phosphorus-rich lake (PO_4^{3-} up to 1.6 mg l^{-1}) well correlated with outbursts of phytoplankton. Phytoplankton consist of several acido-tolerant species of the genera *Coccomyxa*, *Lepocinclis*, *Chlamydomonas* and *Chromulina*. Surface phytoplankton biomass expressed as chlorophyll-*a* varies from 2 to 140 μg l^{-1}. Multicellular zooplankton are almost absent with the exception of *Cephalodella acidophila*, a small rotifer occurring in low numbers. Large red larvae of the midge *Chironomus* gr. *plumosus* were found at the bottom close to the shore, with larvulae in the open water. Developmental stages (protonemata) of a moss, resembling filamentous algae, dwell in the otherwise plant-free littoral zone.

Introduction

A few hundred anthropogenic lakes have formed in the Czech Republic due to the spontaneous flooding of abandoned mines after the termination of opencast mining (Hrdinka, 2007). These lakes often differ significantly from natural ones in morphometric and hydrological properties (Castendyk and Eary, 2009). Extreme examples are the lakes formed after the mining of pyritic shales. In the 19th century, this rock high in sulphides with an admixture of organic carbon (Pašava et al., 1996) was extracted and processed to obtain a supersaturated sulphuric acid (oleum) near Hromnice village. After the termination of mining in 1893, the pit was spontaneously flooded with acid rock drainage thus forming Hromnice Lake. In 1975, the area was declared a natural monument because of its unique status.

Studies of lakes formed after the mining of sulphidic ores with high pyrite content, are known from Spain (Sánchez España et al., 2008; Wendt-Potthoff et al., 2011), the USA (Pellicori et al., 2005), Greece (Triantafyllidis and Skarpelis, 2006), Sweden (Ramstedt et al., 2003), and Czech Republic (Hrdinka and Šobr, 2010). Similar properties have also been observed in lakes after the mining of hard coal and lignite in Germany (Geller et al., 1998; Schultze and Boehrer, 2008; Schultze et al., 2010; von Rohden et al., 2010), Austria (Moser and Weisse, 2011), and France (Denimal et al., 2005). The stratification of pit lakes and the phenomenon of meromixis have been studied *e.g.* by Boehrer and Schultze (2008), who also provided an overview of studies dealing with this topic (Boehrer and Schultze, 2006).

The specific properties of mining lakes waters are often evaluated from long-term perspectives. The aim of this study was to detail the yearly course of the basic physical and chemical properties of Hromnice Lake associated with the meromixis and to identify the processes involved therein. Further, we focused on identifying the organisms present in the lake adapted to extreme conditions and to compare the community of Lake Hromnice with communities of other extremely acidic lakes in Germany and Austria (Nixdorf et al., 1998a,b; Deneke, 2000; Lessmann et al., 2000; Wollmann et al., 2000; Moser and Weisse, 2011).

Because this unique environment has been developing for over 110 years, a comparison of the results with lakes of similar origin might bring new information as to how these specific ecosystems may evolve.

The sections on morphometry, physical and chemical limnology were written by T. Hrdinka and M. Šobr, the section on biology by J. Fott and L. Nedbalová.

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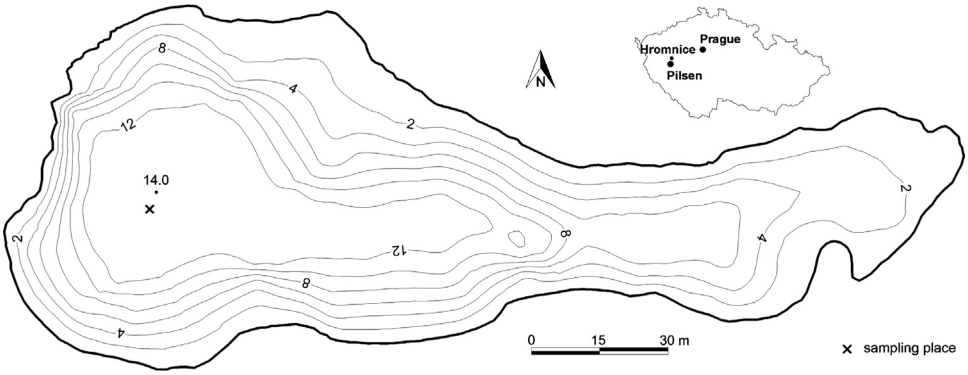

Fig. 1. Bathymetric map of Hromnice Lake (Hrdinka and Šobr, 2010) with the site of physical parameter measurements in the vertical profile and water sampling for chemical analysis marked, contour interval 2 m.

Study site and methods

Geological background and water regime

Hromnice Lake (49°51′02.5″ N, 13°26′39.3″ E) is located in the west of the Czech Republic at the bottom of an approx. 50 m deep mining pit with outer dimensions of 260 m × 150 m (Fig. 1). The lake surroundings are densely forested, with a large area of arable land to the north of the pit regularly slanting towards the lake. The geochemistry of the rock was studied by Pašava et al. (1996), who detected elevated concentrations of sulphur (av 1.61 wt%), organic carbon (av 0.68 wt%) and metals (av 4.15 Fe_{tot} wt%, 0.570 V wt‰, 0.383 Zn wt‰, 0.156 Cr wt‰, 0.093 Cu wt‰, 0.088 Ni wt‰) in a drill hole in close proximity to the site. The lake basin is formed by strongly inclined slopes formed by bare rock and unconsolidated shale debris. The presented morphometric characteristics (Table 1) show that the shape of the lake basin (relative depth, bottom inclination) and wind-protected lake position are factors that can contribute to the formation of permanent lake stratification (Miller et al., 1996; Doyle and Runnells, 1997).

To evaluate the water balance of the lake, which has no surface inlet or outlet, a Solinst 3001 Levelogger was used to record the air pressure compensated water level with an accuracy of 1 mm over a period of 1 year (September 2010–August 2011) at regular 1 h intervals. Further, meteorological data from the Plzeň-Mikulka weather station (mean daily air temperature) and Hromnice rain gauge (daily precipitation) were assessed (Czech Hydrometeorological Institute data). The results (Fig. 2) show a strong subsurface inflow, documented in the prominent increase of water level during the winter season (with relatively lower precipitation) and also a quick response of the water level to causal precipitation throughout the year. The water level fluctuation of 0.86 m during the observation period is equivalent to a lake volume change of 7714 m^3, *i.e.* 13% of the maximum. During the period from December 2010 to March 2011 the lake was permanently covered with ice.

Measurements and sampling

Measurements of the physical properties of the water and sampling for chemical and biological analysis were performed above the deepest point of the lake. Physical properties of water were measured with a YSI 6920 multi-parametric probe at approx. 2-week intervals from September 2010 to August 2011, using 0.25 m steps to a depth of 5 m and further 0.5 m steps down to the bottom. The YSI 6920 probe measures temperature with an accuracy of ±0.1 °C, conductivity with Ni-electrodes compensated to $T = 25\,°C$ ($\pm 10\,\mu S\,cm^{-1}$), dissolved oxygen with an amperometric (Clarke) electrode ($\pm 0.2\,mg\,l^{-1}$), pH with a glass electrode (±0.2 unit) and ORP as the potential between Pt and Ag/AgCl electrodes with an accuracy of ±20 mV (YSI Incorporated, 2009). Water transparency was determined under standard conditions using a Secchi disc (30 cm diameter), and water colour according to the Forel-Ule scale against a Secchi disc at half the transparency depth. The results were visualized using the Kriging interpolation method in Golden Software Surfer 8.

Water samples for chemical analysis were taken in the same time period at approx. 6-week intervals using a 2 L van Dorn sampler (integrating 0.5 m-high water columns) from three horizons at depths of 0.3 m, 5 m and 12 m (the layer approx. 1.5 m above the bottom, excluding the possibility of contamination by sediments). Major ions (Ca, Mg, Na, K, NH_4^+, SO_4^{2-}, Cl^-, NO_3^-, PO_4^{3-}), Fe(II) (bubble-free sample, trace metal grade HNO_3 acidified to pH < 1 on site), total organic carbon TOC (bubble-free sample), pH and selected metals (Fe, Al, Cu, Zn) were determined. Unfiltered samples were immediately transported to the laboratories of the T.G.M. Water Research Institute in Prague, where they were processed within 48 h, with the exception of pH and Fe(II), which were determined immediately. On the first sampling date, sulphidic sulphur S(-II) (sample stabilized with sodium ascorbate) and other metals (Mn, Ni, Cr, Co, Cd) were additionally determined.

Samples for the identification of phytoplankton and determination of chlorophyll-*a* were taken together with samples for chemical analysis using the same sampling technique. An additional sample of phytoplankton and chlorophyll was taken on

Table 1
Morphometric characteristics of Hromnice Lake.

Mean surface level (m a.m.s.l.)	330	Perimeter (m)	535
Area (m^2)	9740	Maximum depth (m)	14.0
Volume (m^3)	60,980	Mean depth (m)	6.3
Maximum length (m)	221	Relative depth (%)	12.2
Maximum width (m)	79	Mean slope of basin (°)	27

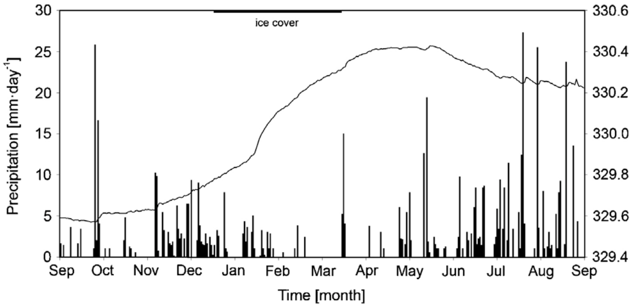

Fig. 2. Water level changes in Hromnice Lake (solid line) and daily precipitation (columns).

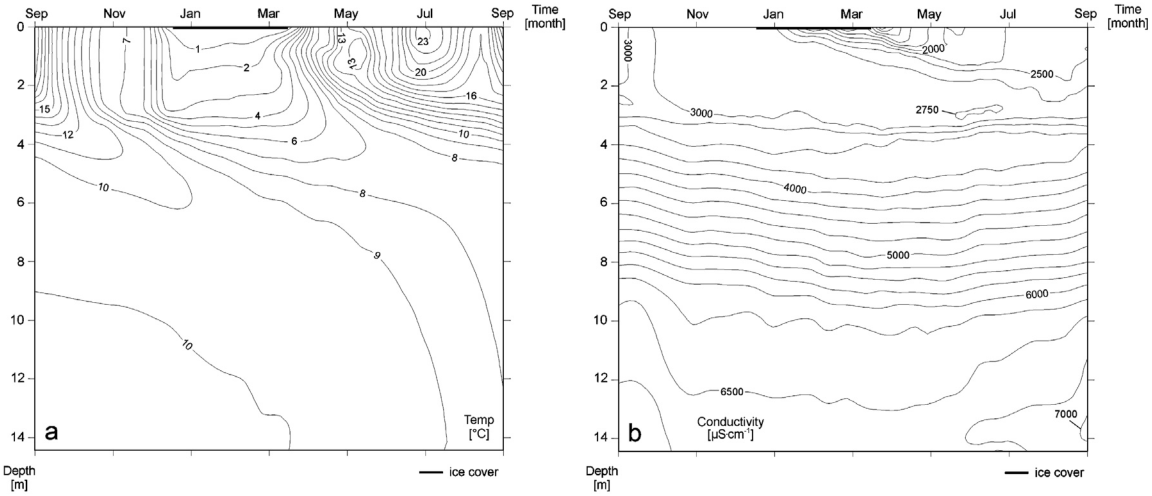

Fig. 3. Year-round course of water temperature (a) and conductivity (b) in vertical profile above the deepest point of the lake.

March 22, 2012. From 2010 to 2011 attempts to sample zooplankton by taking horizontal and vertical hauls by a plankton net 40 μm mesh size were unsuccessful. A successful sampling of small rotifers was carried out on August 16, 2012 with a 6.4 L van Dorn sampler, straining the water through a net with 25 μm mesh size. The invertebrate macrofauna were sampled from the shore with use of a strainer.

Chemical and biological analyses

Several standard analytical methods were used for chemical analysis: pH direct measurement, continuous flow analysis (NH_4^+, NO_3^-), ion chromatography (SO_4^{2-}, Cl^-), ICP-AES (Na, K, Ca, Mg, Al, Cu, Zn, P_{tot}, Fe_{tot}), non-flame AAS (Cu-bottom sample only), Pt-catalysed high temperature combustion method (TOC), spectrophotometric methods with ammonium molybdate (PO_4^{3-}), phenanthroline (Fe(II)) and methylene blue (S(-II)). Samples for ICP-AES and AAS were preliminary acidified to pH < 1 with trace metal grade HNO_3, samples for PO_4^{3-} analysis filtered with a 0.45 μm membrane. Measurement uncertainties were as follows: ±0.2 (pH), 5% (Mg, Na, PO_4^{3-}), 10% (Ca, K, Cu, Zn, SO_4^{2-}, Cl^-, NO_3^-, NH_4^+, Fe(II)), 15% (Al, P_{tot}, Fe_{tot}, S(-II)) and 20% (Cu-AAS).

The live phytoplankton were gently centrifuged (15 min room temperature, less than 1000 rpm) and observed with the use of a microscope, objective lenses 40× and 100×. In *Coccomyxa*, *Lepocinclis* and *Chlamydomonas* no damage to cells took place, but *Chromulina* lost their flagella upon centrifugation. In order to identify *Chromulina*, samples were preserved with Lugol solution immediately before centrifugation. Chlorophyll-*a* was extracted from particles retained on Whatman GF/F glass microfiber filters, 9 mm diameter. The filter was fastened in a Millipore holder connected to a plastic syringe containing 5–50 ml of sample and filtration was carried out by pressing the piston by hand. The volume filtered was adjusted to the concentration of particles so that clogging did not occur and only slight pressure needed to be applied. Then the filter was rinsed with several ml of distilled water from the syringe, put on a filter paper soaked with a phosphate buffer pH = 7.0 and pressed against dry filter paper in order to remove the excessive fluid. Rinsing and buffering the filters before extraction of chlorophyll-*a* are not common practice, but in the present study these precautions were necessary in order to eliminate the acidity that led to lower fluorometer readings. The filters were stored in a freezer in 10 ml screw-cap tubes with 7.5 ml of 90% acetone. Prior to analysis 1.5 ml of methanol was added and the tubes were agitated 2 min in a water bath at 65 °C (Pechar, 1987). After centrifugation chlorophyll-*a* was measured by a Turner TD-700 fluorometer equipped with a non-acidification optical kit. The instrument is designed for analysing low amounts of chlorophyll-*a* and is relatively insensitive to chlorophyll-*b* and chlorophyll degradation products (Welschmeyer, 1994). Identification of phytoplankton and determination of chlorophyll-*a* were carried out in the laboratory of the Faculty of Science, Charles University in Prague.

Since identification of the dominant phytoplankton species by morphology was unsuccessful, we used molecular analysis, using a surface sample from the lake. The sample contained a monospecific population of the desired alga, with no other algal species observed. The cells were harvested by centrifugation and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen). The 18S rRNA gene was amplified using the primers P2 (5′-CTGGTTGATTCTGCCAGT-3′) and P4 (5′-TGATCCTTCYGCAGGTTCAC-3′) (Moon-van der Staay et al., 2000). The polymerase chain reaction (PCR) was done using Taq DNA polymerase (PCR cycle of initial denaturing of 10 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 58 °C, 2 min at 72 °C and a final extension of 10 min at 72 °C). Agarose electrophoresis of PCR products yielded a single band of the expected length. The PCR product was sequenced (Macrogene) using the P2 and P4 primers. The sequences were manually corrected and checked for similarity using the Basic Local Alignment Search Tool (Altschul et al., 1990). HPLC analysis of pigments in a sample dominated by the analyzed species was carried out at the Institute of Microbiology in Třeboň, Czech Republic. The sample was scanned for all major groups of algal pigments (carotenoids, chlorophylls, phycobiliproteins). For a detailed description of the method see Komárek et al. (1999).

Results

Physical variables

The yearly course of water temperature, conductivity and dissolved oxygen in the vertical profile above the deepest point of the lake is shown in Figs. 3 and 4. Fig. 3b shows a substantial mixolimnion (conductivity < 3000 μS cm^{-1}) separated by a sharp chemocline, which lies at a depth of 3–3.5 m throughout the year. Below, at a depth of 3.5–5 m, there is a transitional layer followed by a less distinct yet relatively thick secondary chemocline reaching down to a depth of 9–10 m. During spring, a significant decrease in conductivity (up to 470 μS cm^{-1}) occurred in the upper part of the mixolimnion due to ice melting. This prevented the expected

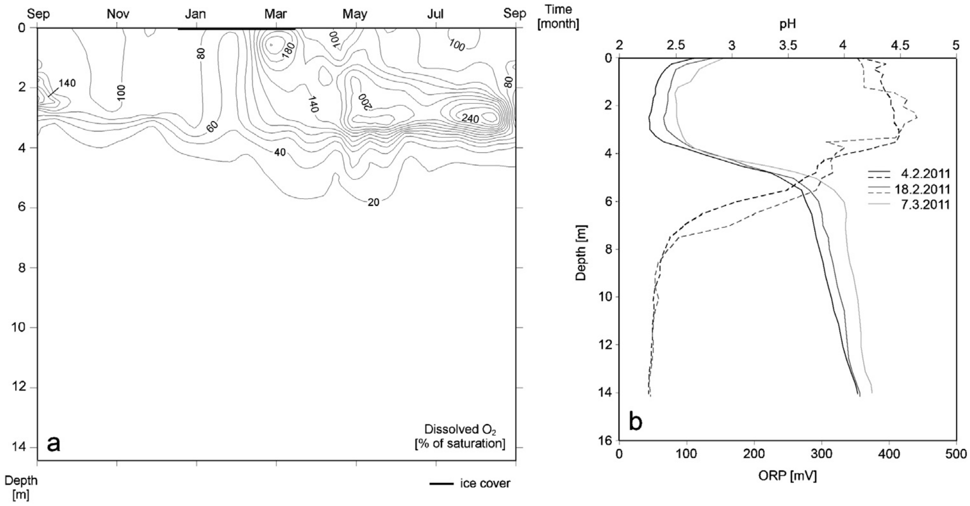

Fig. 4. Year-round course of dissolved oxygen above the deepest point of the lake (a), three representative profiles of pH (solid line) and ORP (dashed line) (b).

spring mixing and a gradual homogenization of the mixolimnion took place for over 5 months (Fig. 3b).

The location of the chemocline at the depth of 3 m corresponds to the winter position of the water temperature anomaly (~4 °C, Fig. 3a), from which the temperature starts to increase gradually downward to the lake bottom (up to 10.3 °C). Also, there is a significant delay in water temperature maxima and minima within the chemocline, which are delayed by up to 6 months at the depth of 5 m, compared to temperatures in the mixolimnion. Further, with regard to the estimated thermal stability of the lower monimolimnion, a gradual decrease in water temperature of 2.2 °C was detected. In autumn 2010, favourable conditions for water mixing in the mixolimnion profile were observed during the isotherm up to the depth of 3 m, lasting for nearly 6 weeks (Fig. 3a).

The yearly course of dissolved oxygen (Fig. 4a) is very closely connected to the position of the chemocline and the transitional layer at the depth of 3.5–5 m. While the most significant changes take place in the mixolimnion, a rapid decrease in dissolved oxygen saturation down to saturation values of 10–20% occurs within the chemocline and the transitional layer. In the bottom layer of the lake, at a depth below 8 m, permanent anoxic conditions (DO < 0.1 mg l^{-1}) were observed. In autumn 2010, the mixolimnion of Hromnice Lake was mixed in its entire volume, resulting in saturation values of 90–110%. After the freezing of the lake surface, there was a temporary decrease in dissolved oxygen to ~60% followed by a significant increase up to 220% (~31 mg l^{-1}) from mid-February to mid-March. This situation was related to intensive photosynthesis of the phytoplankton *Chromulina* sp. just below the ice without snow cover (Fig. 6). Throughout the summer season, considerable oxygen maxima influenced by phytoplankton *Coccomyxa* sp. (Fig. 6) were observed just above the chemocline, with supersaturation levels of 200–270% (~28 mg l^{-1}).

Fig. 4b shows selected pH and ORP profiles measured above the deepest point of the lake. The sudden rise in pH at the depth of 3 m indicates the upper limit of the chemocline, below which reductive processes prevail. The *in situ* measured pH values ranged from 2.3–2.9 in the mixolimnion (higher values were influenced by the presence and melting of ice) to 4.0–4.2 at the bottom of the lake. The ORP in the oxic environment of the mixolimnion ranged between 400 mV and 600 mV throughout the year, which dropped to values from −50 mV to +50 mV (equals Eh ~ 200 mV) towards the bottom. The reductive environment in the deepest layer was indicated by a strong odour of H_2S and confirmed by subsequent analysis (Table 2).

Water transparency ranged from 0.85 m during the autumn mixing to 2.3 m just after ice melting; water colour during the measurements ranged between 17 and 18 (yellow-brown) on the Forel-Ule scale (Wernand and van der Woerd, 2010).

Chemical variables

The chemical composition of water in the Hromnice Lake (Table 2) is primarily a result of dissolution of shale minerals, precipitation of particular Fe(III) compounds and decomposition of organic matter by oxidation-reduction processes. These influence the concentration and form of substances dissolved in the water, depending on the amount of dissolved oxygen, pH and ORP (Blodau, 2006).

In the shallow horizons of the lake surroundings, oxidation of pyrite by means of O_2 and Fe^{3+} dissolved in water prevails (Evangelou, 1995), which causes the release of $SO_4{}^{2-}$ and Fe(II) that concentrate in deeper layers of the lake. In respect of particular sulphate minerals precipitation in the upper oxic layer, the concentration of $SO_4{}^{2-}$ (up to 6410 mg l^{-1}) shows a considerable dependence on the depth (Fig. 5). In the reductive environment below 5 m, Fe_{tot} entirely occurs in the Fe(II) form (Table 2) and is thus in agreement with the results shown in Fig. 4. Dissolved Fe(II) is further oxidized and hydrolysed within the mixolimnion, resulting in minimal concentrations at the surface (~4% of Fe_{tot}) and acidification of the water to pH ~ 2.6. The surface concentration of Fe_{tot}, partially precipitated to reddish-brown sediments close to the lake shore, was also low compared to values from the depth of 5 m and 12 m (up to 2100 mg l^{-1}).

Considering the high biomass of phytoplankton, the concentrations of $PO_4{}^{3-}$, $NO_3{}^{-}$, $NH_4{}^{+}$, and TOC were assessed. Very high concentrations of $PO_4{}^{3-}$ (up to 1.6 mg l^{-1}) were found at the depths of 5 m and 12 m, with relatively low concentrations of ~0.04 mg l^{-1} at the surface (Fig. 5). While $NO_3{}^{-}$ concentrations were up to 24.5 mg l^{-1} near the surface, at the depth of 5 m they were mostly close to the detection limit (1 mg l^{-1}) with an exceptional maximum of 6.4 mg l^{-1} in mid-May, which corresponded to 30% dissolved oxygen saturation (Fig. 4a). An inverse trend was observed in $NH_4{}^{+}$, with average values of 0.07 mg l^{-1} (0.3 m), 0.27 mg l^{-1} (5 m) and 14.5 mg l^{-1} (12 m). The total organic carbon (TOC) concentrations varied between 2–12 mg l^{-1}, with minima at the surface and maxima within the transitional layer at the depth of 5 m.

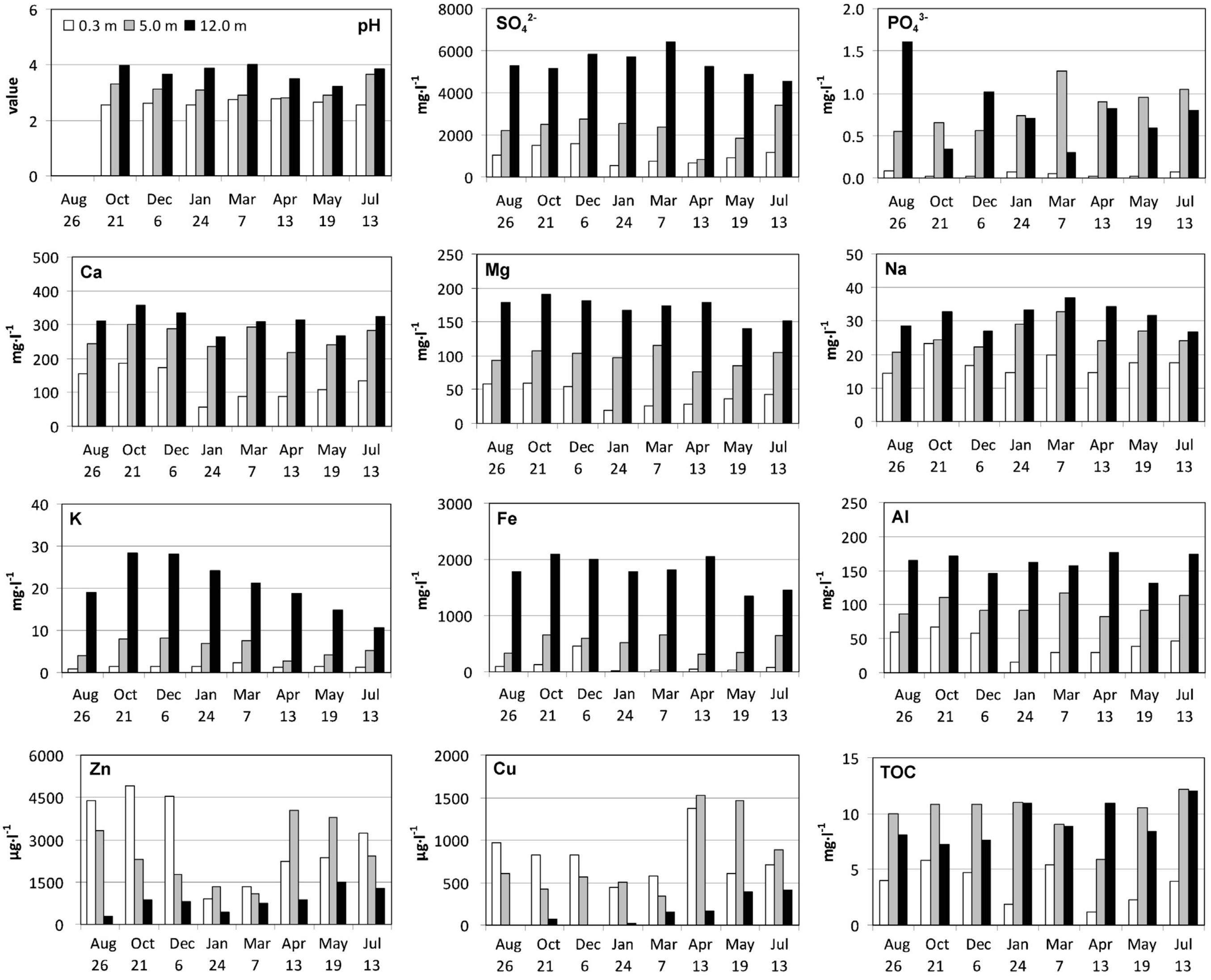

Fig. 5. Year-round development of selected parameters of water quality at depths of 0.3 m, 5 m and 12 m.

The major cations Ca, Mg, Na, and K showed increased concentrations at the depths of 5 m and 12 m with respect to their dilution (ice melting) or partial precipitation in the mixolimnion. Conservative behaviour was shown by Cl^- ions, with low concentrations of 15–30 mg l^{-1} in the entire water column. While most metals showed a concentration increase with depth, the opposite trend was observed for Cd, Cu and Zn. Dissolution of alumino-silicate minerals scattered in pyritic shales results in high concentrations of Al (up to 176 mg l^{-1}); increased concentrations were also observed for Mn, Ni (4.56 mg l^{-1}), Cu (up to 1.52 mg l^{-1}), Co (0.56 mg l^{-1}) and Pb (0.13 mg l^{-1}). As opposed to Fe(II), Mn(II) is more stable in an oxic environment and practically does not oxidize at pH ~ 3 (Takeno, 2005). This is related to more balanced Mn concentrations between the surface (2.8 mg l^{-1}) and the bottom (12.6 mg l^{-1}) of the lake, compared to Fe_{tot} (Fig. 5).

While for most parameters a winter/spring decrease was observed in surface concentrations due to the presence and melting of ice cover, the concentrations of some analytes in samples

Table 2
Selected parameters of water quality at depths of 0.3 m, 5 m and 12 m – mean of 8 measurements with exception of Mn (3), Ni (2) and Co, Cd, Cr, S(-II) (1 measurement), concentrations in mg l^{-1}.

Depth	pH	TOC	SO_4^{2-}	NO^{3-}	NH_4^+	PO_4^{3-}	Cl^-	Ca	Mg	Na	K
0.3 m	2.6	3.6	1020	10.5	0.07	0.04	21	124	40	17.3	1.4
5.0 m	3.1	10.0	2310	1.7	0.25	0.83	17	263	98	25.5	5.8
12.0 m	3.7	9.2	5390	1.7	14.50	0.77	21	310	170	31.4	20.6

Depth	Fe_{tot}	Fe(II)	Al	Mn	Zn	Ni	Cu	Co	Cr	Cd	S(-II)
0.3 m	111	4	43	2.76	2.99	0.56	0.791	0.112	0.030	0.015	<0.01
5.0 m	507	524	98	5.63	2.51	2.23	0.788	0.265	0.053	0.023	<0.01
12.0 m	1790	2017	160	12.60	0.84	4.33	0.154	0.562	0.056	0.002	1.17

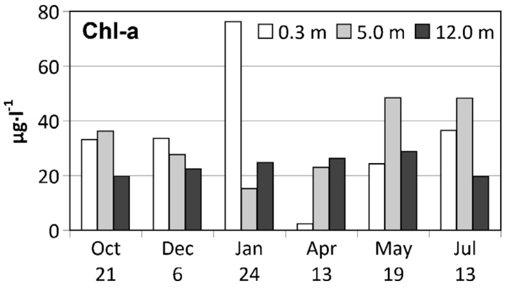

Fig. 6. Concentration of chlorophyll-*a* (2010–2011) at depths of 0.3 m, 5 m and 12 m.

from 5 m and 12 m were surprisingly variable during the year (TOC, PO_4^{3-}, K, Zn, Cu). The possible reasons and uncertainties are discussed further in the text.

Biological variables

The phytoplankton of Hromnice Lake are poor in species but high in biomass. The most abundant species is a coccal green alga of longitudinal shape with a single parietal chloroplast, dimensions 8 μm × 1 μm. Its affiliation to Chlorophyta was demonstrated by the presence of pigments characteristic for green algae: chlorophyll-*a*, chlorophyll-*b*, violaxanthin, lutein and β-carotene. Molecular analysis classified it unambiguously into the class Trebouxiophyceae, and the 18S rDNA sequence (1596 bp) matched closely with several strains of the genera *Coccomyxa* and *Pseudococcomyxa* reported in GenBank (coverage 99%, similarity 99%). Considering the imperfect taxonomy for this group of algae we have chosen the tentative designation *Coccomyxa* sp. However, a more detailed analysis is needed to elucidate its taxonomic position.

Another species common in the lake is a euglenoid flagellate *Lepocinclis* sp., resembling *Lepocinclis buetschlii* or *L. ovum*. Both *Coccomyxa* and *Lepocinclis* occur throughout the year, *Lepocinclis* being most abundant at the 5 m depth. When the lake was frozen (January 25–March 7, 2011) the layer under the ice was inhabited almost exclusively by a chrysomonad flagellate *Chromulina* sp. On March 22, 2012 a bloom of a green flagellate *Chlamydomonas* sp. reaching 140 $\mu g\,l^{-1}$ chlorophyll-*a* was observed. Bacteria were not studied, but long bacterial filaments were occasionally observed together with phytoplankton. A conspicuous feature of the mixolimnetic phytoplankton in Hromnice Lake is the uniform species composition within particular maxima of abundance and the absence of amorphous organic matter (detritus), the only particles being living cells. In fact, microscopic views of centrifuged phytoplankton resemble laboratory cultures. The apparent reason for the lack of detritus is the absence of large algivorous zooplankton. According to values of chlorophyll-*a*, Hromnice Lake is comparable with eutrophic lakes. Most measurements of chlorophyll-*a* from the depths of 0.3 m, 5 m and 12 m fell within the limits 20–50 $\mu g\,l^{-1}$, with extreme values 140 $\mu g\,l^{-1}$ (not included in Fig. 6) and 2.4 $\mu g\,l^{-1}$, respectively. Relatively stable concentrations of about 20 $\mu g\,l^{-1}$ were found frequently at the 12 m depth (Fig. 6).

Green filaments of presumed filamentous algae were sampled from the bottom surface close to the shore in May 2011 and August 2012. However, upon microscopic examination these filaments proved to be developmental stages (protonemata) of a moss, easily recognizable by the diagonal cross walls (Glime, 2007). Their physiological state seemed to be good. We have kept them successfully in laboratory culture with water from Hromnice Lake enriched by the BBM algal cultivation medium. Wooden logs floating in the water were overgrown by periphyton consisting of unidentified green coccal algae. There were no littoral macrophytes.

The only multicellular animals inhabiting the open water were larvulae (first-instar larvae) of the midge *Chironomus* gr. *plumosus* and small rotifers (123 μm × 34 μm) of the genus *Cephalodella*, the latter occurring in an approximate abundance of several individuals l^{-1}. The rotifer was later identified as *Cephalodella acidophila* (Jersabek et al., 2011). Based on micrographs, the identification was confirmed by C. Jersabek. *C. acidophila* was found also in the periphyton together with another small acido-tolerant rotifer, *Elosa worallii*. Ciliata were observed occasionally along with the centrifuged phytoplankton, especially in winter samples rich in *Chromulina*. No Heliozoa have been observed so far.

Large, red-coloured larvae of *Chironomus* gr. *plumosus* were frequently found close to the shore at the bottom surface or in clumps of protonemata. The first-instar chironomid larvae were found in the open water, which is a well-known strategy for their dispersal over the water body (Armitage et al., 1995). Water boatmen (Corixidae) were found occasionally close to the shore but in very low numbers.

Discussion

Physical variables

Several phenomena related to the permanent meromixis were observed in Hromnice Lake. The double layering of the chemocline, although relatively rare, is not unknown in anthropogenic lakes and was found *e.g.* by Ramstedt et al. (2003) in the sulphidic ore Udden pit lake in Sweden. With respect to the length of observation, however, we do not know if the transitional layer separating both chemoclines is only of temporary character. Compared to the Udden pit lake, the transitional layer is very thin, and can thus be assumed to be less stable. The influence of the density gradient in restricting spring turnover due to ice melting in Hromnice Lake was also recorded by Pieters and Lawrence (2009) in Tailings Lake in Canada.

A year-round increase of temperature towards the bottom is a phenomenon typical of meromictic lakes (Denimal et al., 2005; Wendt-Potthoff et al., 2011) and is usually explained by the influence of inflowing groundwater, as observed in Hromnice Lake, or by heat exchange with the underlying rock (Wetzel, 2001). The phenomenon of gradual heat propagation through a chemocline has been described by von Rohden et al. (2010), who showed a temperature delay between both layers of the Moritzteich in Germany of over 4 months. This is related to heat progression under the conditions of limited vertical exchange, where the transport of heat and substances takes place mainly on the molecular level (von Rohden et al., 2010). However, considering the length of measurement in Hromnice and insufficient groundwater data, it cannot be determined with certainty whether the gradual decrease of temperature in the lower monimolimnion is related to the cooling process following the last two winter seasons or seasonal variations in groundwater temperature. In the first case, the cooling of this layer could be delayed by over 1 year.

Poorly oxic/permanently anoxic conditions due to the biochemical oxidation of dead organic matter and Fe(II) are very frequent below the chemoclines of meromictic pit lakes. They have also been observed in the morphometrically and hydrochemically similar, most acidified Austrian lake AML Langau (Moser and Weisse, 2011), but unlike Hromnice Lake, they were not permanent throughout the year, probably due to a thicker mixolimnion and easier oxygen diffusion to greater depths. Two different oxygen maxima were recorded in Hromnice Lake. A causal (although not that strong) relation between the development of phytoplankton and dissolved oxygen below ice is relatively common in eutrophic lakes (*e.g.* Wiedner and Nixdorf, 1998; Phillips and Fawley, 2002), while extremely high levels (>200%) are the domain of shallow, strongly eutrophic lakes in particular (Wetzel, 1966; Alvares Cobelas et al., 1990); they have not been reported in lakes created after the mining of sulphide ores. This phenomenon was facilitated by increased water transparency in the spring/summer season and very likely further enhanced by the uptake of dissolved CO_2 and phosphorus from the nearby chemocline.

Hromnice Lake, with its surface pH ~ 2.6, ranks among very acidic ($2 < pH < 4$, Sánchez España et al., 2008) and extremely acidic lakes ($pH < 2.8$, Nixdorf et al., 1998a). This value is characteristic for surface water of lakes formed after the mining of sulphidic ores (Levy et al., 1997; Pellicori et al., 2005; Wendt-Potthoff et al., 2011) and lignite (Nixdorf et al., 1998a; Lessmann et al. 2000; Moser and Weisse, 2011) with an admixture of pyrite. Within the chemocline, the oxidation of Fe(II) to particular Fe(III) minerals (goethite, schwertmannite, *etc.*) is apparently limited, which reflects in a higher pH. An increase of pH in the chemocline might be further enhanced by reductive dissolution of precipitated Fe(III) minerals by organic carbon (C_{org}) from decay (Isaacson et al., 2006). Practically the same course of pH (2.6–4.2) with respect to depth was observed by Wendt-Potthoff et al. (2011) in the meromictic lake Cueva de la Mora in Spain (Iberian Pyrite Belt), which is also comparable in the vertical pattern of ORP. The Eh values of about ~200 mV observed at the bottom of Hromnice Lake may already facilitate a weak biogenic reduction of SO_4^{2-} to sulphidic sulphur S(-II) (Takeno, 2005), which is in agreement with the results of chemical analysis. It should be noted that redox potentials measured in natural systems may be difficult to interpret since they are not usually in equilibrium. As a result, the coexistence of some oxic and reductive forms of analytes is made possible (*e.g.* NO_3^- and Fe(II) at the bottom) as in the case of Hromnice Lake.

Besides the assumed influence of the phenolics originating from the biodegradation of leaf fallout, the reddish colour of the lake is probably further enhanced by the occurrence of hydrated iron oxides, similar to the Berkeley Pit Lake in Montana (Gammons and Duaime, 2006).

Chemical variables

In Hromnice Lake a kind of chemically induced, crenogenic type of meromixis exists, formed by the underground inflow of mineralized water (Walker and Likens, 1975). According to Boehrer et al. (2009) the lake can thus be classified as iron-meromictic.

In the oxic environment, rich in SO_4^{2-}, precipitation and dissolution of sulphate minerals are the main mechanisms influencing the concentration of dissolved Fe(III) and water pH in the lake. The same phenomenon has been described at many locations after the mining of sulphide ores (Sánchez España et al., 2008) and lignite (Totsche et al., 2003). A considerable decrease of Fe(III) in favour of Fe(II) on the chemocline level similar to Hromnice Lake was observed by Wendt-Potthoff et al. (2011) in Cueva de la Mora. On the contrary, Pellicori et al. (2005) demonstrated considerable depth concentrations of Fe(III) with extremely high ORP, which, however, is rather rare in meromictic lakes. The measured values of $Fe(II) > Fe_{tot}$ are most likely caused by the use of different analytical methods, but since they are still within the uncertainty limits of the methodology, the results can be considered relevant. The concentration of SO_4^{2-} roughly corresponds to the concentrations observed in other lakes formed after the mining of sulphidic ores with water pH ~ 2.5–4.0 (Pellicori et al., 2005; Sánchez España et al., 2008). In contrast, it is about 5–10 times higher than in lakes created after the mining of hard coal and lignite (Denimal et al., 2005; Schultze et al., 2010; Moser and Weisse, 2011).

The high concentrations of PO_4^{3-} observed in the strengthened reductive environment (samples from 5 m and 12 m) are supposed to contribute significantly to the late spring development of phytoplankton at the upper limit of the chemocline (Figs. 4a and 6). On the contrary, relatively low concentrations at the surface can be explained mainly by adsorption of PO_4^{3-} ions on hydrated Fe(III) oxides or precipitation by Fe^{3+} ions producing $FePO_4(s)$ (Pitter, 2009), in addition to assimilation by phytoplankton. These solids subsequently accumulate in the monimolimnion, where they are again dissolved in the anoxic conditions (Kleeberg and Gruenberg, 2005). The NO_3^- and NH_4^+ concentrations reflect prevailing oxic/anoxic conditions in the vertical profile of the lake. The exceptional elevated concentrations of NO_3^- (7.0 mg l^{-1}) at the bottom of the lake were most probably of allochthonous origin (*e.g.* inflow of fresh groundwater from shallow horizons associated with the arable land to the north of the lake) and their presence indicates that denitrification, if any, is very slow given the pH and ORP (van Haandel and van der Lubbe, 2007; Pitter, 2009).

While meromictic pit lakes can generally be considered as oligotrophic with low primary productivity (Doyle and Runnells, 1997), the TOC in Hromnice Lake rather indicates a eutrophic state (Thurman, 1985), which is in line with increased concentrations of chlorophyll-*a* and PO_4^{3-}. The TOC values are comparable to those in lakes formed after the mining of lignite in Central Germany (DOC 3–15 mg l^{-1}, Schultze et al., 2010), where, however, the concentrations of chlorophyll-*a* and PO_4^{3-} were very low and increased DOC originated mainly from the remnants of dispersed lignite. In Hromnice Lake, on the contrary, we assume that besides the carbon bound in pyritic shales, the amount of TOC is significantly influenced by phytoplankton and organic matter from the densely forested surroundings of the lake. With respect to the low content of phosphates in the ore and the absence of municipal pollution, two potential sources of the high concentrations of dissolved phosphorus appear relevant – the arable land to the north of the lake and the biodegradation of accumulated organic matter. As regards the potential trophic state, Hromnice Lake may be compared to the eutrophic lakes Cueva de la Mora in Spain (Wendt-Potthoff et al. (2011) or Lugteich Lake in Germany (Lessmann et al., 2000).

Compared to the bottom samples, the very low K^+ surface concentrations may be explained by the precipitation of particular sulphate minerals (K-jarosite) (Dutrizac, 2008). Additionally, priority uptake by plants for the synthesis of organic matter could also be involved (Pitter, 2009). This may explain the gradual decrease in K^+ during the vegetation season at the depth of 12 m, but the study period was too short to confirm this assumption. Although no detailed survey was conducted, the results indicate the possibility that Cd, Cu, and Zn are partially precipitated by the produced S(-II) (1.17 mg l^{-1}) to insoluble sulphides at the bottom of the lake. The same phenomenon was observed in the sulphide ore Udden Lake by Ramstedt et al. (2003), where, however, the pH in vertical profile reached 4.8–6.4. No similar decrease was observed for Fe(II) and other metals in Hromnice, presumably due to a higher solubility product and relatively low concentration of S^{2-} ions corresponding to given pH values (Pitter, 2009). The concentrations of those metals measured are roughly equivalent to other sites created after the mining of sulphidic ores with water pH ~ 2.5–4.0. In comparison with lakes formed after the mining of lignite (Schultze et al., 2010; Moser and Weisse, 2011), they are by approximately one order higher, with the same pH. Relatively low concentrations of Fe and Cu were also observed in Udden Lake (Ramstedt et al., 2003). Extremely high values of Fe and Al (in $pH < 3$ occurs in toxic form Al^{3+}) along with a high content of Cu are among the main reasons for the absence of higher forms of life in the lake (Besser and Leiby, 2007).

Regarding the variability of parameters from the 5 m and 12 m samples, TOC is most likely related to the dynamics of biodegradation at the upper limit of the monimolimnion, depending on the supply of organic material, yet no clear-cut trend related to the vegetation cycle was found. For PO_4^{3-}, processes connected with phosphorus transport from the nearby arable land may prevail;

Table 3
Selected parameters of four extremely acidic mining lakes. (A) This study; (B) Moser and Weisse (2011); (C) Nixdorf et al. (1998a); (D) Wollmann et al. (2000); (M) meromictic; (D) dimictic.

Lake	Flooding (year)	Area (ha)	Max depth (m)	Mixing	Sampling depth (m)	pH		P_{total} ($\mu g\,l^{-1}$)		Chlorophyll-*a* ($\mu g\,l^{-1}$)	
						Min	Max	Min	Max	Min	Max
Hromnice (A)	1900	1	14	M	0.3	2.6	2.8	<20	80	2	140
					5	2.8	3.7	400	760	15	48
					12	3.2	4.0	330	390	20	29
AML Langau (B)	1965	2	10	M	0–5	2.1[a]	3.7[a]			0.6	7
					7–8	4.2[a]	6.1[a]			80	96
Plessa 111 (C)	1965	10	10	D	Epilimnion	2.6	3.0	<5	6	1[b]	4[b]
					Hypolimnion						21[b]
Lugteich (C)		3–20	5	M	Mixolimnion	2.6	2.8	63	230	104	523
					Monimolimnion	6.0	6.2	33	90		

[a] Taken from Moser and Weisse (2011), Fig. 2.
[b] Taken from Nixdorf et al. (1998a), Fig. 3.

a detailed groundwater analysis would be necessary to elucidate this variability. Moreover, the possible disruptive influence of Fe(II) on PO_4^{3-} spectrophotometric determination could be involved, as discovered by Moser and Weisse (2011). To exclude this possibility, lab tests based on several dilution steps of filtered (0.45 µm) water samples were conducted during the additional sampling on November 14, 2012. The results did not vary much, possibly due to the many times higher PO_4^{3-} concentrations found in Hromnice Lake, which make the measurements more accurate. These results, also confirmed by the ICP-AES analysis of total phosphorus, can be thus considered relevant. The concentrations of Zn and Cu proved to be the most variable. While higher surface values in autumn and lower surface values in winter/spring are probably related to low precipitation or the influence of ice cover, similar dynamics at the depth of 5 m and especially the late spring maxima cannot yet be satisfactorily explained.

Biological variables

We compared the communities of Hromnice Lake with those of other extremely acid mining lakes (Tables 3 and 4), AML Langau in Lower Austria (Moser and Weisse, 2011) and lake Plessa 111 in Lusatia, Germany (Nixdorf et al., 1998a,b; Wollmann et al., 2000). These two lakes were chosen considering their very low pH and the availability of data on their biota. All have surface $pH < 3$, ranking them among Fe-buffered lakes. Unlike the other lakes, Hromnice lies in a deep pit with steep slopes. The Lugteich Lake (Nixdorf et al., 1998a) in Lusatia is similar to Hromnice in having high phosphorus and high surface chlorophyll-*a* (Table 3).

Like in many acidic mining lakes, the communities are very poor in number of species (Table 4). A typical component of the phytoplankton are flagellates: Euglenophyceae (*Lepocinclis*) and Chlorophyceae (*Chlamydomonas*) in all three lakes, Chrysophyceae (*Ochromonas* in Plessa 111 and AML Langau, *Chromulina* in Hromnice). The small non-motile alga *Coccomyxa* (Trebouxiophyceae) occurs in high abundance only in Hromnice Lake. The related species *Pseudococcomyxa simplex* (syn. *P. adhaerens*) is known for growing in laboratories in illuminated bottles with distilled water and with stock solutions used to prepare media for algal cultivation: KCl, KH_2PO_4, $MgSO_4$ (Sládečková, 1959; Braune, 1964). Thus, more species of this group seem to be able to thrive in extreme chemical conditions.

Small non-motile algae are not commonly reported from extremely acid mining lakes. In the highly acidic eutrophic mining lake Lugteich a small (3 µm) round non-motile *Nanochlorum* sp. (Trebouxiophyceae) occurs (Lessmann et al., 2000). Common features of the Hromnice Lake and Lugteich are high phosphorus and chlorophyll-*a* concentrations at the surface (Table 3). In contrast, surface phosphorus and chlorophyll-*a* are low in both AML Langau and Plessa 111. High concentrations of chlorophyll-*a* at 7–8 m in AML Langau (Table 4) are explained by the high attenuation of light and availability of nutrients.

Although protonemata of terrestrial mosses can be cultivated in aquatic media, finding them in Hromnice Lake in a form resembling filamentous algae was a surprise, as reports on such phenomenon are scarce. The moss *Dicranella heteromalla* was reported from acid springs in British Columbia, living in the protonemal stage only (Wehr and Whitton, 1983). Higuchi et al. (2003) found

Table 4
Comparison of biota in three extremely acidic mining lakes. (A) This study; (B) Moser and Weisse (2011); (C) Nixdorf et al. (1998a,b); (D) Wollmann et al. (2000); (NR) not recorded.

Lake	Plankton			Littoral		
	Algae	Heliozoa	Rotifera	protonemata	Chironomidae	Corixidae
Hromnice (A)	*Coccomyxa* (1) *Lepocinclis* (1) *Chromulina* (1) *Chlamydomonas* (1)	NR	*Cephalodella* (1) *Elosa woralli*[a]	present	*Chironomus*	scarce
AML Langau (B)	*Chlamydomonas* (1) *Ochromonas* (1) *Lepocinclis* (1)	*Actinophrys* (1)	*Cephalodella* (1)	NR	NR	NR
Plessa 111 (C, D)	*Chlamydomonas* (3) *Ochromonas* (5) *Lepocinclis* (1) *Trachelomonas* (1)	*Actinophrys* (1)	*Cephalodella* (1) *Elosa woralli* *Rotaria* (1)	NR	*Chironomus*	8 species

[a] Found only in periphyton so far. Number of species per genus in parentheses.

macroscopic mat-forming aggregates of green filaments in acidic rivers (pH 1.9–2.4) in Nagano Prefecture, Japan. Transverse walls of the filaments were sometimes obliquely oriented. The molecular phylogenetic analysis put the plant among Bryopsida, the most similar species being *D. heteromalla*. In nature this plant remained in a prolonged protonemal stage for several growing seasons. The same may apply to protonemata from Hromnice Lake as well; this way of life may be considered as a unique adaptation to the acidic environment. Unidentified green filamentous algae were also reported from the littoral of acidic Lusatian lakes (Lessmann et al., 2000). It would be interesting to ascertain if these filaments are actually moss protonemata or not.

Our first efforts to sample zooplankton in Hromnice Lake led us to believe that the lake did not harbour any multicellular zooplankton. Only after having used dense mesh for concentrating samples was a tiny rotifer of the genus *Cephalodella* found. A small *Cephalodella* was reported from extremely acidic lakes in Lusatia (Deneke, 2000) and identified as *Cephalodella hoodi*. It was later found in AML Langau (Moser and Weisse, 2011) and described by Jersabek et al. (2011) as a new species under the name *C. acidophila*. The authors claimed that the species from Lusatian lakes was conspecific with *C. acidophila* and so is the species from Hromnice Lake. This confirms the assumption that this acido-tolerant species regularly inhabits acid mining lakes, but the habitat from which it dispersed to these historically very young man-made lakes remains unknown. Despite the occurrence of *C. acidophila* in Hromnice Lake (and in the other acid mining lakes), its role in the food web is negligible because of its low abundance. The same applies to another small acido-tolerant rotifer *E. worallii* (Deneke, 2000), inhabiting both Hromnice and Lusatian lakes (Table 4).

Comparing the age of acidic mining lakes in Central Europe (Geller et al., 1998; Moser and Weisse, 2011) Hromnice Lake (age 110 years) is by far the oldest (Table 3). Yet, according to its low pH, zooplankton (ciliates, rotifers) and insect fauna (*Chironomus*, corixids), it is still in the first stage of a hypothetic succession from a reduced, acid-stressed community to a normal species assemblage (Nixdorf et al., 1998b; Kalin and Geller, 1998). The barren shores free of macrophytes reflect this initial stage as well (Pietsch, 1998). Contrary to the efforts to remediate acidic lakes, Hromnice Lake has been protected since 1975 as a geological and historical relic. Another feature worthy of protection is the aquatic community which has evolved in this extreme habitat.

Conclusions

In existence for 110 years, Hromnice Lake is apparently the oldest acidic mining lake in Central Europe. In contrast with other lakes of similar origin it differs mainly in the high concentration of dissolved phosphorus and marked oxygen maxima driven by phytoplankton just above the chemocline. Despite being meromictic Hromnice Lake does not appear to be hydrochemically stable as certain variability of water parameters were observed below the chemocline throughout the year. Its aquatic community does not differ substantially from communities of man-made, extremely acidic lakes in lignite mining districts in Germany and Austria. It deserves protection not only as a geological relic, but also as a site suitable for comparable scientific research of life in extreme habitats.

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