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Phytoplankton in acidified lakes: structure, function and response to ecosystem recovery

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Ph.D. thesis

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Annotation: Key factors and mechanisms influencing species composition and structure of plankton biomass with an emphasis on phytoplankton were studied in two acidified lake districts (Bohemian Forest and Tatra Mountains). Current progress in their biological recovery from acidification was assessed.

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I declare that for publications submitted as a part of this Ph.D. thesis, Linda Nedbalová was an important member of the team, and significantly contributed to data collection, biological analyses and to manuscript preparation.

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I declare that neither this thesis, nor any of the publications attached within, have been submitted for the purpose of obtaining the title of Ph.D. or any other title at another institution.

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3.2. **NEDBALOVÁ, L.**, STUCHLÍK, E. & STRUNECKÝ, O. (2006): Phytoplankton of a high mountain lake (Ľadové pleso, the Tatra Mountains, Slovakia): seasonal development and first indications of a response to decreased acid deposition. *Biologia* **61**/Suppl. **18:** S91–S100. 27

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3.4. VRBA, J., KOPÁČEK, J., BITTL, T., NEDOMA, J., ŠTROJSOVÁ, A., **NEDBALOVÁ, L.**, KOHOUT, L. & FOTT, J. (2006): A key role of aluminium in phosphorus availability, food web structure, and plankton dynamics in strongly acidified lakes. *Biologia* **61**/Suppl. **20:** S441–S451. 55

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1. INTRODUCTION

This thesis is based on five publications dealing with plankton assemblages of lakes in two heavily acidified mountain areas in Central Europe, the Bohemian Forest and the High Tatra Mountains. As both lake districts are the object of long-term systematic ecological research, a unique set of complex limnological data was gathered, enabling a comparison with other affected regions throughout the world and also some attempts at generalisation. My role in the team consisted chiefly in the study of phytoplankton as the key component of the food web. I was mainly concerned with the structure, seasonal development and function of the phytoplankton assemblages, and I also focused on the evaluation of general biological response to the current chemical recovery of the lakes from acidification.

The main questions addressed in this thesis with an emphasis on phytoplankton assemblages are the following:

- What is the recent progress in chemical and biological recovery of the Bohemian Forest lakes from acidification?
- What are the key factors and mechanisms influencing species composition and structure of plankton biomass in the Bohemian Forest lakes?
- What is the role of episodic acidification in shaping both phytoplankton abundance and biomass in a non-acidified high mountain seepage lake (Ľadové Lake, High Tatra Mountains)? Are there any changes associated with the decrease of acid deposition?

2. BACKGROUND OF THE STUDY

2.1. Anthropogenic acidification of lake ecosystems: the case of the Bohemian Forest and Tatra Mountains

In the 1970s and 1980s, anthropogenic acidification of lakes, streams and soils due to atmospheric deposition of sulphur and nitrogen compounds was one of the major environmental threats in large regions of Europe and North America which received high loads of acidifying pollutants (e.g. HENRIKSEN 1980, SCHINDLER 1988, JOHNSON & LINDBERG 1991). Acid sensitive lakes are generally found in catchments with weathering-resistant bedrock, such as granite and quartzite, and poorly developed soils (WRIGHT & HENRIKSEN 1978, WRIGHT & al. 1980). The key role of toxic aluminium in biological damage was widely accepted (HÖRNSTRÖM & al. 1984, DRISCOLL 1985), and current studies have suggested a more complex role of aluminium in ecosystem functioning (e.g. KOPÁČEK & al. 2000a, PUHE & ULRICH 2001). In many lake districts, acidification has caused a drastic reduction of biodiversity followed by the disruption of food web structures (e.g. SCHINDLER 1988).

The mountain lakes in the Bohemian Forest and High Tatra Mountains are situated within ~ 200 km of the German-Polish-Czech border area, known for its large coal resources and numerous power plants as the „Black Triangle". The emissions of SO_2 in this region were the highest in Europe, reaching up to ~ 800 $mmol.m^{-2}.year^{-1}$ during the peak in the late 1980s (BARRETT & al. 1995). Since both lake districts lie on a geologically sensitive bedrock, they were seriously affected by acidification (FOTT & al. 1994).

In the Bohemian Forest, the impact of acid rain on lake water chemistry and biology has been quite well documented, especially in the case of the Černé Lake. The lowest pH (~ 4.5) was observed in the middle of the 1980s, and this pH decline resulted in the disappearance of fish and planktonic cladoceran species from the lake (FOTT & al. 1994, VESELÝ 1994). Concentrations of aluminium in the Bohemian Forest lakes peaked at higher values in comparison with other acidified lake districts, reaching nearly 1 $mg.l^{-1}$ in Čertovo Lake with the dominant proportion being in the labile fraction (FOTT & al. 1994). According to their acidification status, the lakes were divided into three categories: (i) strongly acidified lakes (Černé Lake, Čertovo Lake, Rachelsee, Plešné Lake), (ii) moderately acidified lakes (Prášilské Lake, Kleiner Arbersee) and (iii) slightly acidified lakes (Grosser Arbersee, Laka) (VRBA & al. 2000). Crustacean zooplankton survived the period of the most severe acidification only in the latter two lake groups, and fish are extinct in all lakes (FOTT & al. 1994, VESELÝ 1994, SCHAUMBURG 2000). Therefore, the pelagic zone of the lakes is characterised by simplified food webs with dominance of microbial interactions and by an important proportion of heterotrophic filaments in the plankton biomass (VRBA & al. 1996).

In contrast to the Bohemian Forest, there are fewer direct reports on the beginning of acidification derived changes of lake water chemistry in the High Tatra Mountains (KOPÁČEK & al. 1998). The first data on sulphate and nitrate concentrations in lake water come from 1937 (STANGENBERG 1938), and these background levels increased until the 1980s when the acidification peak was reached, roughly at the same time as in the Bohemian Forest lake district (KOPÁČEK & al. 1998, KOPÁČEK & al. 2004a). Repeated synoptic samplings carried out from 1980–1983 revealed profound changes in the water chemistry of sensitive lakes. Acidified lakes above timberline were characterised by extremely low chlorophyll-*a* concentrations ($< 0.2\ \mu g.l^{-1}$), and by the disappearance of crustacean zooplankton (STUCHLÍK & al. 1985). The sensitivity of particular lakes to acidification and the degree of impact was shown to be determined by the concentration of calcium and magnesium (FOTT & al. 1992), and nitrogen was shown to contribute significantly to decreases in lake water pH (KOPÁČEK & STUCHLÍK 1994). Overall, major factors governing lake water chemistry were bedrock composition and the amount of soils and vegetation in their catchments (KOPÁČEK & al. 2006). With respect to zooplankton status, in comparison with pre-acidification data (e.g. MINKIEWICZ 1914), naturally fishless lakes above timberline were divided into three groups: (i) non-acidified lakes with zooplankton composition unchanged, (ii) acidified lakes, where planktonic crustacean zooplankton disappeared and (iii) strongly acidified lakes, where only the acid-tolerant littoral species *Chydorus sphaericus* (Cladocera) survived. These groups were delimited using the arbitrary limits of an acid neutralising capacity of 25 and 0 $\mu eq.l^{-1}$. The toxicity of aluminium and food starvation due to acidification-induced oligotrophication were identified as key factors limiting the distribution of zooplankton species (FOTT & al. 1994, HOŘICKÁ & al. 2006). The acidification status of lakes was also reflected in a different structure of epilithic diatom assemblages (ŠTEFKOVÁ 2006).

2.2. Phytoplankton in acidified lakes

The phytoplankton is one of the first biological communities to be affected by acidification (SCHINDLER 1987). Changing chemical composition of lake water is reflected in shifts in species composition and biomass (FINDLAY & al. 1999, HÖRNSTRÖM 2002, ANDERSON & al. 2005).

2.2.1. Species composition

The effects of acidification on the diversity and structure of phytoplankton assemblages have been well documented. In general, the deficiency of plankton species biodiversity is one of the most important consequences of the anthropogenic acidification of lake water (ALMER & al. 1978). The phytoplankton of the most acidified oligotrophic lakes is typically represented by a low number of slowly growing flagellates. By means of vertical migration, these species are probably able to facilitate their nutrient supply, which is a more important factor driving taxonomic composition in comparison with acidity as such (HÖRNSTRÖM & al. 1984).

The phytoplankton of circumneutral oligotrophic lakes is typically dominated by nanoplanktonic flagellates from the Chrysophyceae, Chlorophyceae and Bacillariophyceae. In contrast, Dinophyceae and sometimes Cryptophyceae become more important in acidified lakes (DIXIT & SMOL, 1989). One of the most striking changes in affected communities is the disappearance of planktonic diatoms and desmids from lakes (ALMER & al. 1978, HÖRNSTRÖM & al. 1984). The common presence of *Peridinium umbonatum*, *Gymnodinium uberrimum*, *Dinobryon pediforme*, and other small naked dinoflagellates and chrysophycean flagellates are often recorded (ROSÉN 1981). Other species frequently occurring in acidified oligotrophic waters include *Bitrichia ollula* (Chrysophyceae), *Mallomonas* spp., *Synura echinulata* (Synurophyceae) or *Isthmochloron trispinatum* (Xanthophyceae) (HÖRNSTRÖM, 2002).

Large dinoflagellates are typical K-selected species with low growth rates and the ability to exploit low nutrient concentrations (REYNOLDS, 1984). Namely *Peridinium umbonatum* is considered to be characteristic for strongly acidified lakes elsewhere (e.g. ALMER & al. 1974, ALMER & al. 1978, HÖRNSTRÖM & al. 1999, FINDLAY 2003). *Peridinium umbonatum* is known to be very tolerant to high concentrations of toxic aluminium, and in addition it is a superior competitor for nutrients in ultraoligotrophic conditions (YAN 1979). High abundance of *Peridinium umbonatum* is often found in acidified lakes, where crustacean zooplankton is absent or highly reduced. Selective herbivory thus probably does not play an important role in the common dominance of this species in strongly affected lakes (HAVENS & DECOSTA 1985, FOTT & al. 1994). In another large dinoflagellate, *Gymnodinium uberrimum*, tolerance to conditions at low lake water pH can be enhanced by the presence of a thick gelatinous sheath (FINDLAY & KASIAN 1986).

Green algae (Chlorophyta) and cyanobacteria are usually highly reduced in conditions of low pH (ROSÉN 1981, DIXIT & SMOL 1989). However, the green coccal alga *Monoraphidium dybowskii*, for example, is known to be tolerant to high levels of toxic aluminium species, similarly to *Peridinium umbonatum*. In laboratory experiments, the growth of *Monoraphidium dybowskii* was unaffected up to a concentration of 1000 $\mu g.l^{-1}$ with a dominant proportion of labile monomeric aluminium (HÖRNSTRÖM & al. 1985). An extensive lake survey in Sweden revealed that this species is quite common in acidified lakes characterized by higher nutrient levels, and its occurrence is often recorded together with the colonial cyanobacterium *Merismopedia tenuissima* (ROSÉN 1981).

Overall, observed shifts in phytoplankton structure in lakes affected by anthropogenic acidification are the results of an interplay between high acidity, nutrient depletion and aluminium toxicity. However, the increased concentration of toxic aluminium species is considered to be mainly responsible for the disappearance of some phytoplankton species or even groups (HÖRNSTRÖM & al. 1984). The effect of aluminium is sometimes manifested not only by growth reduction, but also by disturbance of cell shape and content, as has been demonstrated by HÖRNSTRÖM & al. (1984) in *Monoraphidium griffithii* and *Synedra* cf. *nana*.

Even a considerable drop in pH together with associated changes in lake water chemistry sometimes does not result in a significant effect on the diversity and taxonomic composition of phytoplankton. In ultraoligotrophic acid-sensitive mountain lakes, severe growth conditions in the preacidification period have selected species, which are apparently able to survive when the lake become acidic. For example, the species composition of the strongly acidified Černé Lake in the Bohemian Forest has remained almost unchanged in comparison with data from 1935–1936 collected by B. Fott (FOTT & al. 1994). The only exception is the disappearance of *Cyclotella* sp. from the lake, which is in agreement with the generally accepted sensitivity of planktonic diatoms to acidification (ALMER & al. 1978). Unfortunately, long-term data on species composition allowing such comparisons are often lacking.

The presence of flagella has been recognised as crucial feature of phytoplankton in mountain lakes, enabling algae to maintain an optimal depth in the water column with regard to light conditions, nutrient concentration and temperature requirements (PECHLANER 1971).

Flagellates thus most frequently represent the dominating fraction of phytoplankton in mountain lakes, irrespective of pH (ROTT 1988, PUGNETTI & BETTINETTI, 1999, TOLOTTI & al. 2003). Due to the lack of nutrients as the result of natural oligotrophy, sometimes combined with oligotrophication caused by acidification (see below), mixotrophy seems to be a succesful life strategy for phytoplankton in mountain lakes characterised by low nutrient input (FELIP & al. 1999, PUGNETTI & BETTINETTI 1999, SIMONA & al. 1999). The ability of mixotrophic nutrition has been demonstrated for several dinoflagellates, chrysophytes and cryptophytes (e.g. PORTER 1988, NYGAARD & TOBIESEN 1993, ISAKSSON 1998). A good example is the genus *Dinobryon* (Chrysophyceae), covering its phosphorus demand by phagotrophy on the single-celled bacterioplankton (BIRD & KALFF 1987). This grazing by *Dinobryon* has a potentially important effect on both the size and structure of bacterial populations (ZNACHOR & HRUBÝ 2000).

2.2.2. Oligotrophication during acidification and phytoplankton biomass

Acidification influences not only the chemical composition of lake water, but also lake trophic status. Oligotrophication is characterised by a drop in nutrient supply and availability, and the precipitation of phosphorus by aluminium has been proposed as one possible mechanism (DICKSON 1980). Contradicting hypotheses concerning oligotrophication during acidification were reviewed by OLSSON & PETTERSSON (1993). JANSSON & al. (1986) suggested that low phosphorus level in acidified lakes is caused by reduced input from the watershed, possibly due to the efficient fixation of phosphorus to aluminium complexes in soils. The internal phosphorus cycle and availability is apparently disrupted as a result of acidification (KOPÁČEK & al. 2000a, BITTL & al. 2001). Due to the natural inactivation of available phosphorus by aluminium in water bodies with elevated aluminium input and a pH gradient between its inlet and outlet (KOPÁČEK & al. 2001, KOPÁČEK & al. 2004b), acidified lakes are generally characterised by low phytoplankton biomass and consequently high transparency (ALMER & al. 1974). However, many acid-sensitive lakes were probably already oligotrophic before the onset of acidification (OLSSON & PETTERSSON 1993).

On the other hand, a whole-lake experimental acidification did not result in decreases in phosphate and phytoplankton biomass (SCHINDLER 1980). Moreover, long-term observations of phytoplankton during acid rain induced acidification have sometimes revealed increases in biomass (HAVENS & DE COSTA 1984, FINDLAY & al. 1999). One explanation of this effect could be the replacement of small species by large ones (FINDLAY & KASIAN 1986), and the dominance of large forms can thus be one of the reasons for high transparency (ALMER & al. 1978). In strongly acidified lakes, however, the reduced ability of aluminium to precipitate reactive phosphorus and resulting increase in nutrient availability was shown to be key mechanism resulting in high phytoplankton biomass (DICKSON 1980). Accordingly, the highest amount of phytoplankton in southern Sweden was recorded in lakes with pH below 4.5 (ALMER & al. 1974). In the period of maximal acid deposition, acidified lakes in the High Tatra Mountains were characterised by extremely low chlorophyll-*a* concentrations, whereas higher values were found both in non-acidified and especially in the strongly acidified lakes (HOŘICKÁ & al. 2006). This distribution of chlorophyll-*a* concentrations (similar to Almer's U-curve for Swedish lakes, ALMER & al. 1974) corresponded to the values of total phosphorus (VYHNÁLEK & al. 1994, KOPÁČEK & al. 2000b).

An extremely high activity of extracellular phosphatases is often observed in acidified lakes (JANSSON & al. 1986, VRBA & al. 1996). Elevated concentrations of aluminium, which act as a competitive inhibitor, were suggested as the reason for this phenomenon (JANSSON 1981). However, BITTL & al. (2001) showed that the inhibitory effect of aluminium could not fully explain the extreme activities of phosphatases in the Bohemian Forest lakes, and suggested that phosphorus immobilization by particulate aluminium could be the complementary cause. Direct microscopic detection of extracellular phosphatases with a fluorogenic substrate (ELFP) showed that their activity is not evenly distributed within the phytoplankton community, and mirror the nutritional status of individual cells. Moreover, the production of phosphatases is very rare in groups with mixotrophic capacities (ŠTROJSOVÁ & VRBA 2006).

2.2.3. Phytoplankton of lakes in the Bohemian Forest and Tatra Mountains

The Bohemian Forest and the Tatra Mountains are the only two regions in the Czech and Slovac Republics where lakes of natural origin occur. These remote lakes have fascinated explorers for more than a century. In the Bohemian Forest, the pioneering limnological study was done by FRIČ & VÁVRA (1898). Detailed phycological investigations of the Černé Lake plankton was carried out by B. Fott, who even described three new species there, *Bitrichia ollula* (Chrysophyceae), *Katodinium planum* and *Katodinium bohemicum* (Dinophyceae) (FOTT 1937, 1938). In 1978, studies of acidification were started by a team from Charles University in Prague, but they focused at the beginning mainly on the chemistry of lake water and zooplankton composition in relation to acid rain driven changes (e.g. FOTT & al. 1980).

FOTT & al. (1994) compared actual data on phytoplankton species composition with that from the pre-acidification period, and came to the conclusion that phytoplankton structure has remained surprisingly stable in spite of drastic changes in lake water chemistry. However, no quantitative study was performed. Further data on phytoplankon including chlorophyll-*a* concentrations were published e.g. in VRBA & al. (1996, 2000) and HEJZLAR & al. (1998). NEDBALOVÁ & VRTIŠKA (2000) investigated the seasonal development and vertical distribution of phytoplankton in four lakes. Species richness of cyanobacteria and algae in selected lakes was also studied (LUKAVSKÝ in WEILNER 1997), and a new species of *Coelastrum* was described from the littoral zone of Černé Lake, Grosser Arbersee and Kleiner Arbersee (LUKAVSKÝ 2006). Other studies focused in detail on interesting interactions and mechanisms, e.g. ZNACHOR & HRUBÝ (2000) on *Dinobryon* grazing on bacteria, ŠTROJSOVÁ & VRBA (2006) on the seasonal and vertical distribution of the phosphatase activity of *Monoraphidium dybowskii* in Plešné Lake or NEDOMA & NEDBALOVÁ (2006) on the chlorophyll content of Plešné Lake phytoplankton.

Although algological investigations of lakes in the Tatra Mountains have a shorter history, the area can be considered as well explored (HINDÁK & KOVÁČIK 1993, HINDÁK 1994). The first mention about phytoplankton can be found in MINKIEWICZ (1914); more detailed data concerning several lakes were included in ERTL & al. (1965) and JURIŠ & al. (1965). An extensive survey was done by JURIŠ & KOVÁČIK (1987), who published a list of species in a large set of lakes. LUKAVSKÝ (1994) observed a decrease in phytoplankton species richness with increasing altitude and decreasing pH. Due to their difficult accessibility, lake research was mostly limited to single observations. The only two studies focusing on the seasonal development of phytoplankton were presented by DARGOCKÁ & al. (1997) and FOTT & al. (1999). In DARGOCKÁ & al. (1997), the influence of spring episodic acidification on the species composition and biomass of phytoplankton in a non-acidified lake was also discussed. VYHNÁLEK & al. (1994) studied chlorophyll and phosphorus concentrations of lake water, which were shown to be related to the acidification status of the lakes. Data on chlorophyll-*a* concentrations in the surface layer of a representative set of lakes were included in the monitoring, and form an integral part of numerous publications dealing both with period of the acidification peak and lake district recovery (e.g. STUCHLÍK & al. 1985, FOTT & al. 1994, KOPÁČEK & al. 2006, STUCHLÍK & al. 2006). Due to very the low trophic status of most lakes, a sensitive fluorometric method was introduced for the determination of chlorophyll-*a* in the framework of regular regional surveys (STRAŠKRABOVÁ & al. 1999).

2.3. Recovery of lakes from acidification

As ecosystems are naturally changing both through internal processes and global changes, the recovery from acidification cannot be simply judged as a return to a state before disturbance (GUNN & SANDØY 2003). According to the European Water Framework Directive, the goal in restoring ecosystems is to obtain "the biological community which is expected in conditions of minimal anthropogenic impact" (EUROPEAN COMMISSION 2000). Other definitions stress the return of key species (e.g. DISE & al. 1994).

The evidence of widespread biological damage both in Europe and Northern America has forced authorities to implement regulations, which have resulted in substantial reductions in the deposition of acidifying compounds (HEDIN & al. 1987, JENKINS 1999), and in a partial recovery of surface waters from anthropogenic acidification (STODDARD & al. 2000, WRIGHT & al. 2005). However, the biological recovery of affected lakes is often delayed or uncertain (KOPÁČEK & al. 2002, JEFFRIES & al. 2003, SKJELKVÅLE & al. 2003).

In the context of current chemical recovery, phytoplankton species, as probably the most sensitive plankton organisms, are likely to react very quickly due to their rapid turnover times (SCHINDLER 1987, FINDLAY 2003). The recovery of phytoplankton species diversity and a shift towards circumneutral assemblages has been observed both in Europe and Northern America (HÖRNSTRÖM 1999, FINDLAY 2003). In Sweden, extensive liming activities led to the restoration of original phytoplankton species composition, with the exception of several species of cyanobacteria and desmids, in lakes, which were previously strongly acidified (HÖRNSTRÖM 2002). Concerning zooplankton, a massive return of *Daphnia longispina* (Cladocera) by hatching from sediment egg-banks was observed in lakes in southern Norway (NILSSEN & WÆRVÅGEN 2002). In acid-stressed lakes near Sudbury (Canada), evidence for relatively rapid biological recovery was reported for plankton, mobile species of benthic invertebrates and some fish populations (KELLER & al. 1992, YAN & al. 1996). However, the restoration of aquatic communities can not be adequately predicted, especially in the case of isolated mountain headwater lakes. The natural dispersal of metazoan fauna is a stochastic process, and the successful establishment of a new population depends on the size of inoculum and both abiotic and biotic factors (CÁCERES & SOLUK 2002). Furthermore, ecosystem resistance due to the presence of invertebrate predators in acidified lakes without fish may prevent rapid biological recovery (NILSSEN & WÆRVÅGEN 2003, WÆRVÅGEN & NILSSEN 2003).

In the Bohemian Forest and Tatra Mountains, the partial reversal in hydrochemistry of acidified lakes due to political and economical changes in postcommunist countries since 1989 has also been observed (KOPÁČEK & al. 1998), but changes in lake water chemistry followed the decreases in nitrogen and sulphur emissions with a certain hysteresis (KOPÁČEK & al. 2002). One significant change in the chemistry of the lakes has been a rapid decrease in concentrations of aluminium (VESELÝ & al. 1998, KOPÁČEK & al. 2006). However, even the full implementation of the Gothenburg Protocol will not be sufficient for the full recovery of the most sensitive lakes (KOPÁČEK & al. 2004a).

Similarly as in other acidified lake districts, biological recovery of mountain lakes in Central Europe has been shown to be significantly lagging behind the reversal in lake water chemistry. In the Bohemian Forest, the first sign of a biotic response has been observed in Černé Lake, where *Ceriodaphnia quadrangula* (Cladocera) reappeared in the open water in the late 1990s (KOPÁČEK & al. 2002). The return of this species was probably possible only due to its survival in the littoral zone during the peak of acidification (FOTT & al. 1994). Recently, the survival of *Daphnia longispina* (Cladocera), which disappeared from several Bohemian Forest lakes a result of acidification, was tested in a bioassay, and the best results were obtained with the water from Plešné Lake. Subsequently, a large scale experiment was conducted by transferring two indigenous crustacean plankton species into this lake. The reintroduction of *Cyclops abyssorum* (Copepoda) was confirmed to be successful after one year, though that of *Daphnia longispina* was not (KOHOUT & FOTT 2006).

In the Tatra Mountains, a significant increase in chlorophyll-*a* concentrations in lake water was observed in 2004 in comparison with 1994 data, which might be due to better phosphorus bioavailability following increases in pH (KOPÁČEK & al. 2006). Concerning zooplankton, the first signs of biological recovery were recorded in the early 2000s, when several extinct species of crustacean reappeared in some acidified lakes. However, acidification still remains the most important factor driving the zooplankton composition in lakes (HOŘICKÁ & al. 2006). The return of most previously extinct littoral Cladocera has also been detected, within ten years of the beginnining of chemical recovery (SACHEROVÁ & al. 2006).