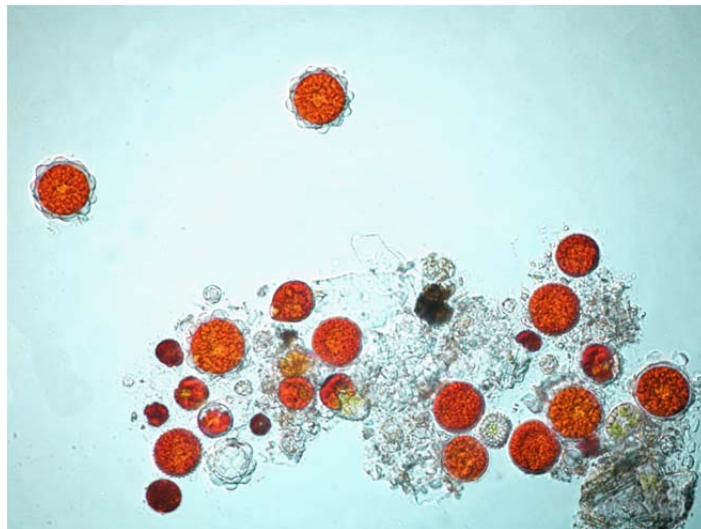


**Charles University**

**Faculty of Science**



**Diversity, ecology and ecophysiology  
of algae and cyanobacteria  
from extreme environments**



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## Preface

This thesis summarises my research work on algae and cyanobacteria from various types of extreme environments that has been undertaken over the last 10 years at the Charles University in Prague and at the Institute of Botany of the Czech Academy of Sciences in Třeboň. Although I feel that my main field is phytoplankton ecology and limnology, I was very happy to meet experts in different disciplines, mainly molecular phylogenetics, taxonomy, biochemistry and geochemistry. Thanks to these collaborations, new horizons have opened up before me, and this is reflected in the choice of publications included in this thesis.

The following text consists of a short introductory section that gives an overview of the main results. The core part of the thesis is formed by selected papers that I have co-authored in which issues relevant to the main topic of the thesis are addressed. The division into three parts reflects the three main types of extreme environments that I have had the chance to study (Antarctic lakes, snow and acidic habitats).

To explain the genesis of this structure, I have to go back in time to my student times, when I started to work on phytoplankton ecology of acidified lakes in the Bohemian Forest and in the High Tatra Mountains in the frame of the projects focused on recovery of the lakes from acidity. The part of the thesis dedicated to various acidic habitats is thus a natural continuation of my student work. Although the focus is again on lakes, it also includes one paper on an unusual anthropogenic habitat – a burning spoil heap. The second part is devoted to snow algae. For the first time, I saw large patches of red snow in 2001 during the sampling of Ladové Lake in the High Tatra Mountains and I became immediately fascinated by these organisms. In the following years, I had the chance to study field samples of snow algae from many other localities mainly in Europe, but also in South America. Finally, in 2008, I was extremely lucky to have the opportunity to start the pioneering limnological research of the Ulu Peninsula on James Ross Island, which is one of the largest ice-free territories in Antarctica, a unique place to study ecological processes shaping biodiversity and adaptations of life to extreme environment. The thesis is thus introduced by the chapter in which I present the results of our research of lakes and their biota in this area.

## Acknowledgements

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I owe much to Josef Elster, who invited me to study lakes on James Ross Island, for the opportunity to work on common projects and for inspiring discussions. Our fieldwork in Antarctica would not have been possible without the safe base of the Johann Gregor Mendel Czech Antarctic Station, which was constructed in 2005–2006. During my stays at the station, I enjoyed the opportunity to conduct fieldwork with numerous colleagues. I am particularly indebted to Daniel Nývlt, Bedřich Mlčoch, Kamil Láska, Radek Vodrážka, Zbyněk Engel, Olga Bohuslavová, Václav Pavel, Peter Váczi and Miloš Barták. Their help and good companionship were highly appreciated.

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I would like to stress at this point the key role of my master and Ph.D. students, who are the first authors or co-authors of many publications included in this thesis. It would never have been completed without them. Lenka Procházková, Katka Kopalová, Adéla Moravcová, Antonín Střížek and Dovile Barcyte, in particular, have kept me alert and inspired by their energy, ideas or simply by asking questions.

The results described in detail in this thesis have been published with many other co-authors. I would also like to thank numerous others who have contributed to this work, but there is not enough room to mention them here. The research leading to this compendium was supported by several grant agencies, especially GA ČR (Czech Science Foundation) and funding programmes of the Ministry of Education, Youth and Sports of the Czech Republic. Financial and other support for different parts of my research is acknowledged in individual papers as appropriate.

At the Department of Ecology and at the Institute of Botany, I enjoyed the company of many colleagues, especially that of Veronika Sacherová and Jana Šnokhousová.

Last, but not least, I am grateful to my parents for their life-long patience and support. I thank Vojta and Zuzana, my husband and my daughter, for lovely companionship and smiles.

## **Life on the edge – the fascinating world of algae and cyanobacteria in extreme environments**

Life on our planet flourishes in various unexpected places, even those where the conditions are at the edge of biological limits and have been until recently considered incapable of supporting life (Seckbach 2000). The organisms found in these habitats are called extremophiles. One of the many definitions of this term says that optimal growth conditions for extremophiles are found beyond the “normal” environments as perceived from an anthropomorphic point of view (Kristjansson & Hreggvidson 1995). In many cases, organisms in extreme biotopes must face multiple stresses, which is reflected in their designation as “polyextremophiles”. However, a large proportion of inhabitants of harsh habitats are often not “true extremophiles”, as their growth optima do not fit to their environment. Whatever their physiological characteristics, organisms from extreme habitats can provide important answers to general questions in the fields of ecology, biogeography, ecophysiology and biochemistry. Because of their adaptations to various types of environments, they have been shown to produce a tremendous diversity of specific compounds, and their thorough study can lead to biotechnological applications (Seckbach & Oren 2007).

The most extreme habitats can be viewed as the closest approximation to the conditions that probably existed when life first appeared on Earth. Moreover, they offer potential analogues for conditions on other planetary bodies (Preston & Dartnell 2014). Because their habitats are often geographically isolated, extremophiles represent excellent model organisms for the study of the dispersal abilities of different microbial groups (Miller et al. 2007, Pinto 2007).

Oxygenic photosynthetic microorganisms occupy a wide variety of extreme environments where visible light as the necessary source for photosynthesis is available. They show a surprising ability to adapt to a wide range of various environmental conditions. These extreme factors can include very high or very low temperatures, pH values, or salinity, lack of water, high concentrations of heavy metals, or very high or low levels of solar radiation (Seckbach & Oren 2007). Cyanobacteria and algae have a special status in extreme environments because of their key role as primary producers and also because of the lack of significant levels of predation. The physiology of photosynthesis and its versatility under various extreme conditions is one of the most important features of their adaptation. Because of their long evolutionary history, they are widely adapted to the extremes that have been associated with the changes that have occurred throughout the geological history of our planet. Extreme environments inhabited by photoautotrophs can be roughly divided into two categories: stable and unstable. The first type hosts well-adapted organisms (e.g. psychrophiles, thermophiles or acidophiles), whereas the second type is characterised by large fluctuations of environmental factors that require different adaptations (Elster 1999).

Although our knowledge of physiological strategies is still limited to a small pool of species, prokaryotic cells seem to be much better adapted to all environmental extremes than eukaryotes. The diversity, versatility, and specialisation of cyanobacteria may help to explain their extraordinary ability to colonise various extreme environments (Seckbach &

Oren 2007). The following list can demonstrate some of the striking examples of extremophilic eukaryotes: the green alga *Dunaliella salina*, which is able to grow close to the upper limit of salinity allowing for life (Oren 2002), the unicellular red algae of the order Cyanidiales that inhabit extremely acidic environments with temperatures from 40 to 56 °C (Pinto 2007) and snow algae from the order Chlamydomonadales, which grow in environments with temperatures near 0 °C and high irradiance levels (Hoham & Duval 2001).

In this thesis, I focus on algae and cyanobacteria in three types of extreme environments that differ markedly in their conditions and community structure: Antarctic lakes, snow and acidic localities. In Antarctica, the availability of liquid water is severely limited because of low temperatures. Therefore, freshwater habitats, i.e. mainly lakes, host most of the known diversity of life in Antarctic terrestrial environments (Vincent 2004). Interestingly, the conditions in Antarctic lakes can be very diverse, and this is reflected in their biota. Melting snow is a special type of low-temperature habitat characterised by multiple extremes and inhabited by a relatively small group of well-adapted microalgae. Cryoflora draws attention not only because of the striking greenish to reddish snow discolorations, but also because of the variety of morphological and physiological strategies used to cope with this harsh habitat (Leya 2012). Finally, acidic habitats represent another type of extreme environment, which can also offer interesting insights into the diversity, ecology and ecophysiology of photosynthetic microorganisms. The papers included in this thesis thus represent a humble attempt to proceed with the answers to some of the important open questions of the research on life in extreme environments, i.e.:

- What is the range of environmental parameters supporting life and what are the factors shaping the diversity and structure of microbial communities in extreme habitats?
- What is the actual diversity of different microbial groups in various extreme habitats?
- What is the proportion of endemic species in extreme habitats? Are there some dispersal limits in microorganisms and do they vary among different taxonomic groups?
- What is the range of physiological strategies in various groups of extremophiles? Which compounds do they produce to cope with their harsh environment?
- What is the potential of strains isolated from extreme habitats that could be successfully used in biotechnological applications and what are the possible target compounds?
- What is the rate of reaction of communities in extreme habitats to environmental changes and what are the itineraries, mechanisms and constraints of these reactions?



## Results

### Chapter I. Lakes on James Ross Island (northeastern Antarctic Peninsula)

#### *Setting the scene*

Although Antarctica is almost completely covered by an ice sheet, one of its characteristic features is a surprising diversity and abundance of lakes (Vincent & Laybourn-Parry 2008). Many ice-free areas with lakes can be found on the Antarctic Peninsula and surrounding islands. Limnological surveys of freshwater lakes in this region have been carried out e.g. on Alexander Island (Heywood 1977), the South Shetland Islands (Ellis-Evans 1996, Toro et al. 2007), and at Beak Island (Sterken et al. 2012), and they have shown a wide range of limnological conditions with nutrient status ranging from ultraoligotrophic to eutrophic. However, the distribution, physical and chemical characteristics and biology of lakes around this region are still poorly understood.

The northern part of the Antarctic Peninsula is considered to have been one of the most rapidly warming parts of our planet over the last 50 years (Vaughan et al. 2003). Polar lakes represent sensitive indicators of environmental changes, and rapid responses of Antarctic lake ecosystems induced by climate changes have already been recorded. These changes have led to profound changes in their functioning (Quayle et al. 2002, Verleyen et al. 2012). There is growing evidence that Antarctic lakes represent valuable centres of biodiversity (Laybourn-Parry & Pearce 1997) with a high proportion of endemic species (Sabbe et al. 2004, Komárek et al. 2008). In connection to recent climate changes, invasions of alien species were recently identified as an important threat to Antarctic biota (e.g., Chown et al. 2012). Therefore, assessment of the current limnological status and biodiversity of Antarctic lakes is urgently needed.

James Ross Island belongs biogeographically to a transitory zone between maritime and continental Antarctic regions (Øvstedal & Lewis-Smith 2001). More than 80 % of the island is covered with ice. Only the northernmost part of the island, the Ulu Peninsula, is significantly deglaciated and represents one of the largest ice-free areas (oases) in the northern part of the Antarctic Peninsula. The Late Pleistocene to Holocene deglaciation of the Ulu Peninsula (Johnson et al. 2011, Nývlt et al. 2014) enabled the evolution of numerous types of lakes. Until now, the study of lakes on James Ross Island has been limited mostly to paleolimnological investigations (Björck et al. 1996). Information about the recent limnological status of the lakes has been limited to several temperature, pH and conductivity measurements recorded during investigations of their cyanobacterial microflora (Komárek & Elster 2008). In the austral summers of 2008 and 2009, we performed the first extensive limnological survey of the ice-free part of the Ulu Peninsula ([Part I-1, Nedbalová et al. 2013](#)). Twenty-nine representative lakes and ponds were classified according to their origin, geomorphological position, stability, and physical and chemical characteristics. Based on these features, six different types of lakes were defined: stable shallow lakes on levelled surfaces at higher altitude, shallow coastal lakes, stable lakes in old moraines, small unstable lakes in young moraines, deep cirque lakes and kettle lakes. Their age was estimated to span between 6500–8000 BP (Johnson et al. 2011) and one century or even a few decades. We observed a significant relationship between lake type and water chemistry. Bedrock, lake

age, morphometry and altitude were identified as the most important factors underlying the high limnological variability. Large differences in nutrient chemistry between old and young lakes were detected. High concentrations of nitrate in several young lakes could not be explained solely by atmospheric input, neither by common processes reported from Antarctica as animal influence or concentration mechanisms nor the presence of nitrate-bearing salts (Toro et al. 2007, Webster-Brown et al. 2010). A possible role of bedrock as alternative nitrogen source was suggested (Morford et al. 2011). To conclude, the lakes on the Ulu Peninsula represent a valuable set of diverse lacustrine habitats in the transitional zone between maritime and continental Antarctica. The results of this survey could serve as baseline information for future limnological investigations in the region of the Antarctic Peninsula, as well as for the study of biodiversity in this rapidly changing part of Antarctica.

Even though the sampling in 2008–2009 was focused primarily on water chemistry, cyanobacteria and microalgae, we also obtained interesting data about other organisms inhabiting the lakes on James Ross Island. Aerobic anoxygenic phototrophic (AAP) bacteria are a specific group of microorganisms that require organic substrates for respiration and growth, but are able to obtain cellular energy from light using bacteriochlorophyll *a*-containing reaction centres. They are common in various limnic habitats (e.g., Mašín et al. 2008); however, the records of their occurrence in Antarctic lakes are scarce (e.g., Labrenz et al. 2009). As a result of the survey of James Ross Island lakes, we provided the first quantification of AAP bacteria in Antarctic lakes (Part I–2, Medová et al. 2016), which revealed that their relative proportion was comparable to that of freshwater lakes in Central Europe (Mašín et al. 2008). Our results thus suggest that this group of organisms may play a significant role in the microbial food webs in polar freshwater ecosystems.

Furthermore, we frequently observed abundant populations of the Antarctic fairy shrimp (*Branchinecta gaini*), which were feeding on microbial mats in the lakes (Part I–3, Nedbalová et al. 2017a). Together with the calanoid copepod *Boeckella poppei*, the fairy shrimp was recorded in shallow lakes located near the coast as well as in inland lakes at higher altitudes (up to 230 m a.s.l.) across the whole ice-free area of the Ulu Peninsula, at Clearwater Mesa and also on the nearby Vega Island (surroundings of Devil Bay and Cape Lamb). The current distribution of *B. gaini* ranges from Patagonia, South Georgia, South Orkney and the South Shetland Islands to halfway down the western side of the Antarctic Peninsula (Hawes 2009). Palaeolimnological data confirmed the presence of *B. gaini* on James Ross Island for a significant portion of the Holocene. Interestingly, it has been considered extinct in this area (Björck et al. 1996). We cannot conclude as to whether *B. gaini* really disappeared from James Ross Island for a certain period. Valuable data could be gained from analysis of further sediment cores from Monolith Lake and other sites on both islands and this research is currently underway. Molecular phylogeography could also provide some insight into the dispersal history of *B. gaini* within its current range. The recent rapid warming of the northeastern Antarctic Peninsula (Mulvaney et al. 2012) might generally promote the occurrence of *B. gaini*. However, the acceleration of desiccation of shallow ponds because of warming might have adverse effect on the future long-term persistence of this species in this region (Hawes et al. 2008).

## *Diversity, ecology and ecophysiology of microflora from James Ross Island lakes*

One of the most limiting factors for organisms in polar ecosystems is the presence of liquid water, and freshwater habitats are therefore considered oases for life in the polar desert (Vincent & Laybourn-Parry 2008). Given the relative isolation of the region and the severe biological constraints, Antarctic microbial ecosystems are of special importance for the study of their biodiversity and evolutionary origins. Food webs in Antarctic lakes are dominated by microbial life, and photoautotrophic microbes are the dominant primary producers (Vincent 2004, Laybourn-Parry & Pearce 2007). In addition to a general ecological interest in their diversity and strategies for adapting to extreme physical and chemical conditions, Antarctic microorganisms represent a potential source of bioactive compounds (e.g., Fogliano et al. 2010).

There is an ongoing discussion about the level of endemism of freshwater cyanobacteria and algae in the Antarctic region. According to the theory of ubiquitous dispersal of free-living microorganisms, it should be low (Finlay & Clarke 1999). However, the repeated development of specific communities with the same species composition, zonation and seasonal periodicity suggests the existence of stable adapted cyanobacterial geno- and morphotypes endemic for Antarctic habitats (Sabbe et al. 2004, Komárek et al. 2008). A study covering a wide geographic area of continental Antarctica also revealed a high proportion of novel and endemic cyanobacterial operational taxonomic units (OTUs) based on analysis of 16S rDNA and ITS sequences (Taton et al. 2006), but some species thought to be endemic to Antarctica have also been discovered in the Arctic (Jungblut et al. 2010). To further demonstrate the controversy on another group of photoautotrophic microorganisms, some studies have indicated that green algal communities were dominated by cosmopolitan taxa (Broady 1996) and a widespread geographic distribution of some coccoid green algae has been recently demonstrated using molecular methods (Hodač et al. 2016). On the contrary, de Wever et al. (2009) suggested that Antarctica was characterised by a distinct microchlorophyte flora, which was probably the result of a long evolutionary history on the continent (“glacial refugia hypothesis”) (Convey & Stevens 2007). Studies on diatoms have suggested a high proportion of endemic morphospecies (e.g., van de Vijver et al., 2010, Kopalová et al. 2011), a notion supported by molecular data, which are, as yet, still fragmentary (Pinseel et al. 2017). Further taxon sampling and a wider implementation of modern molecular methods is apparently required in order to elucidate phylogeographic patterns in Antarctic cyanobacteria and microalgae.

Because of its position between maritime and continental Antarctica, James Ross Island is a very interesting place for biogeographical studies. The northeastern Antarctic Peninsula including the James Ross Island archipelago was already recognised as a distinct bioregion hosting specific assemblages (Terauds et al. 2012). However, the microflora of James Ross Island lakes was rarely studied before the opening of Mendel Polar Station in 2006 (Hansson & Håkansson 1992). During the first seasons after the completion of the station, phycological research was focused on basic floristic and ecological recognition of the area (Komárek & Elster 2008). A study of the diversity and ecological distribution of cyanobacteria by Komárek et al. (2008) revealed the presence of many morphotypes in lakes, with most

of them forming distinct populations and ecologically delimited communities. The majority of the studied taxa did not correspond to known species from other regions. Based on the field sampling of a representative set of lakes on the Ulu Peninsula (see **Part I-1**), we focused on the main taxonomic groups of photoautotrophic microorganisms found in these habitats, i.e. cyanobacteria, diatoms and green algae.

Green algae (Chlorophyta) observed in Antarctic lakes include members of several classes, of which the most common species are from Chlorophyceae and Trebouxiophyceae (Vincent & Laybourn-Parry 2008). In contrast to diatoms and cyanobacteria, this group have remained virtually unstudied for a long time. In the plankton of ice-covered lakes of the Ulu Peninsula, we observed the dominance of coccoid green algae from the genus *Monoraphidium* (Chlorophyceae, Selenastraceae) (**Part I-4, Nedbalová et al. 2017b**). This was a rather surprising finding because phytoplankton in Antarctic lakes are usually dominated by a small number of flagellate species (mostly cryptophytes, chlorophytes or chrysophytes) and non-motile species are rarely dominant (e.g., Izaguirre et al. 1998). Phylogenetic analyses of 18S rDNA together with analysis of ITS2 rDNA secondary structure and cell morphology revealed that the two isolated strains belong to one species that should be described as new. The closest relative of the two Antarctic strains, *M. cf. dybowskii* SAG 2393, was isolated from a calcifying biofilm of a karst creek in Germany (Hodač et al. 2015). This is a further indication that many coccoid green algae could possibly have a wide geographic distribution as was recently demonstrated for the genera *Chlorella* and *Stichococcus* (Hodač et al. 2016). The strains grew in a wide temperature range, but the growth was inhibited at temperatures above 20 °C, indicating their adaptation to low temperatures. The ability to grow in a broad temperature range is believed to be related to the broad temperature fluctuations in the original habitat (Seaburg et al. 1981, Elster 1999). However, Dolhi et al. (2013) demonstrated that microorganisms with temperature requirements similar to our *Monoraphidium* strains occur also in the extreme but stable environment of lakes in the McMurdo Dry Valleys. In fact, a surprisingly low number of "true psychrophilic" algae have been isolated so far (e.g. Morgan-Kiss et al. 2008). The strains were characterised by an extremely high content of polyunsaturated fatty acids (PUFA, more than 70 % of total fatty acids). Steariadonic (6,9,12,15-18:4) and hexadecatetraenoic acid (4,7,10,13-16:4), which have significant pharmacological effects (Guichardant et al. 1993), were identified as the main PUFA. Together with preference for low irradiances and relatively high growth rates, their fatty acid profiles make these isolates interesting candidates for low-temperature biotechnological applications.

The presence of well-developed photoautotrophic mats made up largely of cyanobacteria is often a characteristic feature of Antarctic lakes (Vincent 2000). During our sampling of the Ulu Peninsula lakes, we observed various types of mats with a specific macroscopic structure suggesting the presence of specific assemblages in the littoral zones of the lakes. Besides the dominant cyanobacteria, pennate diatoms represent another important component of the mats. During the ongoing study of benthic diatoms, a well-developed flora of more than 120 taxa (**Part I-5, Kopalová et al. 2013**) was recorded in freshwater habitats of the Ulu Peninsula. It included a large number of new and endemic diatom species belonging to the genera *Luticola*, *Neidium*, *Craticula* and *Halamphora* (e.g., van de Vijver et al. 2010, Zidarova et

al. 2009, Kopalová et al. 2011). Diatom communities present in streams and seepages could be clearly distinguished from those from lakes. The survey further suggested a strong link between the taxonomic composition of lacustrine mats, particular lake types, and their physical and chemical characteristics, with conductivity and nutrients as the two main factors determining species composition. Similar results were obtained at other Antarctic localities (e.g. Sabbe et al. 2004). A transfer function for water conductivity could be also developed, potentially providing a powerful tool for planned palaeoecological studies. The dominant species in the lakes were *Nitzschia* spp., which were later studied in detail. This produced the description of seven new species from this genus based on samples from the James Ross Island lakes (Hamsher et al. 2016). Moreover, the presence of species that are characteristic of continental Antarctica, but absent from all maritime Antarctic localities (*Achnanthes taylorensis*, *Luticola gaussi* and *Craticula glaberrima*) confirmed the transitory character of lacustrine habitats on James Ross Island as already indicated by Komárek et al. (2008) based on the species composition of cyanobacteria.

In further papers, we focused on detailed description of unique photoautotrophic mats in two shallow lakes near Andreassen Point that we sampled for the first time during the limnological survey in 2008–2009 (Parts I–6–9). According to the classification presented in Part I–1, the lakes Green 1 and 2 belong to the category of stable shallow lakes on levelled surfaces, and rank among the oldest in the area, with possible origin in the early Holocene. The name of the lakes was derived from the character of their conspicuous microbial mats in the flat littoral zone. The upper surface of stones was covered by black cyanobacterial mats covered by mucilage that was colonised by clusters of densely agglomerated filaments of a green alga. When the biofilm dried because of evaporation of lake water during the summer period, a net of precipitated micro-fortified mucilage mixed with organic matter and mineral clasts was visible. Moreover, macroscopic inorganic crystal-like structures of unknown nature were observed in the field. This specific mat type thus exhibited some features reminiscent of complex structures formed by microbial mats in deep, perennially ice-covered lakes in continental Antarctica (Wharton et al. 1982, Andersen et al. 2011). This mat type was observed only in the two lakes, although lakes with very similar morphometric and chemical characteristics are found in the area of the Ulu Peninsula (Part I–1).

Using a polyphasic approach (16S rDNA sequencing and morphological and ecological evaluation of both natural material and laboratory strains), we first studied the species composition of the cyanobacterial leather-like black biofilm in the two lakes (Part I–6, Komárek et al. 2012). The biofilm was formed by heterocytous cyanobacteria, and a species new to science (*Calothrix elsteri*) was identified as the dominant one. Several species from the genus *Calothrix* (e.g. *C. parietina*) have already been described in Antarctica (Broady 1986, 1996, Ohtani 1986), but they differ substantially from *C. elsteri* by their morphological characters. Two more heterocytous species were described from the Green lakes: *Hassallia andreassenni* and *Hassallia antarctica*. Whereas *H. andreassenni* was associated with *C. elsteri*, *H. antarctica* was found in stone crevices, only loosely attached to the substrate, which suggested the differing ecological requirements of the two species. All the three species are important components of distinct communities, they have a specialised ecology and must be considered *ad interim* as endemic for Antarctic ecosystems. Interestingly, the mat dominated

by *C. elsteri* contained unusual molecular species of N-acylphosphatidylethanolamine with very-long-chain anteiso branched fatty acids (from C<sub>20</sub> to C<sub>30</sub>, [Part I-7, Řezanka et al. 2009](#)). If we do not consider animal waxes such as lanolin, the presence of these fatty acids has only been reported in cryptoendolithic microbial communities from the McMurdo Dry Valleys (Matsumoto et al. 1992). These abnormal phospholipids thus probably play an important role in the membranes of *C. elsteri* and represent another example of adaptation of lipid composition, enabling growth and survival under the extreme conditions of Antarctica.

Later, we focused on the co-dominant species of the mats in Green lakes, i.e. the branched green alga, which grows in macroscopic colonies producing green spots on the cyanobacterial mat ([Part I-8, Škaloud et al. 2013](#)). Freshwater filamentous green algae with branched thalli are almost unknown in the Antarctic region. They have rarely been recorded from maritime Antarctica and from sub-Antarctic islands with rich phanerogamic vegetation (Broady 1979, Komárek & Komárek 1999, Bonaventura et al. 2006). The co-dominance of a green microalga is unique because mats in Antarctic lakes are most frequently formed by cyanobacteria (Vincent 2000). Molecular sequence data (18S ribosomal DNA and the internal transcribed spacer) of the strain from Green Lake 2 indicated that it belongs to the genus *Hazenia* (Ulotrichales). Based on distinct molecular, morphological, and ecological characters, this alga was described as a new species (*Hazenia broadyi*), closely related to the type strain *H. mirabilis*. So far, it is known only from the two Green lakes.

To conclude, we have shown that the photoautotrophic mats in the Green lakes are formed by a unique microflora. Its dominant components were described as new species that are considered endemic to Antarctica. Finally, we tried to elucidate the nature of the inorganic precipitates formed in association with the mats ([Part I-9, Elster et al. 2016](#)). The spicules' chemical composition and crystal structure corresponded to pure calcite, and we believe that its precipitation is connected with the photosynthetic activity of the mats. This process is probably closely linked to their specific composition, which is unique in the study area. More specifically, we hypothesised that a more rapid photosynthetic rate in *Hazenia* compared with cyanobacteria may induce conditions necessary for carbonate precipitation (Vincent 2000). To our knowledge, the biogenic calcite structures in the Green lakes are quite different to any microbially mediated structures yet described from modern environments (e.g., Couradeau et al. 2011) and also to structures formed by abiotic precipitation (e.g., Vogt & Corte 1996).

#### *Antarctic microalgae as candidates for low-temperature biotechnology*

Polar microalgae have evolved a complex network of adaptation/acclimatisation reactions to survive in low-temperature environments (Elster & Benson 2004, Morgan-Kiss et al. 2006). Therefore, a high potential of photosynthetic microorganisms isolated from polar regions to produce various biotechnologically important compounds can be expected. PUFA in particular are of primary interest to the food, cosmetics and pharmacological industries, and could possibly be obtained from various microalgae (Cepák et al. 2014). Moreover, cold-adapted strains could be grown in winter in temperate regions where current outdoor mass

cultivation operates only during the summer months (May–September), or even in polar regions. So far, most commercial mass cultivation of microalgae has been undertaken in tropical and subtropical areas because of the growth requirements of commercially-grown strains such as *Arthrospira* (*Spirulina*), *Dunaliella salina*, or *Haematococcus pluvialis*, which are slightly thermophilic and mesophilic (Borowitzka 2005).

Therefore, we decided to test one of the cold-adapted strains of *Monoraphidium* (Selenastraceae, Chlorophyta) for PUFA production. These strains, newly isolated from ice-covered lakes on James Ross Island, contained around 70 % PUFA in their fatty acid profiles, with exceptionally high values of stearidonic (18:4) and hexadecatetraenoic acid (16:4) (see **Part I–4**). The strain CCALA 1094 was successfully cultivated in an outdoor 150 l thin-layer photobioreactor in early winter conditions in Central Europe (Třeboň, Czech Republic), where the average temperature was 10.0 °C and the average photosynthetically active radiation (PAR) intensity only 32  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (**Part I–10, Řezanka et al. 2017a**). Only one out of more than 20 studies mentioned in the review by Yee (2016) used cultivation temperatures below 20 °C. The growth rate of CCALA 1094 over the 22 days of the pilot cultivation reached a value of 0.341  $\text{day}^{-1}$  and lipid productivity was 162  $\text{mg l}^{-1} \text{day}^{-1}$  being comparable, or even superior, to values from experiments performed at significantly higher temperatures and irradiances (e.g., Zhao et al. 2014). The high proportion of the 16:4 and 18:4 acids resulted in respective productivities of 27.5 and 43.7  $\text{mg l}^{-1} \text{day}^{-1}$ , which is one order of magnitude higher than previously reported values (Zhao et al. 2014). Together with low temperature and irradiance requirements, these results make this strain unique within Selenastraceae (Yee 2016). It is thus eminently suitable as a source of PUFA that could be used in dietary supplements for livestock and humans. A national patent was recently awarded to the method of PUFA production that uses this strain (Řezanka et al. 2017b), and the strain will be further tested for biotechnological applications.

## Chapter II. Snow

### *Green cryosestic algae – an overview*

Coloured snow is a striking natural phenomenon caused by large numbers of microorganisms, mostly algae (Kol 1968). Snow algae represent a specific, well-adapted group, which developed a physiology optimised to such an extreme environment. Most snow algae belong to the order Chlamydomonadales (Chlorophyta), and the genera *Chloromonas* and *Chlamydomonas* are widely distributed (Hoham & Duval 2001). In a review (**Part II–1, Komárek & Nedbalová 2007**), we summarised information about the biology, diversity, ecology and ecophysiology of green cryosestic algae that was available at the time. They can be divided into two groups according to life form: 1) species with motile stages (Chlamydomonadales) and 2) simple filamentous species. However, members of the latter group lack resting stages and other adaptive features and are now considered to be primarily soil organisms (Stibal & Elster 2005).

The life histories and ecology of several species from the order Chlamydomonadales were studied in detail (e.g., Hoham 1975a, Hoham et Mullet 1977, Hoham et al. 1979). Their

complex life cycle, which includes both flagellated and non-motile resting stages, is crucial for their growth and survival in the harsh snow habitat. The zygospores found in natural samples were previously described as coccoid algae from the genera *Cryocystis*, *Trochiscia* or *Scotiella*. At the date of publication of our review, there were around 20 described chlamydomphycean species that live in snow. However, recent descriptions of new species combine molecular and morphological data and confirm that the diversity of snow algae is apparently still underestimated (Muramoto et al. 2010, Matsuzaki et al. 2014, Remias et al. 2016). Particular attention is paid to the red snow alga *Chlamydomonas nivalis*, which usually dominates snowfields above the tree line. This alga is determined mostly on the basis of typical red spherical cells without any observation of flagella. It is probable that the reddish colouration of snow at alpine sites is caused by other species with red immotile cells. However, Lutz et al. (2016) showed using high-throughput sequencing of 18S rDNA that the red snow algae community composition was highly similar across the Arctic. From this point of view, a wider molecular characterisation of all cryosestic *Chlamydomonas* and *Chloromonas* types is urgent (first prospective results see in Hoham et al. 2002, 2006, Muramoto et al. 2008, Remias et al. 2010, **Part II-7**).

In the second part of the review, the ecological factors in snow and the adaptations of cryosestic algae to this extreme environment are presented. Low temperature is of course the key feature of the snow habitat. Species found in snow can be divided into two groups: psychrophilic (cryophilic), which typically cause snow colouration, and psychrotolerant, which have an optimum temperature higher than 15 °C (Hoham 1975b, Stibal & Elster 2005). In the high mountains, the photon fluence rate can reach up to 6000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  on the snow surface while steep light gradients form because of strong light attenuation in a snow layer (Gorton et al. 2001). Irradiance levels are significantly lower at forested sites (Komárek et al. 1973, **Part II-4**). The major protection against high irradiance levels (both UV and PAR) is apparently fulfilled in snow algae by the accumulation of the secondary pigment astaxanthin. However, there are only few data describing their pigment composition in the field (Bidigare et al. 1993, Remias et al. 2005, Remias et al. 2010, Remias et al. 2016). The protective role of astaxanthin in snow algal cells is complex because this secondary carotenoid is also an effective antioxidant (Tinkler et al. 1994). The water content in cells is reduced when astaxanthin concentration is high, reducing the likelihood of ice crystal formation (Hoham 1992). As protection from UV radiation, some algae contain phenolic compounds (Duval et al. 1999a). However, the production of mycosporine-like amino acids has not been reported for snow algae. Although snow is usually considered an oligotrophic environment, the concentration of nutrients can reach high values, especially at forested sites (Komárek et al. 1973). However, the rapid growth of snow algae can result in nutrient depletion. The relationships between snow algae and their environment are apparently complex and poorly understood. Because of the remoteness of localities with snow algae, there are still few data about the seasonal development of populations, or their geographic distribution and ecophysiology. We have tried to fill some of the gaps in the following papers.



## *New records of snow algae in Europe and South America*

Snow algae can be found in polar and mountain regions worldwide. Their distribution was comprehensively reviewed by Kol (1968), with most records from Europe and North America. Following their relatively recent discovery in the Atlas Mountains in Africa, a continent with no record of these organisms until 1998 (Duval et al. 1999b), snow algae are now known to be found on all of the continents. However, at a more fine geographic scale, our knowledge of the distribution of snow algae remains fractional.

In Bulgaria, cryoseston was recorded for the first time by Kol (1956) in the Rila and the Pirin Mountains. Although the Vitosha Mountains have all necessary conditions for development of cryoseston, i.e., altitude well above 1 000 m a.s.l., and therefore snow fields that regularly persist until late spring or early summer, there have not yet been any records of snow algae. Therefore, we sampled snowfields in this mountain range to study the species composition of cryoseston ([Part II–2, Lukavský et al. 2009](#)). In May 2005, the snowfields were large at the edge of tree line, and were not coloured but with dirty surface. The snow algal community was dominated by zygosporangia of *Chloromonas nivalis* and *Chloromonas brevispina*. Later, in July, only a few small snowfields with characteristic shell-form surface persisted above tree line; one field had a very light pink tint. Zygosporangia of *Chloromonas nivalis* were again dominant, but characteristic alpine species of the snow algae *Chlamydomonas* cf. *nivalis* and *Chloromonas rostafinskii* were also observed in the samples. *Chlamydomonas* cf. *nivalis* was also dominant in late June 2006, forming patches of red snow on snowfields in the alpine zone (2100 m a.s.l.). The Vitosha Mountains thus are a suitable locality for the development of snow algae. Their species composition and general distribution pattern were similar to those of the Krkonoše (Giant) Mountains (Czech Republic), see [Part II–4](#). In a small area, we recorded a high diversity of snow microorganisms. This study represents the first record of cryoseston from the Vitosha Mountains.

Because of their high degree of isolation from temperate and polar regions, glaciated tropical mountains can be expected to host particular cryofloras. Nevertheless, the algae research in the tropics has received little attention. The most extensive study relates to the glaciers of New Guinea (Kol & Peterson 1976), whereas only very little is known about the snow algae species composition in other tropical alpine regions. Moreover, a unique habitat type is disappearing from the tropics along with melting glaciers. In Ecuador, red snow formed by a rich cryosestic community was reported from the Pichincha volcano (Lagerheim 1892), where persistent snow disappeared during the second half of the 19th century, meaning snow algae must be considered extinct on that mountain. The current composition of the Ecuadorian cryoflora thus remained unknown. Examination of the surface melt water from the Iliniza glacier ([Part II–3, Nedbalová & Sklenář 2008](#)) yielded the very first record of the snow alga *Cylindrocystis brébissonii* f. *cryophila* for equatorial regions, and a new record of spherical cysts of the red snow alga *Chlamydomonas* cf. *nivalis*, which was previously reported by Lagerheim (1892). Although snow algae represent only a minor part of the species richness of the Ecuadorian Alps, they further increase the extraordinary ecological diversity of the tropics. Further study of their distribution, life cycles and

ecological requirements is thus urgently needed, particularly given the situation of rapidly vanishing snow and ice conditions.

### *Ecology and ecophysiology*

In the Krkonoše Mountains (Czech Republic), snow algae have thus far been observed by Fott et al. (1978) and Kociánová et al. (1989), who reported the occurrence of both green and red snow. The occurrence of snow algae over a wide altitudinal gradient at sites with contrasting environmental conditions provides a good opportunity to study the autecology of particular species. Based on analysis of samples covering an extensive area that were collected in 2002–2006, we summarised the available data on the distribution of snow algae in this mountain range, and compared selected ecological parameters of sites characterised by differences in elevation and exposure ([Part II–4, Nedbalová et al. 2008](#)). *Chloromonas brevispina* and *Chloromonas nivalis* were reported as the most common taxa. Although these species often grow together under the same environmental conditions, we observed a clear difference in their altitudinal distribution. *Chloromonas brevispina* is a forest species found in shaded or at least partially shaded localities, whereas *Chloromonas nivalis* occurs also in open exposures at higher elevations. Whether the populations of *Chloromonas nivalis* from distinct habitats have diverged at genetic level is currently one focus of our work. However, recently there have been reports of high genetic variability in *Chloromonas* cf. *nivalis* originating from different geographical regions (Muramoto et al. 2008, Remias et al. 2010, see also [Part II–7](#)). The rare red snow species *Chlamydomonas* cf. *nivalis* was restricted to open exposures above the tree line or to avalanche tracks and the slopes of glacial corries. To conclude, we observed a clear pattern in the altitudinal distribution of species and important differences in environmental parameters (e.g., temperature, PAR intensity, and nutrient concentration) among sites. In contrast to the most intensely studied localities, where red snow is dominant, green snow with *Chloromonas* spp. is most abundant in the Krkonoše Mountains. Accordingly, this small mountain range represents an important place for the research of snow algae.

One of the major adaptations of metabolic function influencing growth and photosynthesis at low temperatures is the maintenance of membrane fluidity. In particular, the extent of unsaturation of fatty acids in membrane lipids plays a major role in avoiding membrane rigidification at low temperature, and the importance of this mechanism in the low-temperature adaptation of polar microorganisms is widely recognised (Morgan-Kiss et al. 2006). Although snow algae represent an excellent model for the study of membrane adaptations at low temperatures, there are only few examples of changes in the content of fatty acids as a factor regulating membrane fluidity in this group (e.g., Bidigare et al. 1993, Spijkerman et al. 2012). In a study focused on fatty acid composition of field samples, we isolated unusual short and medium chain polyunsaturated fatty acids potentially enhancing membrane fluidity in the flagellated cells of the snow alga *Chloromonas brevispina* collected in the Bohemian Forest (Czech Republic) ([Part II–5, Řezanka et al. 2008a](#)). A total of 43 fatty acids, predominantly short- and medium-chain PUFA, were identified. The only saturated fatty acid identified was palmitic acid. Moreover, the high content of PUFA with chains

shorter than C16 was extraordinary. Although it is widely distributed in cold-adapted bacteria (Russel 1984), this is the first report of shortened fatty acyl chain length as a cold adaptation of snow algae. The high content of PUFA (more than 75 % of total fatty acids) indicates that they are an important element ensuring cell survival of this species, similarly as in freshwater Antarctic planktic green algae (see **Part I-4.**)

Further data on fatty acid composition in snow algae from the genus *Chloromonas* were obtained in **Part II-6 (Řezanka et al. 2014)**. In three field samples collected in the Krkonoše and Jizera Mountains, the proportion of PUFA also reached high values (59–70 %) with  $\alpha$ -linolenic, hexadecatetraenoic and oleic acids as dominant fatty acids. However, fatty acids with chains shorter than C14 detected in the previous study (**Part II-5**) were not found, which suggests a species-specific mechanism of adaptation to cold environments. We also performed for the first time a lipidomic analysis of snow algae from the field using an LTQ Orbitrap Velos high-resolution mass spectrometer, which resulted in the determination of more than 300 lipid molecular species. Because the genus *Chloromonas* is a major producer of C16 PUFA (Lang et al. 2011, **Part II-5**), particular attention was paid to lipid classes that contained this group of PUFA (both polar lipids and triacylglycerols – TAGs). Using the analysis by silver-ion liquid chromatography and non-aqueous reversed phase liquid chromatography/mass spectrometry (NARP-LC/MS) of TAGs, we also identified regioisomers of C16 and C18 PUFA. TAGs having between 0 and 12 double bonds and contained only C16 and C18 fatty acids with 0 to 4 double bonds were separated. The complex approach used in this study may represent a powerful tool enabling insights into the adjustments of metabolic pathways in microorganisms living in extreme environments.

In the following section, we studied in detail an interesting alga that was described by Kol as *Scotiella tatrae* from pink snow collected at a high mountain site in the High Tatra Mountains (Slovakia) (Kol 1965, **Part II-7, Procházková et al., in press**). An extensive cryobiological study was undertaken in this mountain range that showed high biodiversity in contrast to that in the Krkonoše Mountains (e.g., Kol 1968, Komárek et al. 1973). Many types of *Scotiella*-like cells found in snow were shown to represent the zygotic stage (designated as zygospores or aplanozygotes) of flagellates from the genus *Chloromonas* (e.g., Hoham 1975a); therefore, we felt confident that they belonged to this genus. Because multiple cryptic diversity in the morphologically similar species of *Chloromonas* cf. *nivalis* was suggested by Muramoto et al. (2008), we had to include molecular characterisation to identify the alga correctly. Based on the investigated genes (18S rDNA, ITS1 and ITS2 rDNA, *rbcL*), we have shown that the populations harvested in course of this study are closely related to *Chloromonas nivalis* from the Austrian Alps. Despite the absence of any compensatory base changes (CBCs), the detected difference in the ITS2 sequences of about 5.5 % suggested that a divergence between these algae was ongoing. Slight differences in aplanozygote morphology (number of flanges) between the two organisms were also confirmed. Therefore, we re-designated *Scotiella tatrae* as a subspecies of *Chloromonas nivalis* and proposed a new combination *Chloromonas nivalis* subsp. *tatrae*. Both organisms exhibit similar photosynthetic performance and share similar habitats at open sites above tree line, but differences in astaxanthin accumulation have been found. In a field sample of *Chloromonas nivalis* subsp. *tatrae*, PUFA formed nearly 50 % of total lipids; they were

present mostly in phospholipids and glycolipids. Based on our results, the newly described subspecies thus likely represents a variation of a common cryoflora species with distinct morphology and, to our current knowledge, it is endemic to the High Tatra Mountains.

Because there are many limitations on measurements of the physiological performance of field samples, we also tried to isolate strains from coloured snow that would enable detailed laboratory studies. In **Part II-8 (Lukeš et al. 2014)**, we studied the effect of short acclimation to a wide range of temperatures on growth rate, photosynthetic electron transfer, and lipid and fatty acid composition in a chlamydomphycean strain (CCALA 970) isolated from red snow. The strain showed unusual characteristics with significantly higher growth rates at temperatures of 5 to 15 °C, compared with the mesophilic control *Chlamydomonas reinhardtii* and unprecedentedly high  $Q_A$  to  $Q_B$  electron transfer rate. We hypothesised that this structural flexibility could be made possible by the effect of amino acid changes in the D1 protein combined with the dominance of phosphatidylglycerol in thylakoid membranes. Because of its high melting point, this lipid was suggested as a possible factor in modulating membrane flexibility and hence enhancing chilling resistance (Kaniuga et al. 1998). We proposed that the high phosphatidylglycerol content in the strain CCALA 970 could be used in its production (Kaftan et al. 2013). This study is thus a further confirmation that strains isolated from extreme environments could be useful for biotechnological applications.

### *Biochemistry of astaxanthin and implications for astrobiology*

As mentioned in **Part II-1**, the central role in the protection of snow algal cells against high irradiation levels is played by the secondary carotenoid astaxanthin. Free and esterified astaxanthin is stored in cytoplasmic lipid globules, and its content in dark red cysts of *Chlamydomonas nivalis* can be about 20 times higher than that of chlorophyll *a* (Remias et al. 2005). This ratio may reflect the level of cell maturity during the life cycle (a higher ratio is associated with older cells, Remias et al. 2010). Alternatively, it can indicate differences among clades or taxa. For example, the ratio was three times higher for mature aplanozygotes of *Chloromonas nivalis* subsp. *tatrae* than that of comparable cells of *Chloromonas nivalis* from the Austrian Alps (see **Part II-7**).

Astaxanthin in snow algae is found mostly in esterified form (as monoesters or diesters) (Bidigare et al. 1993). In **Part II-9 (Řezanka et al. 2008b)**, we have reported for the first time that red cysts of *Chlamydomonas nivalis* collected from a high-altitude environment can accumulate astaxanthin diglucoside diesters. This form of astaxanthin with glucose as the only sugar had the highest weight proportion in the cells. Using mass spectrometry with atmospheric pressure chemical ionisation (MS/APCI), 105 molecular species were identified. We have also shown that this form of astaxanthin was associated with unsaturated C16 and C18 fatty acids (see also Lutz et al. 2016). It combines three storage forms in one, i.e., a secondary carotenoid, a fatty acid, and a carbohydrate. Because the cysts of snow algae remain highly photosynthetically active, astaxanthin accumulation could be seen as a metabolic device used to store the photosynthetic energy in a nitrogen-free form (Remias et al. 2010).

In [Part II–10 \(Řezanka et al. 2013\)](#), we combined the analytical methods used in [Part II–9](#) with chiral high-performance liquid chromatography to identify optical stereoisomers of astaxanthin glucoside esters and diesters. On one hand, in photosynthetic organisms, astaxanthin occurs as the nearly pure 3*S*,3'*S* isomer (e.g., Renstrom et al. 1981), while on the other hand, the 3*R*,3'*R* isomer has been identified e.g. in the red yeast *Phaffia rhodozyma* (Andrenes & Starr 1976). We observed a striking difference in the composition of astaxanthin optical isomers in the red snow alga *Chlamydomonas nivalis* collected in geographically distinct regions (the Austrian Alps, the Slovak High Tatra Mountains and the Bulgarian Pirin Mountains). So far, a mixture of all three isomers (3*R*,3'*R*, 3*S*,3'*S* and a meso form – 3*R*,3'*S*; 3*S*,3'*R*) has been reported only in marine animals that obtain astaxanthin through food (Megdal et al. 2009). Furthermore, algae from the Pirin Mountains were characterised by the prevalence of astaxanthin diglucoside diesters (see [Part II–9](#)), whereas the algae from the Alps were dominated by diacyl-astaxanthins suggesting alternative strategies to enhance cell viability at low temperatures. Further analyses are needed to elucidate whether these differences are caused by ecological factors or whether they can be associated with the identity of algae assessed by molecular tools.

In extremely cold environments, life thrives in forms that reflect adaptation to the harsh conditions close to, or beyond, the limits of survival for other organisms. These ecosystems have been proposed as Earth-based analogues to be investigated for their astrobiological significance. The strategies suggested for the search of life on Mars in the framework of the coming missions are tested within the framework of terrestrial scenarios (Preston & Dartnell 2014) and include the deployment of miniature Raman spectrometers (Dickensheets et al. 2000). The advantage of this method is the possibility of detecting *in situ* the key biomarkers of various organisms (e.g., Edwards et al. 2003, Jehlička et al. 2013). Therefore, we decided to test the potential of a miniaturised Raman spectrometer for field detection of the pigments of snow algae at different physiological stages ([Part II–11, Jehlička et al. 2016](#)). Astaxanthin, the major pigment, has been detected in the blooms of *Chloromonas nivalis* and *Chlamydomonas nivalis* at alpine sites in the Krkonoše Mts. (Czech Republic) and in the Ötztal Alps (Austria). We have shown that the handheld Raman spectrometer is a useful tool for fast, direct field estimation of the presence of carotenoids (mainly astaxanthin) within colonies on snow algae. Applying miniature Raman instruments as well as flight prototypes in areas of survival of microbes under extreme conditions is an important stage to prepare for successful deployment of the instrumentation needed for forthcoming astrobiological missions to Mars.

## Chapter III. Acidic habitats

### *New habitats and new species*

Acidification of lakes, streams and soils is a major environmental problem. It changes the chemical composition of water and thereby influences the biological structure and composition of communities. A drastic reduction of biodiversity followed by the disruption of food web structures has been recorded in many lake areas (e.g., Schindler 1988, 1994, Vrba et al. 2003), and the key role of toxic aluminium in biological damage has been widely accepted (Gensemer & Playle 1999). Freshwater ecosystems can be anthropogenically acidified either by atmospheric deposition or by acid mine drainage. Lakes that originated from abandoned metal or coal mines represent an extreme example of acidic habitats with specific morphometric, chemical and biological properties (Castendyk & Eary 2009). In [Part III-1 \(Hrdinka et al. 2013\)](#), we described the specific environment of the most acidified lake (pH ~ 2.6) in the Czech Republic. Hromnice Lake arose 110 years ago as a consequence of pyritic shale open cast mining. It is a permanently meromictic lake with the chemocline lying at a depth of 3–3.5 m. The extreme chemical composition of the lake water is characterised by extreme concentrations of  $\text{SO}_4^{2-}$ , Fe, Al and increased concentrations of other metals, e.g., Mn, Ni, Cu, Co and Pb. The concentration of phosphorus ( $\text{PO}_4^{3-}$ ) ranged from 0.04 mg l<sup>-1</sup> at the surface up to 1.6 mg l<sup>-1</sup> in the deeper layers. The lake supports a low number of phytoplankton species but a high photoautotrophic biomass with a concentration of chlorophyll *a* comparable with that of eutrophic lakes. The most abundant species has been identified as a non-motile green trebouxiophycean alga *Coccomyxa* sp., together with flagellates *Lepocinclis* sp., *Chromulina* sp. and *Chlamydomonas* sp. Regarding zooplankton, only two acid-tolerant rotifers *Cephalodella* sp. and *Elosa worallii* were found in this extreme lake. As the oldest acidic mining lake in Central Europe with specific limnological characteristics and biota, Hromnice Lake deserves its current protection also as a site suitable for research of life in extreme environments.

In the following paper ([Part III-2, Barcyte & Nedbalová 2017](#)), we tried to characterise the coccoid green alga that was recorded as one of the dominant species in the plankton of the extremely acidic Hromnice Lake and tentatively identified as *Coccomyxa* sp. (Trebouxiophyceae) (see [Part III-1](#)). In addition, the dominant component of phytoplankton in the atmospherically acidified Plešné Lake (Bohemian Forest) was studied. Phylogenetic analyses (18S rRNA and ITS2) revealed that the strain isolated from Hromnice Lake belongs to *Coccomyxa elongata*, which is not very commonly reported freshwater species. The strain from Plešné Lake was described as a new species, *Coccomyxa silvae-gabretae*. Interestingly, it appeared to be closely related only to one strain, isolated from an Antarctic lake (de Wever et al. 2009). Based on its morphological characteristics observed in field samples (thick cell wall), this alga from Plešné Lake has been erroneously determined as *Monoraphidium dybowskii* in previous studies (Vrba et al. 2003, Nedbalová et al. 2006). *Coccomyxa* is able to grow in a wide range of habitats including the most extreme ones. Remarkably, it was found as a contaminant in flasks with chemical solutions and in stock solutions used to prepare media for cultivation of algae (Sládečková 1959). Our work represents the first evidence that representatives of this genus are also capable of becoming the dominant planktic algae in the

extreme environments of acidic lakes with an increased supply of phosphorus. There were observed clear differences in cell morphology under different growth conditions. These differences reveal the high phenotypic plasticity of the strains. The ability to change the morphology may help the cells of *Coccomyxa* to survive harsh conditions in two acidic lakes. Our results are consistent with those published recently that suggest that the molecular diversity of the genus is as yet underexplored (Darienko et al. 2015, Malavasi et al. 2016).

Apart from acidic lakes, we have newly investigated a highly unusual acidic habitat – a burning coal spoil heap (**Part III–3, Barcyte et al., in press**). Post-mining sites are important anthropogenic habitats that offer secondary environment for many organisms. Until now, microbiological studies of these sites have focused mainly on bacteria (Kirby et al. 2010). To our knowledge, the only photoautotrophic organism reported from a burning spoil heap was the cyanobacterium *Hapalosiphon cossyrensis*, which was originally described from a hot spring area on Pantelleria Island in Italy (Komárek & Rosa 1957). The first signs of thermal activity in the Heřmanice spoil heap (close to Ostrava, Czech Republic) were observed during the 1950s and 1960s. In 2016, the surface temperature of fume vents reached up to 500 °C and the temperature of fumes decreased with increasing distance from the fumarole zone. Vents with temperatures of 50–55 °C were heavily colonised by bright green mats formed by *Galdieria*-like algae. The mats were growing exclusively on organic material, i.e. tree stumps, bark or branches. The pH of the substrate was 2–3. Molecular markers (rbcL and calmodulin) revealed that the population belonged to the continental European lineage of *Galdieria sulphuraria* (Cyanidiales). This unicellular red alga is known worldwide as a polyextremophile that thrives in environments with low pH, high temperature and often increased metal concentrations (Gross et al. 2001). In our paper, we report a new anthropogenic habitat suitable for this alga. Furthermore, its discovery at a relatively newly established site allows us to better understand the distribution patterns and dispersal abilities of thermoacidophilic Cyanidiales as an ecologically important group. For comparison, we have also studied *Galdieria* strain CCALA 965 isolated in 2000 from a natural, highly acidic, non-thermophilic site in the Czech Republic (Soos National Nature Reserve) and confirmed it to belong to another species, *Galdieria phlegrea*. As we identified more interesting eukaryotic organisms accompanying *Galdieria* on the burning heap (fungi and green algae), our study provides further evidence that highly acidic habitats can accommodate unique communities. However, in December 2016, the Heřmanice site was highly disturbed because of ongoing remediation, and no *Galdieria* has been detected.

### *Insights into ecology of dinoflagellates and diatoms in mountain lakes and streams*

Situated in the region with the highest emissions of SO<sub>2</sub> in Europe and lying on a geologically sensitive bedrock, the Bohemian Forest and the Krkonoše Mountains have been heavily impacted by anthropogenic acidification (Fott et al. 1987, 1994). A significant but slow recovery of water chemistry has been observed in the recent period (see next section, Vrba et al. 2003, Sienkiewicz et al. 2006). In the following papers, we sought a detailed insight into the ecology of two important algal groups in the freshwater habitats of both mountain ranges – dinoflagellates and diatoms.

During the previous research of the Bohemian Forest lakes, we recorded high bulk extracellular phosphatase activity in plankton (Vrba et al. 2006) that is considered a general indicator of severe phosphorus deficiency (Jansson et al. 1988). In [Part III-4 \(Novotná et al. 2010\)](#), we aimed to quantify the phosphatase activity in three dinoflagellate species, occurring in the plankton of three acidified lakes that substantially differed in phosphorus bioavailability because of differences in their phosphorus loading, as well as in concentrations of aluminium and its species (Vrba et al. 2006). We hypothesised significant species-specific responses of phytoplankton to varying phosphorus bioavailability. In order to assess cell-specific activity, we combined the fluorescently labelled enzyme activity (FLEA) assay with image cytometry (Nedoma et al. 2003). The mean cell-specific activity of the three species (*Gymnodinium uberrimum*, *Peridinium umbonatum* and *Gymnodinium* sp.) varied within one order of magnitude among the lakes. Concentrations of aluminium together with pH were identified as significant factors, explaining cumulatively 57 % of the total variability in observed cell-specific phosphatase activity. However, this activity showed an unexpected reverse trend compared with an overall gradient in phosphorus deficiency of the lake plankton. The autecological insight into dinophyte cell-specific activity therefore suggested other factors, such as light availability, mixotrophy, and/or zooplankton grazing, causing further phosphatase activity variations among the acidified lakes.

In another study ([Part III-5, Moravcová et al. 2013](#)), we have investigated the influence of sewage outflows on epilithic diatom assemblages from 16 streams in both mountain ranges. Because mountain streams are among the first recipients of potential anthropogenic pollution, increasing recreational activities are a new threat to the stability of these ecosystems after the peak of anthropogenic acidification. Although extensive research into the impacts of eutrophication on diatoms has already been performed in lowland rivers and lakes (e.g. Rott et al. 1998, Soininen 2002), mountain stream assemblages have received much less attention. In the present study, a total of 53 diatom taxa belonging to 44 genera were identified. Oligo- to mesotraphentic taxa were outcompeted by pollution-tolerant taxa (e.g. *Eolimna minima* and *Planothidium lanceolatum*) as a consequence of sewage pollution. The proportion of affected sites was rather high (~30 %). However, pollution had no significant influence on diatom diversity, which was similar at sites both upstream and downstream of the outflows. Together with water temperature, discharge volume and nitrogen concentrations, pH was identified as a factor with significant influence on species composition, and members of the genus *Eunotia* were confirmed as indicators of acidic sites. Overall, the response of diatom assemblages showed that an increase of recreational activities might significantly alter the ecological status of these vulnerable ecosystems.

#### *Constraints on the recovery from acid stress*

The substantial reductions in the deposition of acidifying compounds have resulted in a partial recovery of surface waters from anthropogenic acidification (Stoddard et al. 2000). However, the biological recovery of affected lakes is often delayed or uncertain (Skjelkvåle et al. 2003, Monteith et al. 2005). The following main constraints preventing



recovery of planktonic and benthic assemblages were proposed: present water quality, dispersal limitation, and community-level barriers including both bottom-up processes and resistance of assemblages established during the acid phase to colonisation of sensitive species (e.g., Monteith et al. 2005, Grey & Arnott 2009). In our research of the glacial lakes in the Bohemian Forest and Krkonoše Mountains, we tried to assess their current limnological status, evaluate the biotic responses of the assemblages and to identify possible constraints preventing their further recovery from acidity.

The significant changes in water chemistry, plankton and benthos composition, as well as extinction of fish in the Bohemian Forest lakes are well documented (Vrba et al. 2003 and references therein). Because of a research tradition dating back more than 140 years, the lakes offer a unique opportunity to assess both chemical and biological recovery from acid stress. 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 the lakes (Vrba et al. 2006). The Bohemian Forest has exhibited the largest changes in acidic deposition in Central Europe (Kopáček et al. 2012), which resulted in a rapid reversal of lake water chemistry (Kopáček et al. 1998, Vrba et al. 2003). However, similarly to other lake districts, the results of a field survey in 2003 indicated that the beginning of biological recovery of the lakes from acidification has been significantly delayed after the chemical reversal (Nedbalová et al. 2006), and this delay has been attributed to continuing adverse effects of aluminium (Vrba et al. 2006).

In [Part III-6 \(Vrba et al. 2014\)](#), we focused on the recovery of one of the most severely acidified of the Bohemian Forest lakes, Lake Rachelsee (Germany). We made use of both historical data and long-term limnological research. The lake was characterised by very low plankton biomass, dominance of heterotrophic filaments and severe phosphorus limitation. Despite gradual chemical recovery of Lake Rachelsee following the changes in acidic depositions in Central Europe since the 1980s, its biological recovery has been delayed (Vrba et al. 2003). Moreover, lake recovery was abruptly reversed in 1999 by a coincident forest die-back throughout the entire catchment, which resulted in elevated terrestrial export of nitrate and ionic aluminium lasting roughly 5 years. This re-acidification episode provided a unique case study, showing plankton recovery in the lake after the rapid decline in nitrate leaching due to natural reforestation of its catchment. We documented both a sudden change in lake water chemistry and plankton biomass structure, such as decreased biomass of bacterial filaments, increased phytoplankton biomass and diversity, and rotifer abundance. The shift from dominance of heterotrophic to autotrophic organisms suggested a substantial release from severe phosphorus stress due to reduced aluminium–phosphorus interaction (Vrba et al. 2006). Hypolimnetic phytoplankton maxima were replaced by epilimnetic ones, reflecting the increase in phosphorus concentration and decrease in light availability. The importance of cyanobacteria and chrysophytes in the 2006–2009 period likely reflected the chemical recovery and replenished dissolved organic carbon level (Graham et al. 2007). To our knowledge, such a rapid change in plankton structure in a lake recovering from acidity has not been previously documented.

As a continuation of our previous work (Vrba et al. 2003, Nedbalová et al. 2006), we tried to evaluate the response of planktonic and littoral assemblages to chemical recovery in the eight Bohemian Forest lakes over a 12-year period (1999–2011) (Part III–7, Vrba et al. 2016). The complex data available allowed us to test which environmental constraints may act in the lakes, and to reconsider the proposed trajectories of biological recovery (Monteith et al. 2005). Despite rapid improvement in the water chemistry of all eight of the studied lakes, only four have partly recovered so far (low-aluminium lakes), while the other four lakes still remain strongly acidic (high-aluminium lakes). All present lake assemblages are dissimilar by 40–90 % from those in the early phase of chemical recovery as a result of species (re)colonisation (ciliates, crustaceans and insects) in the high-aluminium lakes, and considerable species replacement (zooplankton) and (re)colonisation (insects) in the low-aluminium lakes. Phytoplankton remained very similar in the high-aluminium lakes, but changed (loss and/or replacement of some acid-tolerant species) in the low-aluminium lakes. Our data suggested an increasing effect of top-down control and phosphorus regeneration by filter-feeding zooplankton. Aluminium concentrations were identified as dominant factors structuring the assemblages of phytoplankton, rotifers and Nepomorpha, but also affected crustaceans through the carbon : phosphorus ratio of the seston. Both direct (toxicity) and indirect (phosphorus availability) effects of aluminium control biological recovery in the Bohemian Forest lakes. A concentration of 200  $\mu\text{g l}^{-1}$  of total aluminium was shown as the main barrier preventing the high-Al lakes from recovery. Although biotic responses showed important signs of recovery, such as reappearance of some indigenous or acid-sensitive species, decline in eurytopic acid-tolerant species and colonisation by vagile species, the assemblages of all the lakes still suffer from acid stress. Our results also indicate an increasing role of biotic interactions between colonisers and residents and suggest dispersal limitation as another serious constraint on biological recovery.

Even though the glacial lakes in the Krkonoše Mountains (Poland) were also affected by atmospheric acidification, they have received much less attention in comparison with the Bohemian Forest lakes. In 2002, we performed a limnological survey of the lakes Mały Staw and Wielki Staw to assess their current status (Part III–8, Vrba et al. 2008). Low acid neutralising capacity, pH  $\sim$  5 and elevated concentrations of total reactive aluminium ( $\sim$  100  $\mu\text{g.l}^{-1}$ ) suggested high sensitivity of the lakes to acidification. However, the results indicated that some progress in recovery from acidity had taken place since the 1980s (Fott et al. 1987). Plankton composition and abundance in the lakes were typical of acidified mountain lakes whose crustacean fauna were significantly diminished in comparison with the sediment record (Sienkiewicz et al. 2006). Phytoplankton was dominated by flagellates from the classes Chrysophyceae (*Dinobryon*, *Ochromonas*), Dinophyceae (*Peridinium*, *Gymnodinium*) and Chlorophyceae (*Chlamydomonas*). In the shallower and more productive Mały Staw, picocyanobacteria from the genus *Synechococcus* were abundant. The observed differences in water chemistry and biota are probably related to the different characteristics of their whole catchment-lake ecosystems. As the lake ecosystems in the Krkonoše Mountains represent sensible indicators of environmental changes, they should be included among long-term monitored sites to follow their reaction to environmental changes.

## Summary

- In the region of the northeastern Antarctic Peninsula, we have newly described a lake district (Ulu Peninsula, James Ross Island) with high limnological variability that is reflected in a diverse lacustrine biota. A rich microflora composed of cyanobacteria, pennate diatoms and green algae was recorded in the lakes and several new species were described. Specific assemblages with high proportions of endemic species were recorded in the lakes and a transitory character of James Ross Island between continental and maritime Antarctica was confirmed. The production of special lipids with very long chain branched fatty acids was detected in a mat-forming heterocytous cyanobacterium. A newly-isolated, cold-adapted strain of the green alga *Monoraphidium* was successfully tested for the production of polyunsaturated fatty acids in an outdoor photobioreactor during winter conditions in Central Europe.
- Our research of snow algae yielded some new records of these microorganisms at sites in Europe and South America. We have shown distinct ecological requirements of some species, as well as their unusual ecophysiological performance. Their adaptation to the extreme environment of snow was shown to be reflected in the lipid composition, rich in polyunsaturated fatty acids. However, a species-specific response was detected, suggesting diverse strategies of cold adaptation in snow algae. In red snow algae, the secondary carotenoid astaxanthin was shown to occur in a form that has not been described in this group yet. For the first time, all optical isomers of astaxanthin were recorded together in a single microorganism. We have also successfully tested in the field the potential of a miniaturised Raman spectrometer to detect astaxanthin in snow algal cells, which can help in the planning of future astrobiological missions.
- The specific environment of the strongly acidified mining-origin Hromnice Lake in the Czech Republic was described. Similarly to an atmospherically acidified lake (Plešné Lake), the dominant component of its phytoplankton was identified as a green alga from the genus *Coccomyxa*. From Plešné Lake, a new species was described that is so far known only from this locality. A new habitat of thermoacidophilic red algae was discovered – a burning coal spoil heap. The studies of dinoflagellates and diatoms at sites affected by acidification provided detailed insights into their ecology. Thanks to the long-term research on the Bohemian Forest lakes, the most important constraints limiting the lakes' biological recovery from acidity were identified. A rapid change of plankton structure in one of the lakes was observed following a re-acidification episode. We have also recorded the current status of the lakes in the Krkonoše Mountains to enable future comparisons.

## Conclusions and future perspectives

- Although the main types of extreme environments are well characterised, there are still major gaps in the data available on a finer geographical scale for habitats that may harbour significant biodiversity, including new species. This applies not only to remote sites in polar and mountain regions, but also to places that are easily accessible, including recently formed anthropogenic habitats.
- Algae and cyanobacteria from Antarctic lakes and snow and acidic habitats were studied using a polyphasic approach (i.e., a synthesis of morphological, molecular and ecophysiological data) that is crucial to forming a complex understanding of their diversity. The available data suggest that the diversity of photosynthetic microorganisms in extreme environments is surprisingly great, but also apparently still underestimated. Further significant progress can be expected from the critical use of high-throughput sequencing of environmental DNA combined with traditional approaches that include careful microscopic observations of both field samples and laboratory strains. Reliable assessment of current biodiversity is crucial, especially in light of ongoing climate changes that can trigger shifts in community composition, enable colonisation by non-indigenous species and even result in the disappearance of habitats.
- The study of Antarctic algae and cyanobacteria demonstrated a high proportion of potentially endemic species in this region, which suggests the existence of dispersal barriers for microorganisms. In addition, analysis of freshwater diatom flora based on the morphological species concept revealed clear bioregionalisation patterns that are in agreement with classical division of the region into maritime and continental Antarctica. On the other hand, efficient dispersal was proposed for a thermoacidophilic red microalga based on the rapid colonisation of a newly formed anthropogenic habitat in Europe. In any case, more precise and dense field studies in various geographical areas, together with detailed molecular and taxonomic evaluations, are necessary to understand the dispersal and distribution of photoautotrophic microorganisms from extreme environments in more detail.
- The ecophysiological diversity of algae and cyanobacteria from extreme environments is far from understood. Although various categorisations were established to describe it, a continuum of ecophysiological strategies apparently exists, ranging from “true extremophiles” to generalist species. Studies focused on these organisms can provide a valuable insight into the diversity of adaptation and acclimation mechanisms developed by microorganisms and into the general physico-chemical limits of life on Earth.
- The variety of strategies used to cope with extreme conditions is reflected in the production of a multitude of bioactive molecules. Strains from extreme environments are currently considered to be promising producers of various valuable compounds, for example, polyunsaturated fatty acids and pigments. In the temperate zone, cold-adapted algae could be cultivated under winter conditions, which is impossible with current commercial strains. A major effort should be thus dedicated to the isolation of new strains and their biochemical and physiological screening to explore their biotechnological potential.

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## Attached papers

### Chapter I. Lakes on James Ross Island (north-eastern Antarctic Peninsula)

- Part I-1 **Nedbalová, L.**, Nývlt, D., Kopáček, J., Šobr, M. & Elster, J. (2013): Freshwater lakes of Ulu Peninsula (James Ross Island, NE Antarctic Peninsula): Origin, geomorphology and physical and chemical limnology. *Antarctic Science* 25: 358–372.
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- Part I-3 **Nedbalová, L.**, Nývlt, D., Kavan, J., Lirio, J. & Elster, J. (2017): Current distribution of *Branchinecta gaini* on James Ross Island and Vega Island. *Antarctic Science* 29: 341–342.
- Part I-4 **Nedbalová, L.**, Mihál, M.\*, Kviderová, J., Procházková, L.\*, Řezanka, T. & Elster, J. (2017): Identity, ecology and ecophysiology of planktic green algae dominating in ice-covered lakes on James Ross Island (northeastern Antarctic Peninsula). *Extremophiles* 21: 187–200.
- Part I-5 Kopalová, K.\*, **Nedbalová, L.**, Nývlt, D., Elster, J. & van de Vijver, B. (2013): Diversity, ecology and biogeography of the freshwater diatom communities from Ulu Peninsula (James Ross Island, NE Antarctic Peninsula). *Polar Biology* 36: 933–948.
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- Part I-10 Řezanka, T., **Nedbalová, L.**, Lukavský, J., Strížek, A.\* & Sigler, K. (2017): Pilot cultivation of the green alga *Monoraphidium* sp. producing a high content of polyunsaturated fatty acids in low-temperature environment. *Algal Research* 22: 160–165.

### Chapter II. Snow algae

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- Part II-2 Lukavský, J., Furnadzhieva, S. & **Nedbalová, L.** (2009): First record of cryoseston in the Vitosha Mts. (Bulgaria). *Nova Hedwigia* 88: 97–109.
- Part II-3 **Nedbalová, L.** & Sklenář, P. (2008): New records of snow algae from the Andes of Ecuador. *Arnaldoa* 15: 17–20.
- Part II-4 **Nedbalová, L.**, Kociánová, M. & Lukavský, J. (2008): Ecology of snow algae in the Giant Mountains. *Opera Corcontica* 45: 59–68.
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- Part II–11 Jehlička, J., Culka, A. & **Nedbalová, L.** (2016): Colonization of snow by microorganisms as revealed using miniature Raman spectrometers – possibilities for detecting carotenoids of psychrophiles on Mars? *Astrobiology* 16: 1–12.

### Chapter III. Acidic habitats

- Part III–1 Hrdinka, T., Šobr, M., Fott, J. & **Nedbalová, L.** (2013): The unique environment of the most acidified permanently meromictic lake in the Czech Republic. *Limnologia* 43: 417–426.
- Part III–2 Barcyte, D.\* & **Nedbalová, L.** (2017): *Coccomyxa*: a dominant planktic alga in two acid lakes of different origin. *Extremophiles* 21: 245–257.
- Part III–3 Barcyte, D.\*, **Nedbalová, L.**, Culka, A., Košek, F. & Jehlička, J. (in press): Burning coal spoil heaps as a new habitat for the extremophilic red alga *Galdieria sulphuraria*. *Fottea*, doi: 10.5507/fot.2017.015.
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- Part III–5 Moravcová, A.\*, Rauch, O., Lukavský, J. & **Nedbalová, L.** (2013): The response of epilithic diatom assemblages to sewage pollution in mountain streams of the Czech Republic. *Plant Ecology and Evolution* 146: 153–166.
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