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2010 **Kateřina Černá**

Charles University in Prague, Faculty of Science

Department of Botany

Spatial variability and ecology of phytobenthic algal assemblages in peat bogs

Kateřina Černá

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Supervised by: **Dr. Jiří Neustupa**, Department of Botany, Charles University in Prague

Contents

List of papers

This thesis is based on the following four papers, referred to in the text as Papers 1-4:

1. Spatial distribution of algal assemblages in a temperate lowland peat bog
Kateřina Machová-Černá & Jiří Neustupa
International Review of Hydrobiology (2009), 94: 40-56

2. Small-scale spatial variation of benthic algal assemblages in a peat bog
Kateřina Černá
Limnologica (2010), DOI 10.1016/j.limno.2009.11.015 (in press)

3. The pH-related morphological variation of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic
Kateřina Černá & Jiří Neustupa
Aquatic Ecology, (2010), 44: 409-419

4. Diversity and morphological disparity of desmid assemblages in Central European peatlands
Jiří Neustupa, Kateřina Černá & Jan Šťastný
Hydrobiologia (2009), 630: 243-256

Author's contribution
Paper 1. Kateřina Machová-Černá and Jiří Neustupa planned the study jointly, Kateřina Machová-Černá conducted the sampling, species identification, data analyses, and wrote the paper. Jiří Neustupa helped with data analyses and the final revisions of the manuscript.
Paper 3. Kateřina Černá planned the study and wrote the paper. Jiří Neustupa helped with analysis of the geometric morphometric data.
Paper 4. Study was planned jointly. Jiří Neustupa wrote the paper, and conducted the data analysis. Kateřina Černá helped with data sampling, morphometric analyses and data processing; Jan Šťastný contributed to data sampling and species identification.

I hereby declare that I completed this thesis independently, using the listed references, or in cooperation with co-authors of the papers. I did not submit this thesis, nor any part of it to acquire any other academic degree.

As the published co-authors, we, Jiří Neustupa and Jan Šťastný declare the major contribution of Kateřina Černá (Machová) in completing the research and writing the papers, as described above.

…… ……
Jiří Neustupa Jan Šťastný

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1 General introduction

1.1 Phytobenthos in freshwater habitats

Benthic algae play many important roles in freshwater habitats: they are important primary producers in aquatic ecosystems, they behave as chemical modulators as they transform many inorganic chemicals into organic forms (Stevenson, 1996). Moreover, the benthic algae stabilize substrata and could also serve as important habitats for many other organisms. Benthic algal communities in freshwater habitats are mainly dominated by cyanobacteria, green algae, diatoms and red algae. In addition, resting stages and settled cells (capable of photosynthesis) of planktonic algae can be found in the benthos (Poulíčková et al., 2008b). Resting stages can remain viable in sediments for many years (Jewson, 1992; McQuoid and Hobson, 1995; Hašler et al., 2004; Poulíčková et al., 2008a) and, therefore, represent a potential future microautotrophic "stock". Although these algal groups show great evolutionary, genetic, and chemical differences, they share many of the same growth forms, which include: single cells, colonies, and filaments (Stevenson et al., 1996). Obviously, the horizontal and vertical distribution of benthic algae is strongly influenced by the presence of illuminated substrates. In comparison with macrophytes and phytoplankton that could occupy the entire water column, benthic algae are confined to a relatively thin surface sediment layer, within which the concentration of cells can be very high (Wetzel, 1983). Organisms on underwater substrates form heterogeneous and complex associations, and colonize almost all types of substratum in the littoral zone (see, e.g. Poulíčková et al., 2008a). There have been many schemes developed characterizing the benthic habitat of lentic ecosystems that are generally based on light availability to the benthos and are defined by two key regions: (1) the littoral (or photic) zone, and (2) the profundal zone, where light levels are insufficient for primary benthic production. The terms trophogenic and tropholytic are also used, implying an upper (autotrophic), and a lower (heterotrophic) zone, dominated by secondary production and associated with re-mineralization of organic matter, respectively (Round, 1981). A similar scenario exists within rivers and streams (Stevenson et al., 1996). In these systems, the presence of flow and low nutrient capacities of the sediments ensures the fluctuations of habitat parameters. In addition, a stretch of several rivers or streams contains many localized habitat zones, including slow flowing channels and pools, and fast flowing rapids and riffles that elevate the complexity of these systems (Stevenson, 1996).

The high number of algae that make up the autotrophic or mixotrophic components of microbenthos can be separated by their life traits and preferred substrata. Westlake (1980) distinguishes between three groups of phytobenthic communities. One is attached to different substrates and forms dense belts, including young, old and dead cells. The second group consists of numerous filaments or lumps of gelatinous material. The third group includes communities whose members are not strongly attached to the substratum. They are not aggregated and move freely over the substratum, generally the bottom sediments. A summary of the definitions of benthic algal communities, following the terminology published by Round (1956, 1981) and Margalef (1960), and similar to that of Westlake, are presented in Figure1.

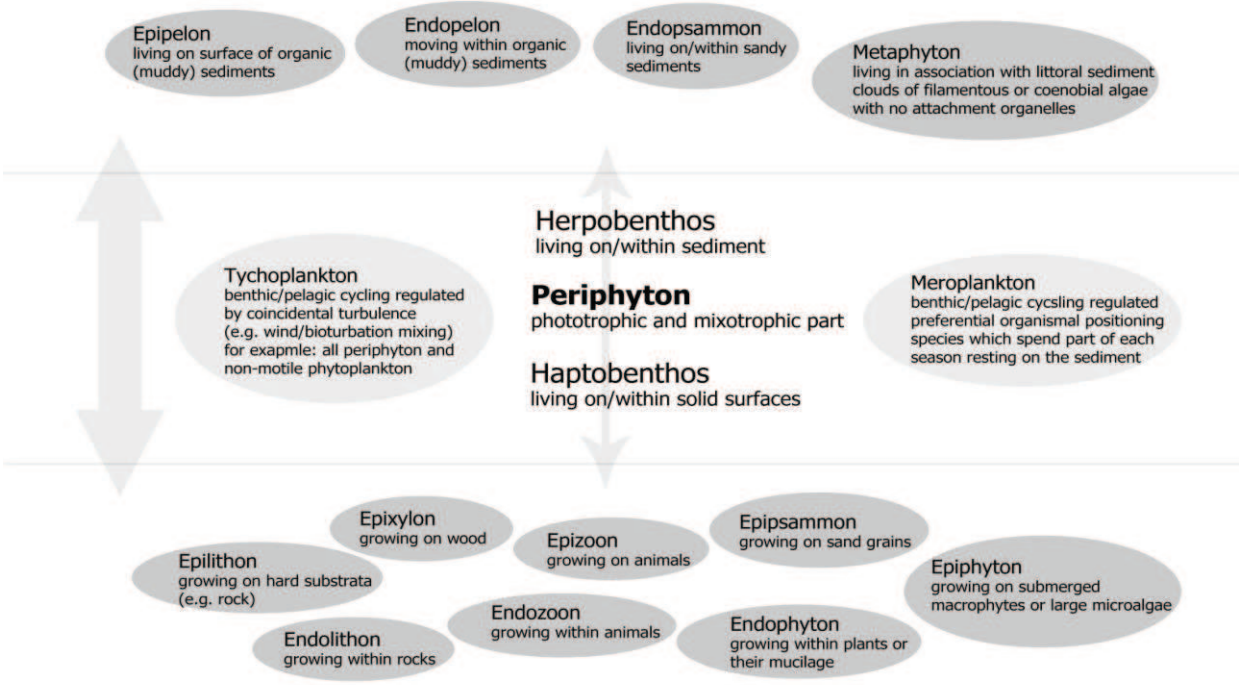

Fig. 1 Schematic showing of benthic algal communities categorised by their preferred substrate. (Amended from Poulíčková et al., 2008b)

Epipelic and epipsammic taxa mostly form mats or films on silt and mud bottoms, and they are typically motile and easily swept away by an increase in current. Epiphytic taxa occur on macrophytes, especially angiosperms, where an epiphytic coating can be detrimental to the host plant (Allan and Castillo, 2007). Unlike epipelic species, epiphytic and epilithic taxa are usually firmly attached by mucilaginous secretions, or via a basal cell and stalk, and thus, they are less likely to be dislodged by the current. Some algal species are in contact with the substrate along the entire cell wall, colony, or filamentous system. This growth form is termed adpressed, and contrasts with erect forms, in which only a basal cell or basal mucilage

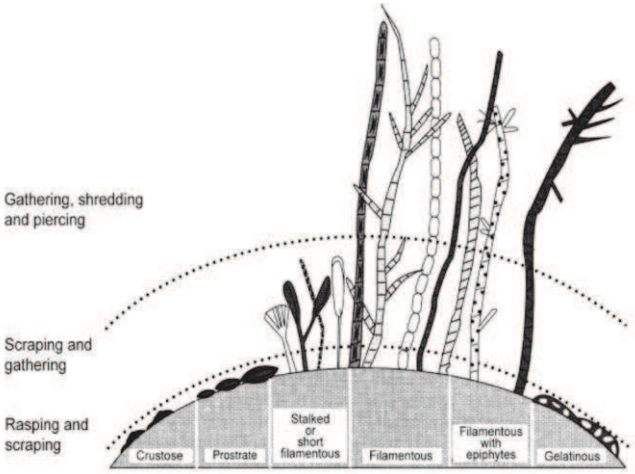

Fig. 2 Hypothetical representations of major growth forms of periphyton assemblages. Different modes of herbivory are expected to be most effective with particular growth forms. (Reproduced from Steinman, 1996)

contacts the substrate (Allan and Castillo, 2007; Fig. 2). Some organisms exist within the benthos and the water column, with migration between the two depending on a number of factors including life histories and susceptibility to resuspension (Poulíčková et al., 2008b). These organisms can be categorized as meroplankton, tychoplankton, or metaphyton (Fig. 1). Meroplanktonic migration is driven by preferential organismal positioning, e.g. diurnal migration (Hansson, 1995) and grazer avoidance (Hansson, 1993); tychoplanktonic distribution is coincidental, i.e. driven by disturbance events (Schallenberg and Burns, 2004); and metaphyton distribution has been strongly linked to acidification (Turner et al., 1995).

The life of phytobenthos in freshwater habitats is driven by many abiotic factors that influence the biomass, diversity and composition of benthic algal assemblages. In streams and rivers the immediate factors that may impact benthic algae involve: light, temperature, water current, substrate, scouring by floods, water chemistry, and grazing (Allan and Castillo, 2007). Biggs (1996) proposed that the features linked to the region and catchment area such as topography, geology, land use, vegetation, and climate affect these proximate variables that consequently regulate the accrual and loss of benthic algal biomass. The level of

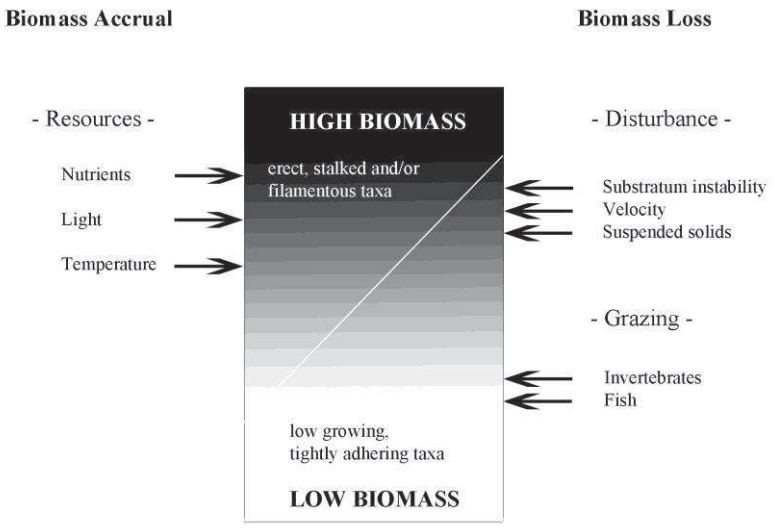

Fig. 3 Summary of the disturbance-resource supply-grazer concept for the control of benthic algal development in streams. The relative balance of "biomass accrual" and "biomass loss" processes is depicted by the width of the triangles that make up the central rectangle. The physiognomy of the community likely to dominate each end of the gradient is also shown. (Reproduced from Biggs, 1996)

resources (light and nutrients), interacting with temperature, influences rates of metabolism and growth, while disturbance (substrate instability and transport, high current velocities) and grazing lead to the dislodgement of algae and biomass loss (Allan and Castillo, 2007). Frequency and intensity of floods can affect processes of colonization and growth, as it influences the availability of propagules, nutrient concentration, water clarity, stream geomorphology, baseflow velocities, substratum size, and density of grazing invertebrates (Biggs, 1996).

In lakes, environmental factors similar to those affecting phytobenthic algal assemblages in streams and rivers could be described: light, turbulence, water chemistry and grazing pressure (Lowe, 1996). Lentic periphyton communities are composed of both autotrophic (algae) and heterotrophic (fungi and bacteria) components that constitute tightly linked and highly structured communities. The algal communities are usually dominated by diatoms, green algae, and cyanobacteria, with occasional occurrence of red algae (Lowe, 1996). The upper eulitoral zone is often dominated by green algae and diatoms, while the sublitoral zone is occupied by cyanobacteria and diatoms (Loeb and Reuter, 1981; Fig. 4). The quantity and composition of algal assemblages is also related to the basin morphometry, as deep lakes with steep sides provide fewer periphyton habitats compared to shallow lakes with gradual slopes. Algae inhabiting the eulitoral zone that are subjected to high-energy wave disturbance are capable of attaching tightly to the substratum, and these areas are inhabited by many species that can be found both in streams and lakes. On the other hand, algal communities that populate the microhabitats below the maximum penetration of the summer thermocline contain a distinct subset of lentic periphyton rich in unique species (Lowe, 1996). The abundance of nutrients plays a strong role in determining the quantity, quality and distribution of periphyton. The inorganic sources originate from atmospheric depositions, surface or subsurface inflow or from sediment release. When the nutrients are abundant, periphyton is light-limited rather than nutrient-limited, due to the subsequent

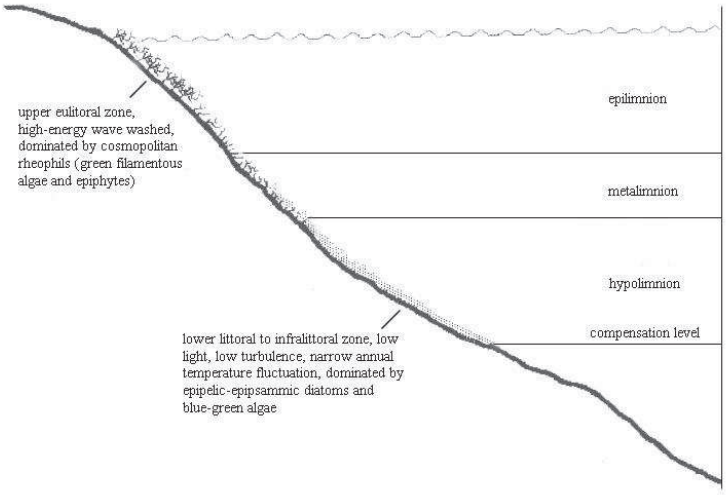

Fig. 4 Periphyton distribution and habitat differences through depth in a thermally stratified lake. (Reproduced from Lowe, 1996)

proliferation of phytoplankton (Hansson, 1992). Also in lakes, disturbance can influence periphytic algal communities, and it can be divided into disturbances that are abiotic mechanical, including turbulence and abrasion; abiotic chemical, involving toxic chemicals; or biotic interference in the form of grazing, i.e. direct consumption or dislodgement of cells from the substratum, or via movement of heterotrophs (Lowe, 1996).

In wetlands, the most important factor is water depth that affects all the other factors (Kadlec, 1979). Shallowness can result in extensive reworking and resuspension of sediments by wind causing high turbidity, and resulting in a water column with dissolved nutrients thoroughly mixed throughout (e.g. Carper and Bachmann, 1984). In addition, in the shallow water the differences in temperature with depth are minor, but the environment of the wetland is less buffered from seasonal temperature changes (Goldsborough and Robinson, 1996). Regular or periodic water level fluctuations affect the availability of substratum and lead to the development of specific algal assemblages. Nutrients come as efflux from the sediments or along with water input from the adjacent lakes, rivers or streams (Goldsborough and Robinson, 1996). The resuspension of sediment by winds increases the turbidity of the water column and decreases the irradiance penetrating the water column. The subsurface light environment changes frequently, and varies with the velocity and direction of prevailing wind, and by the abundance of macrophytes or periodic disruptions of the bottom by benthivorous fish (e.g. Klarer and Millie, 1992). The occurrence and abundance of algae is affected by a high surface area of submersed and emergent macrophytes with their numerous roles in wetland habitat: they provide colonizable substratum for algae, they modify the aquatic light regime, provide both sources and sinks of nutrients and allelochemicals (Elakovich and Wooten, 1989), cause physical abrasion of algal assemblages, habitat for grazers, stabilization of bottom sediments and reduction of turbidity. Wetlands serve as spawning and feeding grounds for numerous vertebrates (planktivores, herbivores, detritovores) promoting resuspension of sediments, destruction of submersed and emergent macrophytes and providing sources and sinks of nutrients (Goldsborough and Robinson, 1996). Using models, there are predicted four quasistable states in wetlands dominated, alternatively, by epipelon, epiphyton, metaphyton, or phytoplankton (Robinson et al., 1996; Fig. 5). The Dry State follows a period of drought or drawdown and is typical with very low water level with dominant epipelic algae (Fig. 5). The biomass of aquatic macrophytes is low in this period but the restricted flow leads to litter accumulation which gives rise to a nutrient-rich environment with high irradiance due to water shallowness (Goldsborough and Robinson, 1996). After flooding, two states can occur depending on the quantity and timing of the water

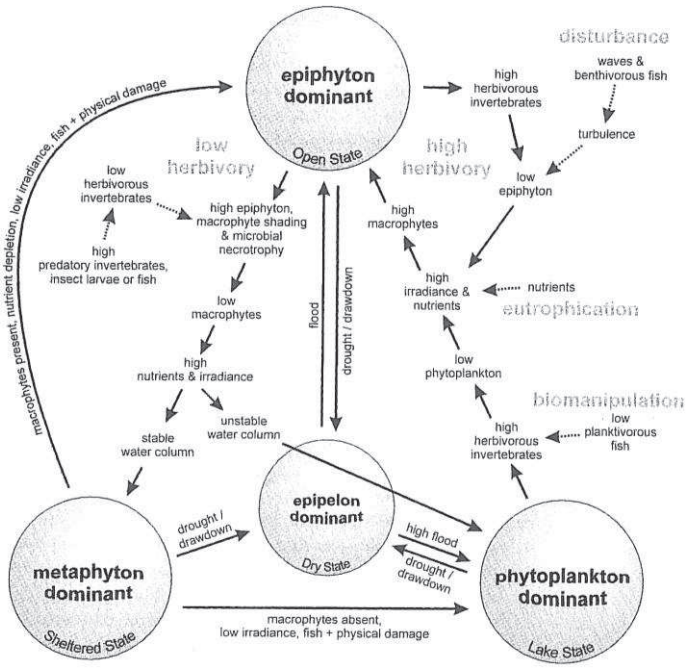

Fig. 5 Predicted trophic interactions occurring in freshwater wetlands, leading to one of four stable states dominated, alternately, by epipelon, epiphyton, metaphyton, or phytoplankton. (Reproduced from Goldsborough and Robinson, 1996)

input. If the water input is slow and shallow the development of aquatic macrophytes occur and these macrophytes serve as colonizable habitats for predominant algal epiphytes (Fig. 5). This state is called Open State and is also characterized by a turbulent water column with variable water column transparency and increased nutrient supply from newly flooded soil (Kadlec, 1979; Schoenberg and Oliver, 1988). Periodic physical disturbance, due to benthivorous fish and other fauna, wave action, and grazing pressure, is an important factor for maintenance of epiphyton dominance (Losee and Wetzel, 1983; Neely, 1994). Conversely, if the water input is too rapid for macrophytes to colonize, or if vegetations is excluded by deep water, a Lake State will occur, which is distinguished by high water and nutrient levels, and a turbid water column with low transparency. The lack of macrophytes as possible colonizable substrates leads to the decrease of epiphyton, and increased wind action leads to the elimination of metaphyton. Consequently, due to reduced competition for nutrients, phytoplankton dominates a Lake State wetland (Goldsborough and Robinson, 1996; Fig. 5). The conditions that typify a Sheltered State wetland are high nutrient loading and a stable water column, as a consequence of shelter provided by macrophytes or by bordering vegetation. A large development of epiphyton leads to the formation of dense mats of metaphyton that carpet the bottom and water surface (Goldsborough and Robinson, 1996; Fig. 5).

A pelagic-based paradigm has historically dominated lacustrine research (Vadeboncoeur et al., 2003). Due to spatial segregation, benthic habitats have often been considered functionally independent from pelagic environments (Carpenter et al., 1996) with parallel communities of microbes, and primary producers and consumers (Vadeboncoeur et al., 2002). However, many recent food web analyses indicate that both benthic and planktonic phytomass

contribute directly or indirectly to the energetic requirements of multiple zooplankton (Rautio and Vincent, 2007) and fish taxa (Bootsma et al., 1996; Vander Zanden and Vadeboncoeur, 2002), and there are some indications that benthic primary production may be more efficiently transferred to higher trophic levels than pelagic phytomass (Hecky and Hesslein, 1995).
The role of benthic primary production is largely unstudied (Lowe, 1996). From the research of Vadeboncoeur et al. (2002), who searched the BIOSIS literature database for publications from 1990 to 1999 to get a representative sample of the frequency with which the two habitats were studied, it was discovered that limnologist measured the phytoplankton productivity 10 times more often than the phytobenthos productivity (Vadoboncoeur et al., 2002). Of 193 studies in which primary productivity in lakes was measured, 91% were concerned with phytoplankton productivity alone, 4.5% only phytobenthos productivity, and 4.5% primary productivity in both habitats (Fig. 6a). Bacterial productivity was reported in 53 papers, of those 89% measured bacterioplakton productivity, 7.5% measured only benthic bacterial productivity, and 3.5% measured both benthic and planktonic bacterial productivity (Fig. 6b). Of 59 studies quantifying invertebrates in lakes, 73% made measurements for only zooplankton biomass or productivity, 15% only for zoobenthos, and 12% for invertebrates in both habitats (Vadeboncoeur et al., 2002; Fig. 6c). The literature shows that benthic organisms can contribute substantially to whole-lake production, that there are common energetic links across habitat boundaries, and that the importance of benthic communities is a function of lake size (Vadeboncoeur et al. 2002). Benthic primary production is a potential source of autochthonous production available to consumers and heterotrophic bacteria. Although the distribution of production between benthic and pelagic habitats within any producer group may vary along water-column nutrient gradients, it will also depend on the relative size of each habitat. It has long been recognized that benthic and pelagic primary producers have the potential to compete for light and nutrients (Sand-Jensen and Borum, 1991). However, the perception that a phytoplankton gradient is equivalent to an ecosystem-level primary productivity gradient

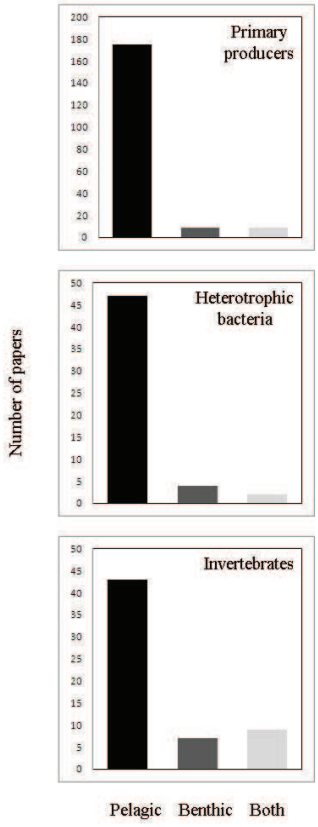

Fig. 6 Frequency of publication on benthic versus pelagic habitats for primary producers, heterotrophic bacteria and invertebrates base on the publications from 1990 to 1999. (Reproduced from Vadeboncoeur et al., 2002)

persists in the absence of any comprehensive analysis of periphyton responses to eutrophication in lakes. Experimental and comparative evidence has shown that: (1) phytoplankton sequester water column nutrients more rapidly than periphyton because periphyton uptake is constrained by boundary layer kinetics (Riber and Wetzel, 1987; Reuter and Axler, 1992); (2) periphytic algae have access to sediment-associated nutrients and regulate availability of those nutrients to phytoplankton (Hansson, 1990); and (3) phytoplankton attenuate light, limiting periphyton production (Hansson, 1992). These competitive interactions set up the possibility for inverse relationships between these two primary producer functional groups across eutrophication gradients (Sand-Jensen and Borum, 1991; Havens et al., 2001), and fertilization experiments demonstrate a compensatory decline in periphyton production in response to increased phytoplankton biomass (Vadeboncoeur et al., 2001). Phytoplankton and periphyton productivity are expected to be positively related to the availability of water-column nutrients. However, at a certain point periphyton productivity is expected to decline because phytoplankton blooms reduce light penetration to benthic surfaces. Although there is a general transition from benthic to pelagic domination of primary productivity along the eutrophication gradient, alternate stable states can occur at intermediate nutrient concentrations (Scheffer et al., 1993). At a given TP concentration, shallow lakes with large littoral zones can alternate between clear-water states with high densities of macrophytes and periphyton and low phytoplankton concentration, and turbid states with low macrophyte density and high levels of phytoplankton (Scheffer et al., 1993).

1.2 Spatial distribution pattern of algal assemblages

Spatial variation in distribution, abundance and composition of species is important for our understanding of the diversity patterns of biological communities. In particular, knowledge of the spatial structure of benthic assemblages underlies the understanding of ecological processes such as succession, colonization, niche differentiation, and competition (Ricklefs and Schluter, 1993). Moreover, identifying the spatial pattern of species composition in water ecosystems is crucial for evaluation of their biodiversity and formulation of conservation strategies (Coleman, 2002). Therefore, spatial analysis has recently become a rapidly growing field in benthology and aquatic ecology.

The factors influencing algal distribution could be also related to spatial scale, and we are able to identify general factors that are significant for individual spatial scales in marine and freshwater habitats. Saburova et al. (1995), who studied spatial distribution of sandflat microphytobenthic communities, defined the main factors relating to scale as: on a microscale

(up to $2m^2$), biotic interspecies interactions are the most important; on the mesoscale (up to $18m^2$), distribution is mainly determined by the granulometric composition and a complex of abiotic conditions in the sediments; and on the macroscale (up to $10{,}000m^2$), distribution depends upon the emersion period during low tide. Similar results were obtained by Méléder et al. (2007) who studied microphytobenthic assemblages of a macrotidal flat. They found that hydrodynamics related globally to the occurrence of spatio/temporal biotic or abiotic gradients, whereas oyster beds, and ridge and runnel features appeared to be local spatial structuring factors. To the macroscale´s structuring factors could be also added the texture and composition of rocky substratum according to findings of Rindi and Batelli (2005). In freshwater habitats, the differences in species composition on a macroscale (kilometers and greater) largely correlate with basic chemical variables (e.g. hardness, Mg, Ca, conductivity, SO_4, total solids, conductivity, total P), geographic location and spatial-dispersal factors (Soininen and Könönen, 2004; Soininen et al., 2004; Charles et al., 2006).

On a mesoscale (tens of meters or greater), spatial heterogeneity of algal assemblages seems to be determined by several factors, including: marine habitat abiotic conditions, granulometric composition of substrate (Saburova et al., 1995), phenological patterns and input of propagules (Santelices,1990; Rindi and Guiry, 2004), variations in recruitment (Menge et al., 1993), and substratum heterogeneity (Archambault and Bourget, 1996). In rivers, factors related to water quality, such as BOD (biological oxygen demand), P, NH_4 and turbidity were the most important pressures driving heterogeneity of algal assemblages on an intermediate spatial scale (Charles et al., 2006). In addition, heterogeneity of diatom communities was shown to be induced by changes in light and current regimes, grazing, successional stages and variation in substratum (Peterson and Stevenson, 1989; Ledger and Hildrew, 1998; Soininen, 2003).

Small-scale biotic interactions, such as competition, grazing, colonization processes (input of propagules), variations in recruitment, or low movement ability affected composition of algae species both in marine and freshwater habitats on a scale of centimeters or greater (e.g. Saburova et al., 1995; Rindi and Cinelli, 2000; Hillebrand et al., 2001; Coleman, 2002; Rindi and Batelli, 2005). Coleman (2002) examined the high variation in assemblages of marine turfing algae on a small spatial scale (10cm). These findings, which were temporally consistent, appeared to be due to small-scale ecological processes. Rindi and Cinelli (2000) and Rindi and Batelli (2005) discussed the importance of small-scale differentiation in Mediterranean intertidal algal assemblages. They stressed the importance of substratum

heterogeneity, as well as colonization processes (input of propagules), competition and variation in recruitment.

At the same time, physical and chemical parameters creating small-scale patchiness of microhabitat (Underwood and Chapman, 1996), substrate complexity and heterogeneity (Komárek, 2003), and water current effects (Passy, 2001) also play an important role. Archambault and Bourget (1999) found increasing abundance of benthic marine algae, from smooth to rough, and more heterogeneous surfaces, on small scales. It was revealed by Benedetti-Cecchi (2001) that a significant proportion of horizontal variation in marine littoral benthic algal and invertebrate communities could be explained on the scale of 10s of centimeters, and related to physical processes of the environment. Passy (2001) revealed current velocity as the major factor controlling diatom distribution in streams, and only a minor influence for other, mostly biotic, factors in shaping diatom communities. Conversely, Soininen (2005) reported that the current velocity did not strongly shape diatom communities in turbid rivers. Soininen and Könönen (2004), also studied diatom benthic communities in many boreal streams and found that environmental factors, principally conductivity, total phosphorus content, and water color were related to algal distribution.

Knowledge of the spatial distribution of species in phytobenthos is important prior to identification and description of their diversity. In order to describe the ecology, occurrence patterns, or abundance of species of any assemblage, it is necessary to determine the scale of its variation. If, for example, small-scale variation goes undetected, differences due to impacts may be confused with differences due to natural spatial variability (Underwood, 1993; Coleman, 2002). That is, if the spatial scale sampled is greater than the scales of natural spatial variation then effects may be assumed that do not really exist; the perceived impact simply being a result of small–scale spatial variation (Coleman, 2002). Spatial patterns of species diversity provide important clues about the underlying mechanisms that regulate biodiversity and are central in the development of biodiversity theory (MacArthur and Wilson, 1967; Brown, 1995; Hubbell, 2001). Assumptions regarding the spatial scaling of biodiversity are a fundamental component of conservation biology, and are frequently used to identify local- and global-scale priority conservation areas (Desmet and Cowling, 2004; Ferrier et al., 2004).

1.3 Algae in a low pH environment

Wetlands are characterized by three major features: (1) shallow water or saturated soils, (2) anaerobic soils, and (3) unique flora and fauna adapted to environmental conditions in

wetlands. There are many different kinds of wetlands that are characterized by different hydrologies (source(s) of water and duration and timing of flooding), geomorphological setting (flats, basins, slopes, channels, etc.), vegetation (submersed aquatic beds, emergents, mosses, shrubs, trees), soils (mineral, peat), and water chemistries. Wetlands can be classified based on their hydrology, vegetation, and/or geomorphology and are found on every continent except Antarctica, and in almost every climatic zone on these continents (van der Valk, 2006; Fig. 7). Because no detailed inventories of the world's wetlands have ever been done, only

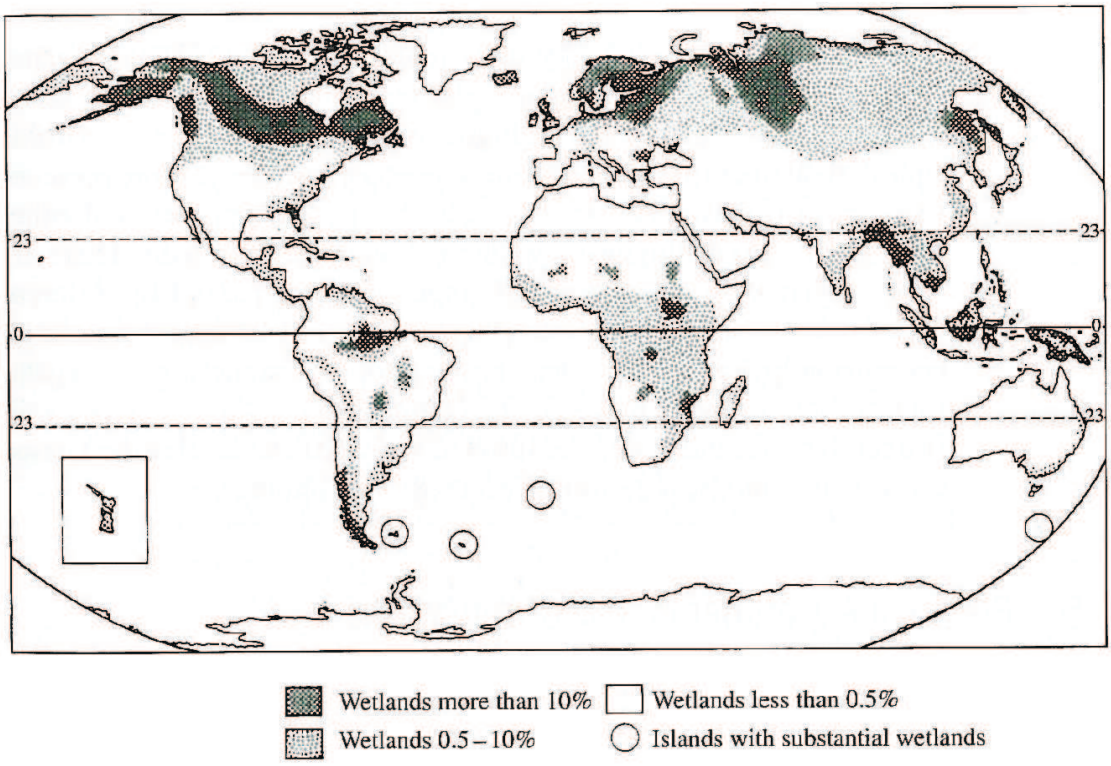

Fig. 7 Global distribution of wetlands. (Reproduced from Williams, 1990)

rather crude estimates of the total area of wetlands are available (Mitsch and Gosselink, 2000). The polar/boreal region is estimated to have about 2.5 x 10^6 km^2, the sub-boreal/temperate region about 1.0 x 10^6 km^2, and the subtropics/tropics 2.0 x 10^6 km^2, for a total of approximately 5.5 x 10^6 km^2 in the entire world; overall, it is estimated that about 5% of the land surface of the earth is covered with wetlands (van der Valk, 2006).

Peatlands represent special kinds of transitional, amphibious ecosystems with hydro-terrestrial habitats, where organic matter tends to accumulate because of the waterlogged, often poorly aerated, conditions (Rydin and Jeglum, 2006). Two complex environmental gradients are responsible for the distinction of main peatland types. One is linked to wetness and aeration, and the other is a combination of pH, calcium content, and base saturation

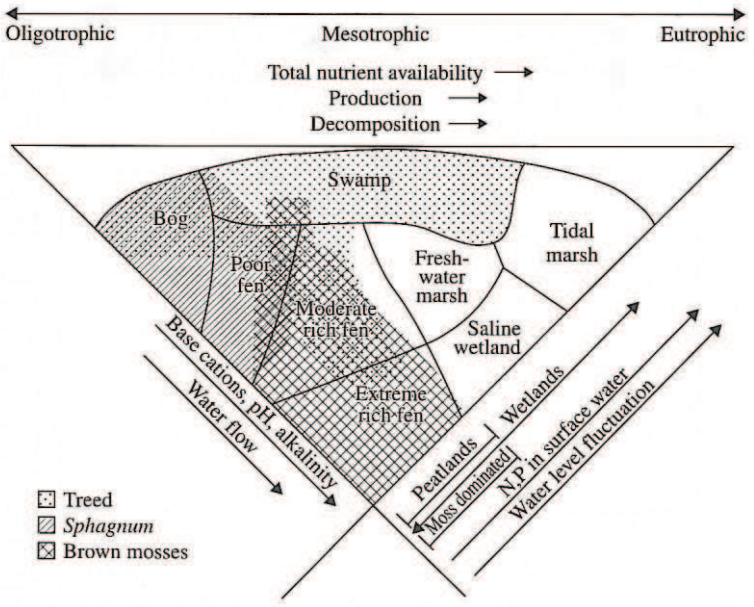

Fig. 8 The relationship between wetland types and major water chemistry, biotic, and hydrological gradients. (Reproduced from Zoltai and Vitt, 1995)

(Rydin and Jeglum, 2006; Fig. 8). The moisture-aeration regime depends on the position of the water table, on the pore structure of the peat, the fraction of the total pore spaces filled with water versus air, and on the oxygen content of the water. The chemical regime can be segregated into two factor groups. One is the variation in pH, linked also to electrical conductivity, calcium content, and base richness. The other is availability of plant nutrients. As in most terrestrial ecosystems, nitrogen is a key nutrient, but the scarcer phosphorus and potassium are more often limiting in peatlands than in mineral soils (Rydin and Jeglum, 2006). When the peat surface is connected with, or has passed over or through, mineral parent materials these sites are termed as minerogenous to indicate that water is added to the peatland from the surrounding mineral soil. Peatlands with a surface isolated from mineral-soil-influenced groundwater will receive water by precipitation only. These peatlands are called ombrogenous. To emphasize the chemical effects on the site we refer to minerogenous peatlands as minerotrophic, nourished by mineral soil groundwater. Correspondingly, we refer to ombrogenous peatlands as ombrotrophic, nourished by precipitation (and airborne dust; Vitt, 2006). Peatlands are found in every continent with the exception of Antarctica. The total peatland area according to data from the Global Peatland Database of the International Mire Conservation Group, which refers to areas with at least 30cm of peat thickness, is now estimated to be 4.16×10^6 km^2 (about 3% of the globe's total land cover; Rydin and Jeglum, 2006; Fig. 9). According to recent estimations, at least 80% of peatlands are in areas with northern temperate or cold climates, 15-20% are tropical or subtropical, and only a few are in temperate or cold climates of the southern hemisphere. Large tropical peatland areas are found in the Amazon basin, in south-east Asia (Indonesia, Papua New Guinea, and Malaysia), and in the Congo River basin. Russia and Canada each have roughly a third of the global

Fig. 9 Peatland area per country. The estimates are from the Global Peatland Database of the International Mire Conservation Group and refer to areas with peat depth >30cm. The circles in some large countries are not located at the actual largest peat areas, e.g. Alaska in the USA and Tierra del Fuego for Argentina. (Reproduced from Rydin and Jeglum, 2006)

peatlands, and together with the USA and Indonesia they contain 85% of all peatlands (Rydin and Jeglum, 2006).

According to environmental conditions, four high-level ecosystem classes were identified - *marsh*, *swamp*, *fen* and *bog* (National Wetland Working Group, 1997; Fig. 10). *Marshes* are characterized by standing or slowly mowing water with submergent, floating-leaved, or emergent plant cover. They are permanently or seasonally flooded with intermittent exposures. The rooting zone generally remains in nutrient-rich water for most of the growing season. Bottom surfaces may be mineral glacial drift, aquatic sedimentary deposits, or precipitates of inorganic compounds or organics. Marsh habitats have little peat, which means that most vascular plants are rooted in the underlying mineral soil from which they can take up nutrients. The principal complex factors influencing biotic variation within marshes are water level (flooding, drawdowns), and in some places disturbance by wave or current energy. Three physiognomic groups of marsh are distinguishable: *open water marsh*, *emergent marsh*, and *meadow marsh* (Rydin and Jeglum, 2006; Fig. 10). *Swamps* are forested or sometimes thicketed wetlands. They have minerogenous water that may come from watercourses or the underlying soil or lateral groundwater throughflow. They have standing or gently flowing water in pools or channels, or subsurface flow. The water table is usually well below the surface, so that the surface layer is aerated and supports the roots of trees or other tall woody

plants. Substrates are organic-mineral mixtures, or shallow to deep peat (in which wood is a large component). The most important complex factors within the swamp are nutrient regime, pH-base richness, moisture-aeration, and light. The main physiognomic groups of swamp are *conifer swamp forest*, *hardwood swamp forest* (deciduous or evergreen), and *thicket swamp* (Rydin and Jeglum, 2006; Fig. 10). *Fens* are minerotrophic peatlands with water table slightly

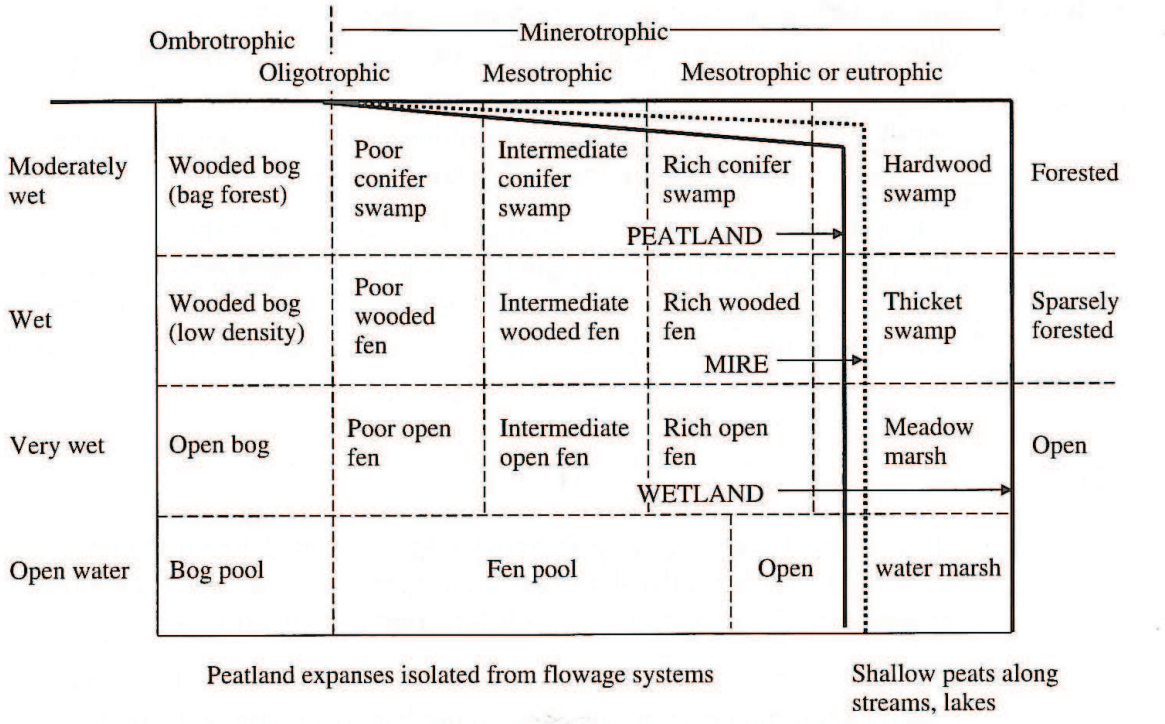

Fig. 10 A general scheme to define the position of broad wetland types in an ordination based on the two most important environmental gradients. Wetness, or distance between vegetation surface and water table, varies along the vertical axis, and the complex gradient with variation in pH, base saturation, and nutrient status is depicted along the horizontal axis. Wetland is an even broader category than shown here, since it includes various habitats of shore and shallow waters. (Reproduced from Rydin and Jeglum, 2006)

below, at, or just above the surface. Usually there is slow internal drainage by seepage, but sometimes with oversurface flow. Peat depth is usually greater than 40cm, but sometimes less. Two broad types are: *topogenous* fen with flat water tables that is located in terrain basins with no outlet, a single outlet, or both inlets and outlets; and *soligenous* fen that is sloping, with directional water flow through the peat or on the surface. The main complex factors are nutrient regime, pH-base richness, and moisture-aeration (and to some extent light). The various physiognomic groups of fen are *open fen* and *wooded fen* (with tree cover, or a sparse tall shrub cover; Rydin and Jeglum, 2006; Fig. 10). *Bogs* are ombrotrophic peatlands with the surface above the surrounding terrain or otherwise isolated from laterally moving mineral-rich soil water. Some bogs are convex in shape (raised bogs), but bogs can also be quite flat or sloping, with slight rises at the margin that isolate them from incoming

minerogenous water. The peat is usually more than 40cm deep. The main complex factors influencing biotic variation are moisture-aeration and light. Because they are nourished only through precipitation, there is less local chemical variation than among the fens. Bogs are extremely nutrient poor and strongly acidic. The main physiognomic groups are *open bog* and *wooded bog* (Rydin and Jeglum, 2006; Fig. 10).

Among aquatic ecosystems, peat bogs are rare environments, the development of which requires particular climatic conditions: low temperature, high humidity and high, evenly distributed precipitations (Mataloni, 1999). According to Wetzel (1981), the microflora of peat bogs is especially interesting because of its remarkably high diversity and its degree of adaptation to acidic conditions. By its peculiar chemistry *Sphagnum* has the ability to acidify the substrate, to survive in extremely nutrient-poor habitats, and to resist decay. As the *Sphagnum* plant grows it continuously creates cation-exchange sites. The uronic acids located at these sites release hydrogen ions, and instead cations in the mire water are taken up, which leads to acidification of the environment. Uronic acids and a number of phenolic compounds contribute to decay resistance in *Sphagnum* (Verhoeven and Liefveld, 1997). Peat bogs are typical for specific environmental conditions, among which pH is a key factor related to low conductivity and availability of nutrients, influencing occurrence and diversity of microorganisms populating these habitats. These specific conditions lead to physiological and morphological adaptations of organisms that allow them to cope with this environment.

A pH of 4.0 is a physically stressful habitat for many taxa due to such factors as increased metal toxicity or reduced bicarbonate availability, the latter being an important source of carbon for algae. Also, phosphorus concentrations are lower in an acidic environment, potentially affecting taxa richness (Pals et al., 2006). Highly acidic pH can be damaging to cell walls as it weakens hydrogen bonds in the cellulose strands comprising the walls, and can result in uncontrolled cell expansion (Gross, 2000). In addition, cells in these conditions may be placed under stress by the need to maintain the neutral pH of their cytoplasm as the H^+ ions continuously penetrate their plasmatic membrane (Gimmler and Weis, 1992). Several adaptive physiological mechanisms of microalgae living in low pH conditions were suggested (Gimmler, 2001; Gerloff-Elias et al., 2005), e.g. maintaining a positive membrane potential and a positive charge outside the plasmatic membrane (Remis et al., 1994), decreasing permeability of protons through the plasmatic membrane, or sustaining active proton pumping (Gross, 2000; Gimmler, 2001).

Additionally, morphological adaptations of microalgae to conditions of extremely low pH were also proposed (Nixdorf et al., 2001). Generally, cells need to have low surface-to-

volume ratios (S/V ratio) in order to minimize the stress posed by H^+ ions penetrating their walls and membranes. Clonal strains of protists with versatile cell shape are known to rapidly manipulate their S/V ratios by expanding their cells (Weisse and Stadler, 2006; Weisse et al., 2007). Similarly, the microalgal strains with a cell wall may simplify their cell morphology in subsequent generations in order to adaptively manipulate their S/V ratio (Neustupa and Hodač, 2005; Neustupa et al., 2008). The morphologically elaborate cells of many desmid species have high S/V ratios in comparison to other microalgae (Padisák et al., 2003). This feature has been considered advantageous in oligotrophic conditions as it maximizes the surface area available for nutrients uptake (Coesel, 1982). However, at the same time Coesel (1982) mentioned that desmids inhabiting highly oligotrophic and acidic localities (e.g. elevated boreal peat bogs) tended to have comparatively lower S/V ratios than desmids from assemblages in localities with higher pH. In extremely acidic conditions, the pH may act as the critical environmental factor driving shape features of desmid cells. Species with a lower S/V ratio may have higher fitness under low pH conditions, because of their lower surface area and reduced exposure to the acidic environment. However, pH of natural localities varies as a result of different biotic and abiotic processes (Rydin and Jeglum, 2006). Photosynthesis and respiration of algae and other photosynthetic organisms cause diurnal changes in water pH and carbon/oxygen availability. During respiration CO_2 is released into the water, causing the pH to decline. Conversely, during photosynthesis CO_2 is utilized by photosynthetic organisms and the pH increases (Odum, 1956; Edwards and Owens, 1965). Also, pH levels are often variable within one locality. This variability is influenced by the moisture-aeration regime (Rydin and Jeglum, 2006), or microhabitat types in the area (e.g. tychoplanktonic communities of the open water of peat-pits, benthic communities of the water-filled shallow hollows, or emergent communities in mossy vegetation; Coesel, 1982; Mataloni, 1999).

1.4 Application of geometric morphometrics in biology

Currently, geometric morphometrics (GM) has became an important investigative tool for the characterization and illustration of morphological variation and quantifying of the plasticity of individual species or populations. In contrast to conventional morphometric methods, geometric morphometric data are able to represent variation in shape as a whole in an investigated data set. The higher statistical power of geometric morphometric methods compared with traditional measurements to discriminate biological objects has been demonstrated many times in different organisms (e.g. Rohlf, 2000; Monteiro et al., 2002;

Beszteri et al., 2005). And more recently, geometric morphometrics have been widely utilized in taxonomy, ecology and distribution of various groups of organisms.

Taxonomical studies used geometric morphometric data to discriminate between traditionally or confusingly delimited species, very often in relation to phylogenetic data. In algal taxonomy geometric morphometrics methods have became a powerful tool able to record the morphological variation that was previously undetectable using traditional morphometric data and under optical, and eventually electron, microscopy. There are only a few studies concerning algal taxonomy. Beszteri et al. (2005) used conventional and geometric morphometric approaches to clarify the taxonomic identity of a centric diatom morph, which showed an intermediate valve morphology between that of typical specimens of *Cyclotella* (*C. meneghiniana* Kützing and *C. scaldensis* Muyleart & Sabbe). The morphometric analyses were used to determine: (1) whether morphological variation of these morphs was continuous, or whether there were distinct morphological groups, and (2) how effective the alternative morphometric approaches were in answering this question. Both approaches proved informative, and their results complemented each other, supporting the conclusion that three distinct size-reduction series were present in the samples investigated. Because the different morphs occurred sympatrically, the authors suggest that they probably belong to three reproductively isolated species. Neustupa and Němcová (2007) studied the patterns of variation in the shape of silica scales of the freshwater algal flagellate *Malomonas striata*. Two data sets were investigated: individual worldwide reported scales and the scales of a single population from Trnová pond in the Czech Republic. Striking similarity of the two morphospaces was revealed indicating the importance of variation related to the position of individual scales on the cell body. There was a higher variability in the worldwide scales set, and the variation in scale morphology in relation to infraspecific identification was revealed. Verbruggen et al. (2005) presented a combined molecular and morphometric approach toward *Halimeda* taxonomy using a selection of specimens representing the five natural lineages within the genus. A broad range of anatomical structures was measured. Molecular data were used to delimit species groups. Segment morphological characters proved fairly good predictors for species membership, but anatomical variables yielded the best results. The good performance of morphometric taxon predictors offers perspectives, not only for future taxonomic case studies within problematic species complexes, but also for thorough examinations of the rich fossil record of *Halimeda*. Kynčlová et al. (2010) used a polyphasic approach to clarify the species concept of *Synura petersenii* utilizing ITS regions analysis of clonal cultures from different localities. This analysis divided strains into six groups that were

also confirmed by CBCs and hemi-CBCs analysis. Furthermore, a morphological analysis revealed unambiguous differences in features of the scale structure among these six clades. Along with historical taxonomic information, Kingston and Papas (2009) used shape analysis as a tool in determining taxon identifications of *Fragilariforma constricta*, *F. constricta f. stricta*, and *F. lata*. Results from shape analysis indicated that *Fragilariforma constricta/F. constricta f. stricta* shape groups may encompass two different species. *Fragilariforma lata* shape groups also may comprise two different species. Conventional and geometric morphometric methods were applied on sympatric nature populations of two pennate diatom species *Reimeria sinuata* and *Gomphonema tergestinum* (Fránková et al., 2009). Although both species differ in their autecology and distribution, they occurred at the same sites and exhibited high morphological variation. Landmark-based geometric morphometrics provided better discrimination of the species, correlating with their traditional taxonomic delimitation and type material. Pichrtová and Němcová (2008) examined morphological variation of silica-scales in four *Mallomonas* species (*M. calceolus, M. kalinae, M. flora* and *M. striata*). The main trends in morphological variation were associated with the width, length and shape of the V-rib, while the proximal border region was found to be more stable. Geometric morphometric-based analyses did not corroborate the classification of these species into traditionally defined sections. Neustupa and Šťastný (2006) studied 14 central European species of the genus *Micrasterias*. All the analyzed cells were correctly placed in their appropriate species clusters on the basis of geometric morphometric data. The width of the polar lobe associated with depth of the incisions between lateral lobules was the dominant morphological trend in the data investigated. Another study concerning the genus *Micrasterias* was performed by Neustupa and Škaloud (2007), who conducted GM analyses on cultured populations of five *Micrasterias* species together with the 18S rDNA sequence analyses of these species. The phenetic comparisons demonstrated the overall great similarity of morphometric indicators extracted from isolated polar lobe data and 18S rDNA genetic distances. The phylogenetic analysis revealed clustering of the *Micrasterias* sequences into two clades, which correspond to qualitative patterns in the morphological variation of isolated polar lobe data.

In ecology, GM methods helped to answer the questions concerning morphological variation of various parts (e.g. wings, gills,) or whole organisms in relation to environmental conditions. Debat et al. (2003) studied phenotypic plasticity of wing shape and size of *Drosophila simulans* across the entire range of viable developmental temperatures. In agreement with previous studies, size clearly decreased when temperature increased. The

allometric component basically revealed a progressive, monotonous variation along with the temperature. Surprisingly, nonallometric shape changes were highly similar at both extremes of the thermal range, suggesting that stress, rather than temperature per se, is the key developmental factor affecting wing shape. In the later study, Debat et al. (2008) used *Drosophila melanogaster* isofemale lines derived from wild flies collected on both slopes of the canyon to investigate the effect of developmental temperature upon the different components of phenotypic variation of a complex trait: the wing. Combining geometric and traditional morphometrics, they found only limited evidence for a differentiation among slopes. Investigating simultaneously phenotypic plasticity, genetic variation among isofemale lines, variation among individuals and fluctuating asymmetry, they could not identify a consistent effect of the stressful conditions encountered on the south-facing slope. The prevailing structuring effect was that of the experimentally manipulated temperature that clearly influences wing mean size and shape. Variability, in contrast, was not consistently affected by temperature. The direct, indirect and total effects of two environmental variables, water flow and dissolved oxygen on several morphological traits of nine populations of the African cyprinid was studied by Langerhans et al. (2007). They revealed that both variables directly influenced relative gill size, body shape and caudal fin shape. Indirect effects also played an important role. Clabaut et al. (2007) studied the relationship between ecology, morphological diversity and phylogeny of African cichlids. The influence of phylogeny on similarity of shape was found to be slight. The analyses of ecological traits on shape conclude that body shape is most strongly predicted by feeding preferences and the water depth at which species occur. Furthermore, the morphological disparity within tribes indicates that even though the morphological diversification associated with explosive speciation has happened in only a few tribes, the potential to evolve diverse morphologies exists in all tribes. Quantitative data support the existence of extensive parallelism in several independent adaptive radiations in Lake Tanganyika. Georgakopoulou et al. (2007) studied whether the temperature experienced by fishes at early developmental stages can influence their phenotype at subsequent stages. They reported that body shape and most of the meristic characters were significantly affected by the temperature of the environment. Herrel et al. (2007) dealt with morphological basis for the sexual dimorphism in bite force of a lizard species. Their results show that the lizard is indeed dimorphic in body and head size and that males bite harder than females. The GM analyses show distinct differences in skull shape between males and females, principally reflecting an enlargement of the jaw adductor muscle chamber for a given body and head size. Thus, the observed dimorphism in bite force is not

merely the result of an increase in head size, but involves distinct morphological changes in skull structure and the associated jaw adductor musculature. Morphometric analysis of ostracod shells reveals no directional trends but a significant co-occurrence of peaks in means of morphological variables with those in inferred salinity may indicate a direct response of the organisms to environment (ecophenotypes; Roberts et al., 2002). Roy et al. (2007) used genetic, body color and geometric morphometric data collected on 118 fish to test if coloration is the initial cause of divergence in the radiating *Telmatherina* genus. Results reveal that all *Telmatherina* previously described in this system can be categorized into three mitochondrial lineages, and that coloration is only weakly associated with early divergence. Clade-specific body shapes, however, likely adapted to microenvironments are key to the initial divergence in this system. Data also show that although colorations were not likely instrumental in seeding divergence in these fish, they appear to have developed in parallel within each clade. Gomez et al. (2008) explored the mechanisms promoting selection on corolla shape in the generalist crucifer *Erysimum mediohispanicum* Polatschek (Brassicaceae). They found that the main pollinators of *E. mediohispanicum* (large bees, small bees and bee flies) discriminate between different corolla shapes when offered artificial flowers without reward. Importantly, different pollinators prefer different shapes. They also found that flowers with narrow petals (those preferred by bees) produce both more pollen and nectar than those with rounded petals. Finally, different plant populations were visited by different faunas. As a result, they found spatial variation in the selection acting on corolla shape. Selection favored flowers with narrow petals in the populations where large or small bees are the most abundant pollinator groups. The study suggests that pollinators, by preferring flowers with high reward, exert strong selection on the *E. mediohispanicum* corolla shape. The geographical variation in the pollinator-mediated selection on *E. mediohispanicum* corolla shape suggests that phenotypic evolution and diversification can occur in this complex floral trait even without specialization. Frederich et al. (2008) investigated an ecomorphological approach in the trophic morphology of eight species of damselfish belonging to different trophic guilds. Geometric morphometrics were used to quantify size and shape variations in four skeletal units. Differences in skeletal shape are mainly related to improving the robustness of some skeletal parts (broad hyomandibular, short and high mandibles). This highlights likely differences concerning feeding by a cutting or scraping method. Finally, no strong correlations exist between size and shapes in the eight studied species. Size difference among species having a very similar shape could be viewed as a factor optimizing resource partitioning.

In algal ecology GM methods are also used. Neustupa and Hodač (2005) studied the changes in shape of marginal coenobial cells of populations of *Pediastrum duplex* Meyen *var. duplex* cultivated at 11 different pH levels and revealed that the morphological trends were related to both size and pH. Temperature-related morphological variation of cultured *Micrasterias rotata* strain and natural populations of *M. rotata* collected in different seasons were studied by Neustupa et al. (2008). They observed that as temperature increased, the population size of cultured *M. rotata* generally decreased and shape of the individual temperature groups differed significantly. The shape variation related to temperature was similar to the size-related change in shape. Natural populations of *M. rotata* were consistently similar to the low temperature cultured populations throughout the season. Potapova and Hamilton (2007) used conventional morphometrics and geometric morphometric approaches to describe the variation of frustular morphology within the *Achnanthidium minutissimum* species complex. It was shown that some historically recognized taxa are morphologically distinct, while others are difficult to differentiate. Morphometric analyses revealed six morphological groups, although it was impossible to draw clear boundaries among them, but these morphological groups differed significantly in their ecological characteristics and could be recommended as indicators of water quality. Řezáčová-Škaloudová et al. (2010) investigated the effect of temperature on scale shape using landmark-based geometric morphometric methods in two synurophycean species. They found statistically significant differences in the shape and size variation of silicate structures corresponding to temperature changes, although a substantial part of shape variation was associated with the position of scales on the cell. They also revealed a tendency for scale size to be reduced with increasing temperature; significant differences were found both in size of scales and in length of bristles or spines.

Geometric morphometrics methods could be also used to describe diversity or spatial patterns of distribution of organisms. Morphological disparity has increasingly been used as an alternative measure of biological diversity based on the shape features of organisms. Roy et al. (2001) reported that there is increasing evidence that trends in species richness might not match trends in other biodiversity metrics, such as morphological diversity. Data from a large group of Indo-Pacific gastropods show that the species richness of a region is a poor predictor of the morphological diversity present there. Areas with only a few species can harbor an impressive array of morphologies and, conversely, morphological diversity in the most species-rich regions is no higher than in regions with half their taxonomic diversity. The species isolation hypothesis of genital evolution or the sexual selection and pleiotropy

hypotheses clarifying geographic variation in genital morphology were investigated by Holwell (2008). He tested these predictions in the praying mantid genus *Ciulfina* (Mantodea: Liturgusidae) using elliptic Fourier analysis. He found significant levels of geographic variation in the genital morphology of four *Ciulfina* species irrespective of the relative proximity of different populations to contact zones with other species. These results reject the species isolation hypothesis, and instead support either the sexual selection or pleiotropy hypotheses to explain patterns of genital evolution in this genus. Neustupa et al. (2009) investigated the species diversity and morphological disparity of benthic Desmidiales in Central European peatland pools. The low-pH localities generally supported a more variable species composition and had high disparity values, irrespective of their rather low species diversity, than did slightly acidic to neutral localities. Interestingly, partial morphological disparity (measuring the contribution of a sample to the overall morphological variation) did not correlate with species diversity. These results indicate the relative importance of mountain peat bogs for the total morphological diversity of Desmidiales within the region that could not be ascertained solely from species diversity data.

2 Aims of the thesis

This thesis concerns two main issues: spatial distribution of microalgal assemblages in the environment of temperate peat bogs, and determination of morphological plasticity of selected desmid species (*Euastrum binale, Staurastrum hirsutum*) under different pH-levels using geometric morphometrics.

The principal aims can be summarized as follows:

1. to evaluate seasonal and spatial patterns of the distribution of algal assemblages in a temperate lowland peat bog. Paper 1.
2. to describe the small-scale spatial patterns of the distribution of algal assemblages in two different microhabitats of a raised bog. Paper 2.
3. to investigate the range of phenotypic and morphological plasticity of selected desmid species under different pH-levels. Paper 3.
4. to evaluate species diversity and disparity of desmids in Central European peat bogs using methods of geometric morphometrics and their relation to environmental conditions. Paper 4.

3 Thesis outline

3.1 Seasonal and spatial pattern of the distribution of algal assemblages

The spatial pattern of the distribution of algal assemblages was studied in the homogenous environment of a lowland peat bog within one year (May, August, October) on different spatial scales (10cm, 1m, 10m). Various statistical analyses were utilized. The seasonal dynamics were exhibited by an increase in diversity, and a decrease in dominance from May to October, with significant differences in species composition. The significant influence of distance, microhabitat type, and conductivity on maintaining the similarity of species composition was proved on the scales of 1m and 10m, but not on the scale of 10cm. The small-scale processes (colonization and niche differentiation), microhabitat type, geographic distance and conductivity were found to be the main factors influencing the distribution of algal assemblages in a temperate lowland peat bog environment.

3.2 The small-scale spatial distribution of algal assemblages

The second study concurs with the previous, and the aim was to study in greater depth the spatial pattern of distribution of algal assemblages on a very small scale (10cm), and the

effect of artificial barriers on species composition. Two transects within different microhabitat types in a raised bog environment were chosen for the study. Samples were taken in a peat bog along linear transects on a scale of 10cm, and water chemistry was examined. In contrast to the previous study, the pattern of spatial autocorrelation along the studied transects was observed. The distribution of algae along both transects was influenced by both spatial distance and environmental conditions in similar proportions. Differences in species composition in various parts of the transects were observed, but this pattern was primarily related to the abundance of species, rather than to their presence/absence in samples. Similarity in species composition correlated with spatial distance and environmental parameters in both microhabitat types.

3.3 The morphological plasticity of selected desmid species under different pH-levels

The pH-related morphological variation was investigated in two acidophilic desmid species (*Euastrum binale* and *Staurastrum hirsutum*) utilizing geometric morphometric methods. The clonal strains isolated from acidic habitats were cultivated in a pH gradient and the plasticity of ensuing populations was quantified and illustrated by the general Procrustes superimposition of landmarks placed along the outline of cells, and subsequent statistical analyses of shape data. In both species, we observed a robust effect of pH on the morphology of cells. In *Staurastrum hirsutum* the pH-related morphological change was accompanied by a decrease in the size of cells cultured at a higher pH. Conversely, in *Euastrum binale*, cell size did not differ in relation to pH, but cell shape was characterized by a deepening of the incisions between cell lobes at higher pH. In both species, cell complexity (indicated by its surface-to-volume ratio) was positively correlated with increasing pH.

3.4 Diversity and morphological disparity of desmid assemblages

The species diversity and morphological disparity of benthic desmids in Central European peatland pools was investigated. The disparity of samples, the average cell complexity and morphospace of cells were related to species diversity data and to the abiotic factors. The species diversity was positively correlated with pH and conductivity, and negatively correlated with total nitrogen concentration. The results showed that the low-pH localities generally supported a more variable species composition and had high disparity values, irrespective of their rather low species diversity, than did slightly acidic to neutral localities. Interestingly, partial morphological disparity (measuring the contribution of a sample to the overall morphological variation) did not correlate with species diversity. These

results indicate the relative importance of mountain peat bogs for the total morphological diversity of *Desmidiales* within the region that could not be ascertained solely from species diversity data.

Paper 1

Spatial distribution of algal assemblages in a temperate lowland peat bog

Kateřina Machová-Černá & Jiří Neustupa

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KATEŘINA MACHOVÁ-ČERNÁ* and JIŘÍ NEUSTUPA

Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 12801 Prague, Czech Republic; e-mail: kaca.cerna@gmail.com

Research Paper

Spatial Distribution of Algal Assemblages in a Temperate Lowland Peat Bog

key words: spatial autocorrelation, spatial scale, phytobenthos, Mantel test

Abstract

Samples of phytobenthos were collected during three different seasons in 2005 along a linear transect of a lowland peat bog at various spatial scales (10 cm, 1 m, 10 m) to investigate the seasonal dynamics, diversity, and factors influencing the spatial patterns of microalgal communities. Non-metric multidimensional scaling (NMDS), similarity percentage (SIMPER) analyses, ANOSIM, Mantel tests and diversity indices were used to analyze the data. Seasonal dynamics were exhibited by an increase in diversity, and a decrease in dominance from May to October, with significant differences in species composition. Mantel tests showed the significant influence of distance, microhabitat type, and conductivity on maintaining the similarity of species composition on scales of 1 m and 10 m. The small-scale processes (colonization and niche differentiation), microhabitat type, geographic distance and conductivity were found to be the main factors influencing the distribution of algal assemblages. We conclude that these factors are related to winter disturbance, and the consequent colonization and subsequent niche differentiation.

1. Introduction

Knowledge of the spatial structure of benthic assemblages underlies the understanding of ecological processes such as: succession, colonization, niche differentiation, and competition (RICKLEFS and SCHLUTER, 1993). Moreover, identifying the spatial pattern of species composition in water ecosystems is crucial for evaluation of their biodiversity and formulation of conservation strategies (COLEMAN, 2002). Thus, spatial analysis has recently become a rapidly growing field in benthology and aquatic ecology.

Small-scale differences in species composition and abundance were found to be significant for several organismal groups, primarily in marine environments. UNDERWOOD and CHAPMAN (1996) described the differences in abundance of intertidal snails and barnacles, on scales of centimeters to 1 to 2 meters by their ecological responses to small-scale patchiness of a microhabitat. Also in 1999, ARCHAMBAULT and BOURGET found increasing abundance of benthic marine algae from smooth, to rough and more heterogeneous, surfaces on small scales. It was demonstrated a few years later by DOWNES *et al.* (1998) that both niche differentiation and higher substratum complexity correlated with invertebrate species richness and abundance in a perennial, upland stream.

* Corresponding author

COLEMAN (2002) examined the high variation in assemblages of marine turfing algae on a small spatial scale (10 cm). These findings, which were temporally consistent, appeared to be due to small-scale ecological processes. Others, RINDI and CINELLI (2000) and RINDI and BATELLI (2005) discussed the importance of small-scale differentiation in Mediterranean intertidal algal assemblages. They stressed the importance of substratum heterogeneity, as well as colonization processes (input of propagules), competition and variation in recruitment. It was revealed by BENEDETTI-CECCHI (2001) that a significant proportion of horizontal variation in marine littoral benthic algal and invertebrate communities could be explained on the scale of 10 s of centimeters, and related to physical processes of the environment. SABUROVA *et al.* (1995) defined the main factors influencing spatial distribution of sandflat microphytobenthic communities in relationship to scale: on a microscale (up to 2 m^2), biotic interspecies interactions are the most important; on the mesoscale (up to 18 m^2), distribution is mainly determined by the granulometric composition and a complex of abiotic conditions in the sediments; on the macroscale (up to 10,000 m^2), distribution depends upon the emersion period during low tide. Similar results were obtained by MÉLÉDER *et al.* (2007), who studied microphytobenthic assemblages of a macrotidal flat. They found that hydrodynamics related globally to the occurrence of spatio/temporal biotic or abiotic gradients, whereas oyster beds and ridge and runnel features appeared to be local spatial structuring factors.

Most of the studies conducted in marine ecosystems referred to small-scale variation as a result of substrate heterogeneity and biotic processes (colonization, competition). Increasing patchiness of benthic assemblages was positively correlated with diversity (UNDERWOOD and CHAPMAN, 1996; ARCHAMBAULT and BOURGET, 1999).

In freshwater benthic habitats, data on small-scale algal differentiation are less numerous. Recent studies concentrated on regional or large-scale processes of individual catchment areas (CHARLES *et al.*, 2006), water bodies (PALS *et al.*, 2006) or running waters (*e.g.*, PASSY, 2001; SOININEN, 2005). The spatial organization of benthic invertebrate communities in two oligotrophic lakes was characterized by STOFFELS *et al.* (2005). They found that the small-scale structure was typically driven by substrate heterogeneity. Many authors concentrated on spatial distribution of diatoms, especially in streams. PASSY (2001) revealed current velocity as the major factor controlling diatom distribution in streams, and only a minor influence for other, mostly biotic, factors in shaping diatom communities. Conversely, SOININEN (2005) reported that the current velocity did not strongly shape diatom communities in turbid rivers. SOININEN and KÖNÖNEN (2004), also studied diatom benthic communities in many boreal streams and found that environmental factors, principally conductivity, total phosphorus content, and water color were related to algal distribution. In addition, a clear spatial configuration of algal distribution, within distinctly different communities in different parts of the country, was revealed (SOININEN *et al.*, 2004).

However, data on the spatial distribution of algal assemblages in stagnant freshwater habitats are missing. Therefore, in this study we concentrated on a Central European lowland peat bog, where we investigated the spatial structure of phytobenthos and periphyton along a linear transect. The aims of this study were to: (1) describe the algal species composition of the locality, and its diversity and dynamics throughout the year, (2) identify the distribution and patterns of spatial autocorrelation of assemblages, and describe factors influencing this pattern.

2. Materials and Methods

The study area is located in the Břehyně-Pecopala National Natural Reserve and Ramsar locality, Czech Republic (50°34′N, 14°42′E). The central part of the reserve is occupied by Břehyňský fishpond (area 90 ha) created in the first half of the 13th century. The pond is surrounded by a huge area of sandstone-based lowland peat bogs that gradually turn into semi-artificial wetland pine forests (ČERMÁK

and MRKVA, 2003). Since the 1970s the time of the Soviet occupation of the former Czechoslovakia, the reserve was part of a large military area established around the Ralsko Soviet military airport and rocket base. At that time, a system of drainage ditches was dug in parts of the wetland. However, today these linear ditches are functionless, and partly overgrown with peat bog vegetation that impedes the current such that they become filled with stagnant water.

The ditch we examined is situated in the southern part of the reserve, and its length is approximately 400 m. It is partly overgrown with mosses (*Sphagnum* spp.), and the depth of the water column varies from 10 to 30 cm. The pH of the water ranged between 3.8 and 4.5, its conductivity ranged between 80 and 170 μS cm^{-1}.

Samples of phytobenthos were collected in three different seasons in 2005: spring: May 12th, summer: August 30th, and autumn: October 28th. They were collected along a transect of the entire 400 m lengthwise axis of the ditch. We collected 40 samples separated by a distance of 10 m from each other, 21 samples 1 m apart, and 20 samples taken 10 cm from each other (Fig. 1). The samples taken for analyses at each site consisted of 10 ml of phytobenthos and periphyton. Conductivity, temperature and pH at each sampling site were measured using a Hanna portable combined pH/conductometer. In addition, the microhabitat type of each site was recorded. We distinguished five physiognomically discernible microhabitat types: (1) epipelic phytobenthos on a fine detritus bottom, (2) benthos dominated by a submerged *Sphagnum* biomass, (3) periphyton within submerged *Sphagnum* tussocks filling-up the water column, (4) periphyton within half-emersed *Sphagnum* tussocks (up to 5 cm above water level), and (5) periphyton within emersed *Sphagnum* tussocks. At individual sampling sets, the microhabitats were differentially represented, as a result of seasonal fluctuation of the water level.

The samples were fixed with Lugol's solution in the field, and later, examined under an Olympus BX 51 light microscope and identified to the lowest possible taxonomic level. Diatoms were identified in mineralized samples mounted on Naphrax permanent slides (HOUK, 2003). The identification was based on standard taxonomic monographs (Süsswasserflora von Mitteleuropa, Binnengewässer, Diatoms of Europe, *etc.* – for references see *e.g.*, ŠEJNOHOVÁ *et al.*, 2003). Semiquantitative estimates of algal populations were deduced from slides, and individual species were classified into three categories: (1) up to 1% of individuals revealed, (2) up to 50% of individuals, and (3) more than 50% of individuals of a particular assemblage (KINROSS *et al.*, 1993; FAUCONNIER, 1995; GAISER and JOHANSEN, 2000; HUSA *et al.*, 2004; KOMÁREK and SUKAČOVÁ, 2004). Approximately 500 cells per sample were observed.

Non-metric multidimensional scaling (NMDS; KRUSKAL, 1964) was used in each set of samples to display the species' structural composition data using the PRIMER® (Plymouth Routines In Multivariate Ecology, PRIMER-E Ltd., Plymouth, UK) software package. To reduce the chance of local optima, 100 random starts were carried out in 2- and 3-dimensional analyses (CLARKE and WARWICK, 2001).

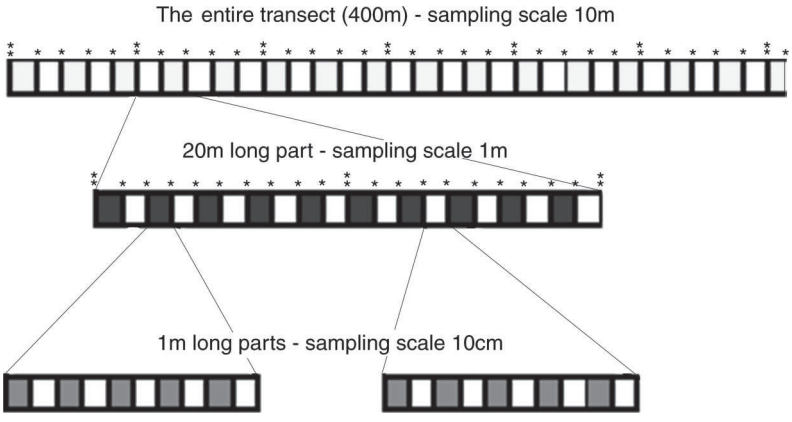

Figure 1. The sampling design indicating position of individual samples along the linear transect. Stars indicated sample set on 10 m scale compared to corresponding sample set on 50 m scale and sample set on 1 m scale compared to corresponding sample set on 10 m scale.

In all cases, the 3-dimensional solutions had slightly lower stress values than the 2-dimensional ones, but we utilized the 2-dimensional data because of the superior representation of sample position. In all analyses, we used Bray-Curtis similarity index (BRAY and CURTIS, 1957; CLARKE, 1993; MÉLÉDER *et al.*, 2007). To retain the contribution of each species according to its semi-quantitative abundance, we made no data transformations (CLARKE *et al.*, 2006).

The species composition of different microhabitats within and between individual seasonal sets of samples was compared using a non-parametric two-group ANOSIM test based on Bray-Curtis similarity index, which is a non-parametric distribution-free analogue of one-way ANOVA (CLARKE and GREEN, 1988; CLARKE, 1993). The procedure was carried out in PAST software (ver. 1.62; HAMMER *et al.*, 2001) with 10,000 permutations used.

Subsequently, we identified species responsible for the individual microhabitat types using the SIMPER (similarity percentage) routine of PRIMER® (CLARKE and WARWICK, 2001; MÉLÉDER *et al.*, 2007). All species observed were included in the analysis. The SIMPER analysis compared the average contribution of each species to the average Bray-Curtis similarity within a group. The SIMPER procedure also examined community patterns defining inter-group dissimilarity.

Additionally, community structure was studied using diversity indices that were calculated for all microhabitat samples in individual season sets throughout the collection period. We used species number, Shannon index, H' (SHANNON and WEAVER, 1949) that takes into account the number of individuals as well as number of taxa (it varies from 0 for communities with only a single taxon to high values for communities with many taxa, each with few individuals), and Pielou's evenness (or equitability), J' (PIELOU, 1969, 1975) that was calculated as Shannon diversity divided by the logarithm of number of taxa (this expresses how uniformly individuals are distributed among the different species, and its values are the opposite of dominance values). Differences between calculated diversity indices were evaluated using permutation *t*-test in PAST software (ver. 1.62; HAMMER *et al.*, 2001) with 10,000 permutations used. Identical analyses as those described above were used to evaluate seasonal dynamics in species composition.

The effect of spatial autocorrelation in species data along the studied transect in individual sample sets was evaluated using two-matrices and partial Mantel tests (MANTEL, 1967; SMOUSE *et al.*, 1986). We tested the mutual relationships among five different matrices: (1) matrix of spatial distances between pairs of sites along a transect, (2) matrix of similarity in species composition (Bray-Curtis similarity index), (3) matrix of differences in pH values between sites; (4) matrix of differences in conductivity values between sites, (5) matrix of similarity in microhabitat type (1 designates the same microhabitat type for a compared pair of samples, *e.g.*, both samples collected from emersed moss tussocks, 0 designates a different microhabitat type; MCCUNE and GRACE, 2002). Mantel tests were conducted for individual spatial scales (10 cm, 1 m, 10 m, and 50 m – this last including every fifth sample taken on 10 m scale) in each sample set using *zt* software (ver. 1.0; BONNET and VAN DER PEER, 2002).

Finally, we tested the species diversity of various pairs of sample sets to ascertain on which scale the highest species diversity was detected during the sampling period. Differences in species diversity were evaluated by permutation tests on Menhinick diversity index (MAGURRAN, 2004) using R 2.3.1 routine (R Core Development Team, 2006). In total, 10,000 permutations were used in diversity testing. All the corresponding sets were tested on individual scales across the seasonal sampling sets. In addition, we tested within-season diversity differences in samples taken in the various microhabitat types on the 10 cm scale, and in all of the 40 samples taken 10 m apart *vs.* eight samples taken 50 m apart (every fifth sample taken on 10 m scale) from the corresponding transect, and in the 21 samples taken on the 1 m scale *vs.* three samples separated by 10 m (Fig. 1).

3. Results

3.1. The Structure in Species Composition

The structure in species composition, based on non-metric multidimensional scaling (NMDS), revealed that the differences in microhabitats clearly accounted for the greatest portion of the variation in species composition of individual sets of samples (Fig. 2). In May (Fig. 2a), the periphyton samples from submerged *Sphagnum* tussocks were separated from two epipelic microhabitats (epipelic phytobenthos on a fine detritus bottom, and benthos

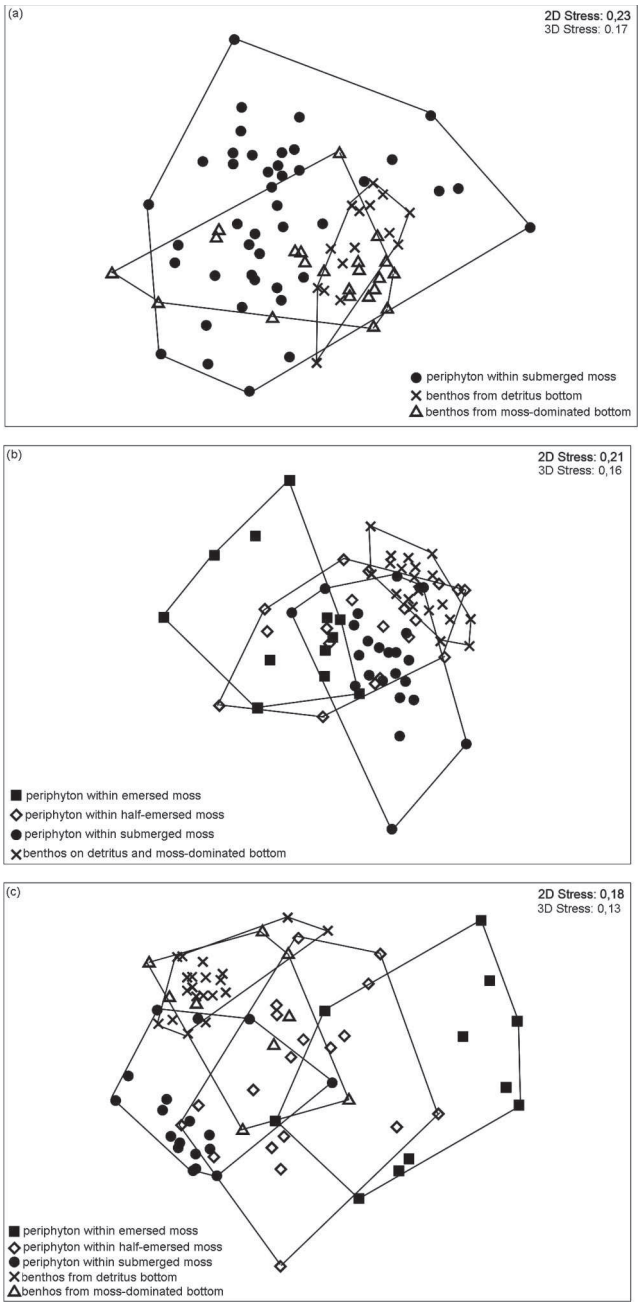

Figure 2. (a–c) Non-metric multidimensional scaling (NMDS) of samples collected in individual seasons of the year – (a) May, (b) August, (c) October. (emersed moss = periphyton within the emersed *Sphagnum* tussocks, half-emersed moss = periphyton within the half-emersed *Sphagnum* tussocks, submerged moss = periphyton within the submerged *Sphagnum* tussocks filling-up the water column, bottom with moss = benthos dominated by the submerged *Sphagnum* biomass, bottom with detritus = the epipelic phytobenthos on the fine detritus bottom).

dominated by a submerged *Sphagnum* biomass). In August and October, we observed a clear separation of the two sample sets; on one side samples that were collected from the emersed *Sphagnum* tussocks (that developed as a result of decreasing water level), and were distinct from the epipelic microhabitats on the opposite side of the sample position plot (Fig. 2b, c). The samples taken from half-emersed and submerged *Sphagnum* tussocks were located in between these two.

3.2. Microhabitat Differentiation

The statistical significance of within-season differences in species composition between all tested microhabitat pairs was confirmed by the non-parametric ANOSIM tests for all three seasonal sets (Table 1). If we used the values of R-statistic from the ANOSIM analysis as the scale factor, the difference between identical pairs of microhabitats generally increased throughout the sampling period. At the same time, statistically significant differences in between-season species composition of individual microhabitat types were detected in most cases, with only two exceptions in the emersed and submerged *Sphagnum* tussocks species composition that showed no statistically significant difference between August and October (Table 1).

The SIMPER analyses detected species identifying individual microhabitat types (Table 2). The algal assemblages from individual microhabitats did not essentially differ in species composition, but rather, in the relative abundance of these species. The algal periphyton growing within the emersed *Sphagnum* tussocks was characterized by the dominance of

Table 1. Comparison of species composition of microhabitats within and between seasons calculated through the use of two-group ANOSIM tests. Values of R statistic are represented. *: $P < 0.05$. **: $P < 0.01$, ***: $P < 0.001$. (Comparison between species composition of benthos on fine detritus bottom and benthos on moss-dominated bottom in August was omitted because of low number of samples collected at the second microhabitat mentioned. Non-significant values are in bold.)

Pairs of microhabitats compared within seasons	May	August	October
Periphyton of emersed moss × Periphyton of half-emersed moss		0,22**	0,18**
Periphyton of emersed moss × Periphyton of submerged moss		0,44***	0,83***
Periphyton of emersed moss × Benthos on moss-dominated bottom		0,81***	0,74***
Periphyton of emersed moss × Benthos on fine detritus bottom		0,81***	0,93***
Periphyton of half-emersed moss × Periphyton of submerged moss		0,15***	0,4***
Periphyton of half-emersed moss × Benthos on fine detritus bottom		0,39***	0,65***
Periphyton of half-emersed moss × Benthos on moss-dominated bottom		0,32***	0,36***
Periphyton of submerged moss × Benthos on moss-dominated bottom	0,16**	0,37***	0,54***
Periphyton of submerged moss × Benthos on fine detritus bottom	0,22***	0,37***	0,62***
Benthos on fine detritus bottom × Benthos on moss-dominated bottom	0,104*	–	0,48***

Microhabitats compared among seasons	May × August	May × October	August × October
Periphyton within emersed moss			**0.026**
Periphyton within half-emersed moss			0.1*
Periphyton within submerged moss	0.18***	0.15**	**0.065**
Benthos on fine detritus bottom	0.28***	0.4***	0.23***
Benthos on moss-dominated bottom	0.42***	0.32**	0.21*

Table 2. SIMPER analyses – tables represented species typical for individual microhabitat types and seasons. S_i(%) – percentage contribution of individual species to intra-group similarity, $S_i : SD(S_i)$ – contribution of individual species to intra-group similarity to standard deviation of the contribution ratio.

Periphyton within emersed moss			**Periphyton within half-emersed moss**			**Periphyton of submerged moss**		
	S_i(%)	$S_i : SD(S_i)$		S_i(%)	$S_i : SD(S_i)$		S_i(%)	$S_i : SD(S_i)$
Eunotia exigua	21.99	5.81	*Cryptomonas* sp.	13.64	2.78	*Eunotia exigua*	12.39	1.91
Cryptomonas sp.	15.9	2.48	*Eunotia exigua*	13.54	2.53	*Eunotia bilunaris*	11.01	2.50
E. paludosa	15.45	2.30	*Eunotia bilunaris*	10.68	4.10	*Cryptomonas* sp.	9.84	1.95
E. bilunaris	13.83	2.53	*Eunotia paludosa*	7.72	1.34	*Mougeotia* sp.	9.6	1.82
Brachysira serians	5.89	1.49	*Mougeotia* sp.	5.5	1.18	*Merismopedia glauca*	5.79	1.25
Eunotia glacialis	3.89	0.68	*Brachysira serians*	4.62	1.59	*Chroococcus obliteratus*	5.69	1.26
Euglena sp.	3.32	0.92	*Merismopedia glauca*	3.9	1.02	*Cylindrocystis brebissonii*	4.15	0.94
Mougeotia sp.	2.8	0.68	*Frustulia saxonica*	3.48	1.07	*Brachysira serians*	3.82	0.98
Pinnularia rupestris	1.68	0.50	*Cylindrocystis brebissonii*	3.36	1.03	*Staurastrum punctulatum*	3.78	1.18
Others	15.25		*Euglena* sp.	3.26	1.18	*Frustulia saxonica*	3.62	1.02
			Chroococcus obliteratus	3.25	0.83	*Euastrum binale*	3.44	0.68
			Staurastrum punctulatum	3.2	1.05	*Eunotia paludosa*	2.91	0.56
			Eunotia glacialis	2.09	0.64	*Chroococcus minor*	2.81	0.64
			Merismopedia angularis	1.99	0.77	*Binuclearia tectorum*	2.42	0.69
			Others	19.77		Others	18.73	

Benthos on fine detritus bottom			**Benthos on moss-dominated bottom**		
	S_i(%)	$S_i : SD(S_i)$		S_i(%)	$S_i : SD(S_i)$
Eunotia bilunaris	7.91	4.19	*Eunotia exigua*	8.79	2.12
Merismopedia glauca	7.72	3.31	*Eunotia bilunaris*	8.68	2.87
Frustulia saxonica	7.19	3.68	*Mougeotia* sp.	7.78	2.25
Eunotia exigua	6.67	2.45	*Merismopedia glauca*	7.02	2.21
Mougeotia sp.	6.45	2.35	*Brachysira serians*	6.41	1.68
Merismopedia angularis	6.37	2.19	*Frustulia saxonica*	6.15	1.78
Brachysira serians	6.32	1.69	*Merismopedia angularis*	5.94	1.74
Cylindrocystis brebissonii	5.03	1.27	*Cryptomonas* sp.	5.78	1.66
Croococcus obliteratus	4.81	1.39	*Cylindrocystis brebissonii*	4.18	1.05
Cryptomonas sp.	4.63	1.84	*Chroococcus obliteratus*	4.09	1.02
Staurastrum punctulatum	3.46	1.76	*Euglena* sp.	2.94	1.24
Pinnularia biceps	3.2	1.29	*Pinnularia rupestris*	2.91	0.83
Pinnularia viridis	3.18	1.74	*Synura* sp.	2.8	1.46
Euglena sp.	2.84	1.50	*Pinnularia viridis*	1.85	0.89
Staurastrum simonyi	1.96	0.83	*Pinnularia biceps*	1.8	0.79
Euastrum binale	1.74	1.01	*Tabellaria flocculosa*	1.78	0.91
Others	20.52		Others	21.1	

May			**August**			**October**		
	S_i(%)	$S_i : SD(S_i)$		S_i(%)	$S_i : SD(S_i)$		S_i(%)	$S_i : SD(S_i)$
Eunotia exigua	13.35	2.32	*Eunotia exigua*	11.12	1.98	*Eunotia exigua*	11.94	1.91
Eunotia bilunaris	11.41	2.52	*Eunotia bilunaris*	9.48	2.79	*Eunotia bilunaris*	10.76	3.15
Mougeotia sp.	9	1.70	*Cryptomonas* sp.	9.11	1.70	*Cryptomonas* sp.	10.58	1.77
Cryptomonas sp.	8.64	1.83	*Mougeotia* sp.	6.61	1.52	*Mougeotia* sp.	6.98	1.42
Cylindrocystis brebissonii	6.04	1.08	*Chroococcus obliteratus*	5.82	1.37	*Brachysira serians*	6.52	1.86
Merismopedia glauca	5.53	1.31	*Merismopedia glauca*	5.75	1.31	*Merismopedia glauca*	5.5	1.07
Frustulia saxonica	4.13	0.98	*Brachysira serians*	5.15	1.41	*Frustulia saxonica*	4.7	1.40
Brachysira serians	3.67	0.88	*Frustulia saxonica*	4.08	1.05	*Chroococcus obliteratus*	4.55	1.02
Pinnularia rupestris	3.33	0.88	*Eunotia paludosa*	3.79	0.70	*Cylindrocystis brebissonii*	3.89	1.01
Euglena sp.	3.31	1.14	*Euglena* sp.	3.7	1.40	*Staurastrum punctulatum*	3.68	1.21
Chroococcus obliteratus	3.13	0.78	*Cylindrocystis brebissonii*	3.11	0.99	*Eunotia paludosa*	3.52	0.57
Eunotia paludosa	3.08	0.54	*Chroococcus minor*	3.08	0.66	*Eunotia glacialis*	2.66	0.73
Staurastrum punctulatum	2.55	0.82	*Merismopedia angularis*	2.85	0.80	*Synura* sp.	2.4	0.90
Dinobryon sociale	2.4	0.84	*Staurastrum punctulatum*	2.71	0.98	*Merismopedia angularis*	2.22	0.67
Synura sp.	2.28	0.82	*Synura* sp.	2.2	0.93	*Euglena* sp.	1.85	0.75
Others	18.15		Others	21.44		Others	18.25	

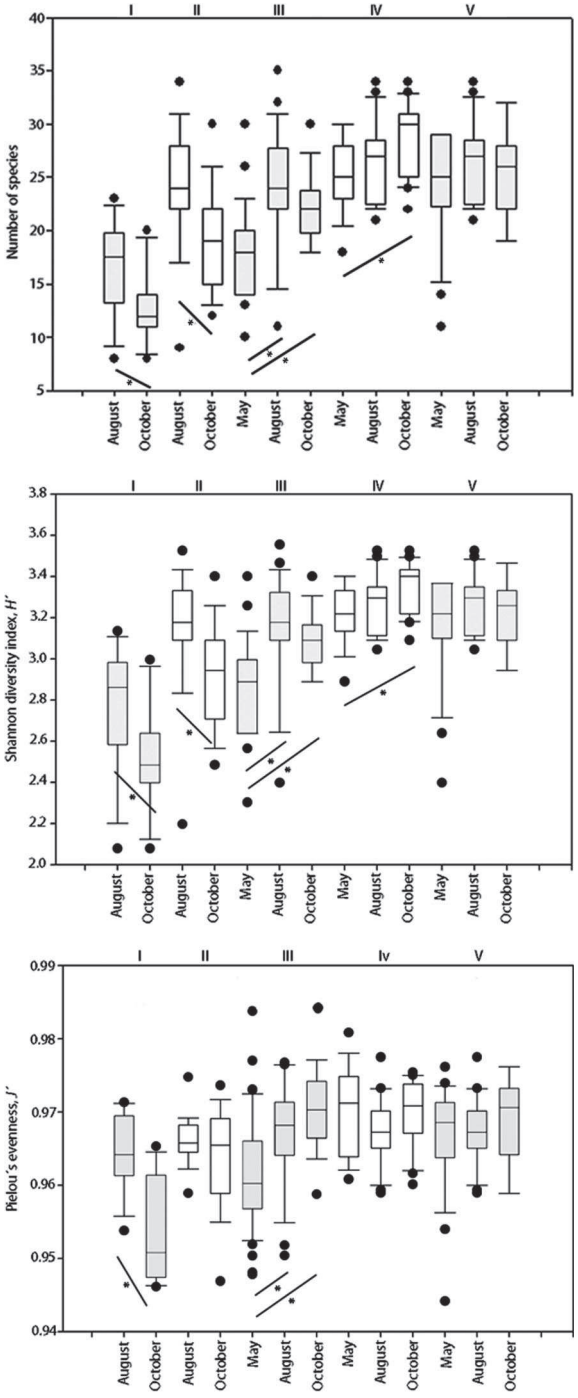

Figure 3. Different diversity indices calculated for individual microhabitat types in different seasons: number of species, Shannon diversity index, *H′*, Pielou´s evenness, *J′*. Significant differences between different diversity indices are indicated with stars and pairs are joined with lines. (I – periphyton within the emersed *Sphagnum* tussocks, II – periphyton within the half-emersed *Sphagnum* tussocks, III – periphyton within the submerged *Sphagnum* tussocks filling-up the water column, IV – the epipelic phytobenthos on the fine detritus bottom, V – benthos dominated by the submerged *Sphagnum* biomass).

the single species *Eunotia exigua* (BRÉBISSON) RABENHORST which, along with a few other diatom species, formed the assemblage. In other microhabitat types, diatoms also made up a significant part of the assemblage, but cyanobacteria, desmids, flagellates and filamentous green algae were also found in abundance. The highest average within-group similarities were detected in benthos of a fine-detritus bottom, and in periphyton growing within emersed *Sphagnum* tussocks (Table 3). These two microhabitat types also exhibited the highest inter-microhabitat dissimilarity (60.7%); they were distinguished from each other by the dominance of a few diatom species in the periphyton microhabitat, and the presence of desmids in the fine-detritus benthos. Nevertheless, the two benthic microhabitats were the most similar in their species composition.

The box-plots of the diversity indices illustrated different seasonal trends in individual microhabitats (Fig. 3). In emersed (I), and half-emersed (II), *Sphagnum* tussock microhabitats the numbers and diversity of species significantly decreased between August and October, and the decrease of evenness was statistically significant only for emersed *Sphagnum* tussocks. In submerged *Sphagnum* tussocks (III) a similar pattern was identified for species numbers and species diversity: an increase in August compared to May, with a subsequent decrease in October (the values were significantly different between May and August and May and October). In the microhabitat with a fine detritus bottom (IV), the number and diversity of species increased throughout the year, as indicated by statistically significant differences between indices values from May to October. In the microhabitat type (V), dominated by *Sphagnum* biomass on the bottom, no statistically significant changes in diversity indices were detected (Fig. 3).

3.3. Seasonal Dynamics

Seasonal changes in species composition were evaluated by two-group ANOSIM tests of samples taken in May, August and October. We found statistically significant differences in species composition between May and August ($R = 0.17$, $P < 0.0001$), May and October ($R = 0.145$, $P < 0.001$), and August and October ($R = 0.038$, $P < 0.01$). The SIMPER analyses of between-season samples resulted in a pattern similar to that of microhabitats – the individual species typifying the seasons were largely the same, but there were differences

Table 3. SIMPER analyses – tables represent average similarity within a group (microhabitat type/season; numbers in bold) and between groups (microhabitat types/season). I – periphyton within emersed *Sphagnum* tussocks, II – periphyton within half-emersed *Sphagnum* tussocks, III – periphyton within submerged *Sphagnum* tussocks, IV – benthos of fine-detritus bottom, V – benthos of moss-dominated bottom.

	May	August	October		
May	**52.9**				
August	50.97	**52.35**			
October	50.26	48.23	**53.14**		
	I	II	III	IV	V
I	**58.06**				
II	47.68	**55.4**			
III	54.25	48.17	**53.39**		
IV	58.48	50.39	50.41	**55.8**	
V	60.7	50.73	50.35	42.07	**62.33**

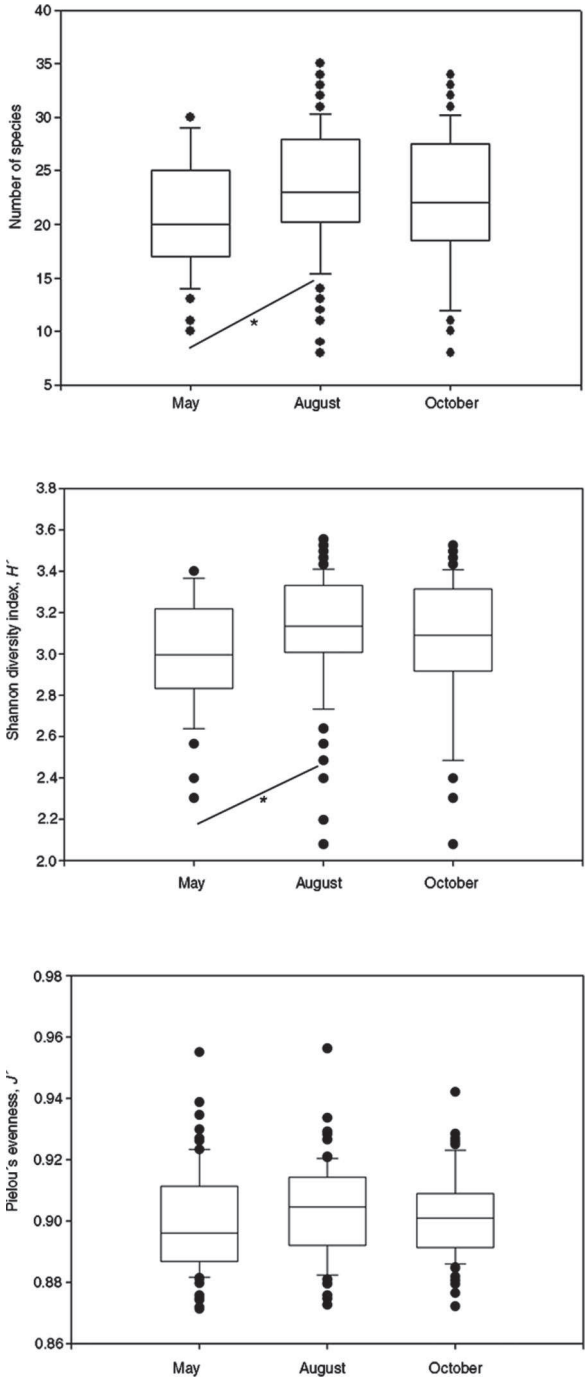

Figure 4. Different diversity indices calculated for individual seasons in the year: number of species, Shannon diversity index, H', Pielou's evenness, J'. Significant differences between different diversity indices are indicated with stars and pairs are joined with lines.

Table 4. Results of simple and partial Mantel tests calculated through the use of different types of matrices at different scales. *: $P < 0.05$. **: $P < 0.01$, ***: $P < 0.001$. (Three numbers at each scale indicated results from individual seasons: May/August/October. Where results are not presented – these pairs of matrices were viewed as unreasonable to calculate because of same type of microhabitat of compared samples. Covariables are indicated in italics.)

Scale	50 m	10 m	1 m	10 cm benthos	10 cm submerged moss
Similarity index × distance	–0.6**/–0.77***/–0.84***	–0.68***/–0.61***/–0.61***	–0.44***/–0.09/–0.15*	–0.5***/–0.28*/0.2	–0.13/–0.39*/–0.26
Similarity index × pH	0.14/–0.29/0.12	–0.14*/–0.09/–0.12	–0.002/0.05/–0.21*	–0.17/0.1/–0.09	0.18/–0.14/–0.1
Similarity index × conductivity	–0.37*/–0.38*/–0.17	–0.43***/–0.41***/–0.4***	–0.07/0.14/0.1	–0.003/0.06/–0.16	0.02/0.004/0.21
Similarity index × microhabitat	0.06/0.15/0.33	0.18**/0.27***/0.34***	0.23*/0.22**/0.32***	–	–
pH × distance	–0.08/0.46*/0.09	0.06/0.17**/0.2**	0.25**/0.06/0.25**	0.25/0.5*/–0.22*	0.08/0.1/0.07
pH × conductivity	–0.09/0.66**/0.52*	0.11/0.49***/0.51***	0.04/0.36*/–0.02	0.1/0.24/–0.19	0.22/0.86***/0.22
pH × microhabitat	–0.44/–0.79/–0.25	–0.12**/–0.15**/–0.1**	–0.14/–0.02/0.07	–	–
Conductivity × distance	0.44*/0.55*/0.3	0.45***/0.6***/0.52***	0.16/0.007/–0.04	0.43*/–0.06/–0.14	0.2/0.21/0.05
Conductivity × microhabitat	–0.17/–0.22/–0.27	–0.2**/–0.16**/–0.17***	–0.01/0.008/0.04	–	–
Microhabitat × distance	–0.17/–0.05/–0.27	–0.09*/–0.13**/–0.26***	–0.25**/–0.04/–0.067	–	–
S. index × distance × pH	–0.59**/–0.75***/–0.86***	–0.68***/–0.61***/–0.61***	–0.45***/–0.1/–0.1	–0.5***/–0.29/0.19	–0.18/–0.37*/–0.25
S. index × distance × conductivity	–0.52*/–0.73**/–0.83***	–0.6***/–0.5***/–0.52***	–0.43***/–0.09/–0.15*	–0.51***/–0.3*/0.19	–0.16/–0.39*/–0.27
S. index × distance × microhabitat	–0.59**/–0.77***/–0.82***	–0.67***/–0.6***/–0.58***	–0.4***/–0.09/–0.18*	–	–
S. index × microhabitat × distance	–0.05/0.17/0.2	0.16**/0.25***/0.24***	0.14/0.22**/0.34	–	–

in their abundance (Table 2). However, there were distinct seasonal trends in the occurrence of various algal groups. Cyanobacteria, autotrophic flagellates (*Euglena mutabilis* SCHMITZ, *Dinobryon sociale* EHRENBERG) and filamentous green algae (*Mougeotia* sp., *Ulothrix* spp., *Microthamnion kuetzingianum* NÄGELI) species decreased in numbers and abundance. On the other hand, the abundance and number of species of desmids and diatoms increased. The average within-group similarities remained consistent in all seasons (52.9–53.14%), while the inter-season dissimilarities varied between 48.23 and 50.97% (Table 3).

The seasonal course of the algal assemblages as a whole, as indicated by diversity indices, showed a similar pattern of diversity that increased during the year (Fig. 4). Furthermore, a statistically significant increase in the number and diversity of species was detected between May and August. Changes in evenness were insignificant.

3.4. Spatial Autocorrelation

Correlation between spatial distance and similarity in species composition among samples was detected on 50 m, 10 m and 1 m scales (Table 4). However, this correlation on a 1 m scale was non-significant in August. Significance of spatial distance on these scales was generally confirmed by partial Mantel tests with effects of pH, conductivity, and microhabitats controlled for (Table 4). Correlation of external factors (pH, conductivity, microhabitat type) with spatial distance was mostly insignificant on 50 m and 1 m scales; however, the correlation between conductivity and microhabitat type were typically significant on the 10 m scale. Correlations between similarity in species composition and distance for samples taken on a 10 cm scale were insignificant, with exceptions in May (benthic samples) and August (samples in submerged moss microhabitat; Table 4).

3.5. Spatial Structure of Diversity

Using diversity comparisons, we evaluated the variation in species diversity with increasing spatial distance. Diversity did not differ significantly between seasonal samplings on different scales from 10 cm to 50 m, nor indeed, along the entire transect. Correspondingly, diversity did not differ among the 10 cm samplings collected in different microhabitats. Diversity of samples taken along transects of various scales was found to be significantly higher on the smaller scale only in the single case of May collections from 10 m *vs.* 50 m sets (permutation $P = 0.0019$). In all other cases (10 m *vs.* 50 m in August and October, and all the 1 m *vs.* 10 m seasonal sets) no statistically significant difference in diversity was found.

4. Discussion

Most of the 82 taxa of cyanobacteria and algae identified in this study were common lowland peat bog taxa (COESEL, 1986; BORICS *et al.*, 2003; NOVÁKOVÁ, 2007). The relatively low number of species encountered is likely related to extremely low pH-levels (3.5–4.5), or to seasonal water level fluctuations. There certainly were several coccoid green algal species that were left unidentified because of their tremendously complicated and confusing species concepts and cryptic diversity (JOHN and MAGGS, 1997; FAWLEY *et al.*, 2004). The high proportion of desmids and conjugates observed correlates with their reported preference for lower pH (COESEL, 1982; MATALONI, 1999), even though a pH level of less than 4.5 is reported to limit occurrence of most these species (COESEL, 1983, 1998).

The NMDS plots illustrate that the pattern of species was principally related to the differences in microhabitat types. The differences in species composition among microhabitats

were demonstrated by ANOSIM and Mantel tests. Abiotic factors were considered important in determining the different algal species components in freshwater benthic microhabitats (*e.g.*, SABUROVA *et al.*, 1995; DOWNES *et al.*, 1998; ARCHAMBAULT and BOURGET, 1999; RINDI and CINELLI, 2000; STOFFELS *et al.*, 2005). The emersed *Sphagnum* tussocks (occurring in summer and autumn, as a result of a decrease in water level) are typical due to acidity stress and desiccation, and consequently because of decreased competition (COESEL, 1982; MATALONI, 1999). We also detected low diversity in this microhabitat, with a decrease in diversity and dominance of species throughout the sampling period, not observed in other microhabitats. The assemblages were mainly composed of diatoms (especially a single dominant species: *Eunotia exigua*). In higher water levels, the submerged *Sphagnum* tussocks, the moss-biomass bottom and the fine-detritus benthos contained a greater number of species (including desmids) and greater evenness than the emersed *Sphagnum* microhabitat, thus indicating a more consistent abundance of species. In general, our report of different algal species composition in different freshwater benthic microhabitats corresponds with those of previous studies (COESEL, 1982, 1986; MATALONI, 1999).

We found seasonal variations in species composition accompanied by an increase in species diversity and a decrease in dominance over the course of the sampling period. Seasonal dynamics of microphytobenthic species was found significant in lakes (HAWES and SMITH, 1994; ABERLE and WILTSHIRE, 2006; O´REILLY, 2006), streams and rivers (POWER, 1992; PETERSON and STEVENSON, 1992; WERNER and KÖHLER, 2005), as well as in peat bogs (HAYWARD, 1957; DUTHIE, 1965; ŁAŹNIEWSKA, 2001). However, the higher difference of spring algal species composition from the rest of the season was not detected in studies investigating phytobenthos of large water bodies or rivers (ABERLE and WILTSHIRE, 2006; GIORGI *et al.*, 2005). We believe that this may be due to disturbance related to winter temperatures below freezing; these would have a greater impact on shallow wetland localities than on considerably larger or faster moving bodies of water (IYOBE and HARAGUCHI, 2005). Thus, the spring algal assemblages reflect the early succession stage with many r-strategists shifting the species composition to a relatively stable summer/autumn stage. Furthermore, the results of diversity measures validated the role of winter disturbance and assemblage succession. Increasing levels of environmental stress have historically been considered to decrease diversity, species richness and evenness, and increase dominance (CLARKE and WARWICK, 2001). However, CONNELL (1978) and HUSTON (1979) suggested that a greater amount of disturbance lead to species elimination by stress and, consequently, less diversity. Conversely, in situations with low disturbance, species diversity may be limited by competitive exclusion of species. Then, a slight increase in disturbance levels leads to an increase in competition, resulting in increasing diversity. In our samples, species diversity was lowest, and the dominance was highest in May, possibly as a result of winter disturbance. In August, the species diversity had increased, perhaps due to niche differentiation and the level of medium disturbance (PADISÁK, 1993). Although, by October, diversity slightly decreased, most likely as a result of increased competition in low disturbance conditions, according to HUSTON (1979).

Similarly to RINDI and BATTELLI (2005) and SOININEN (2003), we determined that on a scale from 1 m upwards there was significant spatial autocorrelation in all the seasonal data sets. In addition, the correlation on large spatial scales was stronger in October. We conclude that the spatial structure in autumn was more developed, durable and resilient due to maturity and stability of communities that also harbored species other than pioneers. Conversely, the 10 cm scale did not show spatial autocorrelation in most samples, thus agreeing with results of KOMÁREK (2003) and COLEMAN (2002), who found the small-scale differences along transects of benthic algae non-significant up to a limit of tens of centimeters. The 10 cm distance between samples might, therefore, be considered as the minimal spatial limit for species composition differences in benthic microalgal assemblages, especially in studies based on morphological species concepts. However, in our study, the 10 cm data in

spring benthic microhabitat and summer submerged moss microhabitat were spatially autocorrelated. Similarly to species composition data, we propose the effect of spring succession following winter disturbance of the benthic community as a possible explanation. The low ability of propagules to disperse (UNDERWOOD and CHAPMAN, 1996), or the lower dispersal capacity of whole organisms (HILLEBRAND *et al.*, 2001) was suggested as the cause of spatial autocorrelation. Alternatively, the niche-based approach assumes that individuals have different fitness according to different environmental conditions (SOININEN, 2007). In this situation, spatial autocorrelation of species composition correlates primarily with similarities in their local environmental characteristics (abiotic factors – as *e.g.*, in RINDI and BATELLI, 2005; CHARLES *et al.*, 2006 – or biotic factors *e.g.*, COLEMAN, 2002; UNDERWOOD and CHAPMAN, 1996). This model presumes that dispersal limitations do not matter (at least within an investigated region), and so the species pools of individual localities are more or less identical. However, in our data the spatial distance significantly affected species composition at different scales, especially in spring, even after the effects of important environmental factors (pH, microhabitat type and conductivity) were removed. This clearly indicates that the dispersal limitation effect could play a significant role, at least in early succession stages of phytobenthic communities, even on small spatial scales.

In conclusion, the small-scale processes, microhabitat type, geographic distance and conductivity were found to influence species composition of benthic assemblages in the shallow peat bog habitat. Spatial distance was the prime factor, especially in spring, when the patchiness of a benthic community was at its greatest, possibly as a consequence of niche colonization following winter disturbance. The significant spatial effect found on larger scales corresponds well to data reported by others.

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Paper 2

Small-scale spatial variation of benthic algal assemblages in a peat bog

Kateřina Černá

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Small-scale spatial variation of benthic algal assemblages in a peat bog

Kateřina Černá *

Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ 12801, Prague 2, Czech Republic

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ABSTRACT

Spatial patterns on a very small scale (10 cm), and the effect of artificial barriers on the composition of phytobenthic algal assemblages along two transects within different microhabitat types were investigated. Samples were taken in a peat bog along linear transects on a scale of 10 cm, and water chemistry was examined. The distribution of algae along both transects was influenced by both spatial distance and environmental conditions in similar proportions. Differences in species composition in various parts of the transects were observed, but this pattern was primarily related to the abundance of species, rather than to their presence/absence in samples. Similarity in species composition correlated with spatial distance and environmental parameters in both microhabitat types. I concluded that, given a homogenous environment on a small scale, spatial distribution of algae is affected by both the environmental conditions of the microhabitats and their dispersal limitations. Moreover, an artificial barrier constituted an obstruction for water and nutrient flow, as well as algal migration, and had an impact on species composition.

Introduction

Variation in distribution, abundance and composition of species in relation to environmental conditions or spatial scale are important for our understanding of the ecology and diversity of organisms. We are able to identify general factors that are significant for individual spatial scales in marine and freshwater habitats. On a macroscale (kilometers or greater) differences in species composition of marine algal assemblages are principally influenced by hydrodynamics (Méléder et al., 2007), or by the texture and composition of rocky substratum (Rindi and Battelli (2005)); in freshwater habitats they largely correlate with basic chemical variables (e.g. hardness, Mg, Ca, conductivity, SO_4, total solids, conductivity, total P), geographic location and spatial-dispersal factors (Soininen et al., 2004). On a mesoscale (tens of meters or greater), spatial heterogeneity of algal assemblages seems to be determined by several factors, including: marine habitat abiotic conditions, granulometric composition of substrate (Saburova et al., 1995), phenological patterns and input of propagules (Santelices, 1990; Rindi and Guiry, 2004), variations in recruitment (Menge et al., 1993), and substratum heterogeneity (Archambault and Bourget, 1996). In rivers, factors related to water quality, such as BOD (biological oxygen demand), P, NH_4 and turbidity were the most important pressures driving heterogeneity of algal assemblages on an intermediate spatial scale (Charles et al., 2006). In addition, heterogeneity of diatom communities was shown to be induced by changes in light and current regimes, grazing, successional stages and variation in substratum (Ledger and Hildrew, 1998; Soininen, 2003). Small-scale biotic interactions, such as competition, grazing, colonization processes (input of propagules), variations in recruitment, or low movement ability affected composition of algae species both in marine and freshwater habitats on a scale of centimeters or greater (Saburova et al., 1995; Rindi and Cinelli, 2000; Hillebrand et al., 2001; Coleman, 2002). At the same time, physical and chemical parameters creating small-scale patchiness of microhabitat (Underwood and Chapman, 1996), substrate complexity and heterogeneity (Komárek, 2003), and water current effect (Passy, 2001) also play an important role.

Knowledge of the spatial distribution of species in phytobenthos is important prior to identification and description of their diversity. In order to describe the ecology, occurrence patterns, or abundance of species of any assemblage, it is necessary to determine the scale of its variation. If, for example, small-scale variation goes undetected, differences due to impacts may be confused with differences due to natural spatial variability (Underwood, 1993; Coleman, 2002). That is, if the spatial scale sampled is greater than the scales of natural spatial variation then effects may be assumed that do not really exist; the perceived impact simply being a result of small-scale spatial variation (Coleman, 2002). Spatial patterns of species diversity provide important clues about the underlying mechanisms that regulate biodiversity and are central in the development of biodiversity theory (Hubbell, 2001). Assumptions regarding the spatial scaling

* Tel.: +420221951647; fax: +420221951645.
E-mail address: kaca.cerna@gmail.com

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of biodiversity are a fundamental component of conservation biology, and are frequently used to identify local- and global-scale priority conservation areas (Desmet and Cowling, 2004).

This study follows our previous study (Machová-Černá and Neustupa, 2009) concerning spatial distribution of algae in a lowland peat bog. In that study, we found that the spatial heterogeneity of algal assemblages was mainly influenced by seasonal succession related to winter disturbance, microhabitat type, spatial distance between samples and conductivity. The pattern of spatial autocorrelation was observed on scales of 1 m and greater, but it could not be established on a scale of 10 cm; this concurs with the results of Coleman (2002) or Komárek (2003). Consequently, in the present study I focused on the spatial structure of algal assemblages in the homogenous environment of an acidic mountain peat bog, and only on a scale of 10 cm; my primary concern being the effect of artificial barriers in the natural environment on the species composition of algal assemblages. I attempted to determine if there is a difference in spatial structure of benthic algal assemblages on a small scale in a lowland and mountain peat bog. I utilized similar statistical methods in order to compare these results. Therefore, I asked the following questions: (1) Can I identify alterations in species composition of algal assemblages caused by artificial barriers two years after revitalization? and (2) Are there differences in the small-scale spatial structure of benthic algal assemblages in two distinct microhabitat types (epipelon and periphyton within Sphagnum moss)?

Materials and methods

The locality examined, "Mlynářská slať" peat bog, is situated in the Bohemian Forest National Park in the south of the Czech Republic (49°0′26″N, 13°28′26″E). This peat bog is surrounded by a pine grove and largely overgrown with spruce. In the past, the locality was intensively used in forestry and many channels were excavated to drain the water. In 2004, a revitalization process took place wherein the drainage channels were artificially blocked by sheet pile walls to retain the natural height of the water and to prevent water outflow.

Samples of cyanobacteria and algae were collected on 24th September 2006 from two drainage channels with different microhabitat types: phytobenthos from the fine-grain bottom of the first drainage channel, and periphyton within the *Sphagnum* biomass overgrown the second channel surface. Samples were taken along linear transects 3 m long in epipelon, and 5 m long in the moss biomass; these were divided in the middle by a sheet pile wall (Fig. 1). Sampling sites were 10 cm apart, and sampling was conducted either with a glass pipette (from the bottom), or by squeezing (for the moss). The pH and electrical conductivity were measured in the field using Hanna portable combined pH/conductometer. Concentrations of total nitrogen and total phosphorus were determined colorimetrically with a continuous flow analyzer (FIA-STAR, Tecator, Sweden). Total nitrogen (TN) was determined following the reduction of all nitrogen forms in the sample to nitrates with perchloric acid. Subsequent reduction of nitrates to nitrites was achieved in a Cd–Cu column and reaction with sulfanilamide and N-(1-naphthyl)-ethylenediamine producing a final pink color that was detected at 540 nm. Total phosphorus concentrations (TP) were estimated following the mineralization of the samples by persulphate (Grasshoff et al., 1983) that converted all phosphorus forms into dissolved reactive phosphorus, and its concentration was subsequently estimated using the standard phosphomolybdenum complex method (Parsons et al., 1984). The final intensive blue color was quantified at 690 nm. Humic acid concentrations were established spectrophotometrically, if acidic conditions, after their extraction to pentanol and then to NaOH, according to TNV 757536 (2003).

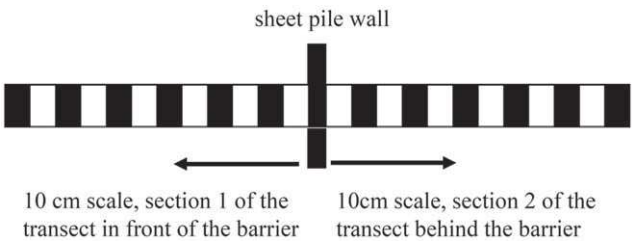

Fig. 1. The sampling design indicating positions of individual samples along the linear transect. Samples were collected on a 10 cm scale on both sides of an artificial barrier in drainage channels on "Mlynářská slať" peat bog (Czech Republic) in September 2006.

Algal species were identified to the lowest possible taxonomic level, within three days of collection without fixation, by Olympus BX51 light microscopy. Diatoms were identified in mineralized samples mounted on Naphrax permanent slides (Houk, 2003). For each sample, one microscope slide was prepared to observe living cells for algae identification, and one prepared to observe and identify diatoms. The identification of algal taxa was based on standard taxonomic monographs (Süsswasserflora von Mitteleuropa, Diatoms of Europe, etc. – for references see e.g. Veselá, 2009). Semiquantitative estimates of algal populations were deduced from microscopic examination, and species were classified into three categories: (1) up to 1% of cells revealed, (2) up to 50% of cells found, and (3) more than 50% of cells of a particular assemblage detected by light microscopy (Kinross et al., 1993; Komárek and Sukačová, 2004). Approximately 500 cells per sample were examined.

Non-metric multidimensional scaling (NMDS) was used to display the major patterns in the species' composition data using the PRIMER® (Plymouth Routines In Multivariate Ecology, PRIMER-E Ltd., Plymouth, UK) software package. One hundred random starts were carried out in 2- and 3-dimensional analyses to reduce the chance of local optima (Clarke and Warwick, 2001). In both cases, the 3-dimensional solutions had lower stress values than the 2-dimensional ones, but we utilized the 2-dimensional data because of its superior representation of sample position. Resemblance of species composition between sites was assessed by Bray–Curtis similarity index (Bray and Curtis, 1957; Méléder et al., 2007). To retain the contribution of each species, according to its semiquantitative abundance, we made no data transformations (Clarke et al., 2006). The possible role of the sheet pile walls on differences in species composition on either side of the barrier was evaluated using non-parametric two-group ANOSIM tests based on Bray–Curtis similarity index performed with PAST software (ver. 1.81; Hammer et al., 2001) using 10,000 permutations. Subsequent SIMPER (similarity percentage) analyses, carried out in PRIMER®, were used to identify species typical in specified sections along the transect, and species responsible for the differentiation between the two divisions of the transects (Clarke and Warwick, 2001; Méléder et al., 2007). The effect of spatial distance and abiotic parameters on the similarity of algal composition along the transect was evaluated using simple and partial Mantel tests (Mantel, 1967; Smouse et al., 1986). Mantel tests were calculated for the entire length of transects in benthic and moss substratum types, and independently for both sides of the transect separated by the sheet pile wall. The similarity matrix was calculated from paired comparisons of species composition between two samples using Bray–Curtis similarity index, and matrices of environmental distances were composed of unsigned differences among values of variables for all possible pairs of samples. The significance of correlations was tested with simulation of 10,000 randomizations. Mantel tests were performed using *zt* software (ver. 1.0; Bonnet and Van der Peer, 2002). The effects

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of environmental (pH, conductivity, total nitrogen, total phosphorus, humic acids) and spatial parameters (geographical distance of samples from the beginning of transect and location on the transect) on species composition were evaluated using linear ordination techniques, including redundancy analysis (RDA) and partial RDA (Lepš and Šmilauer, 2000). Percentage of variation in species data explained by environmental and/or spatial factors was partitioned according to Borcard et al. (1992). Ordination methods were performed in CANOCO for Windows version 4.5 (ter Braak and Šmilauer, 1998, 2002).

Results

Measured abiotic parameters are presented in Table 1. Generally, pH was higher in the benthic microhabitat, but all other chemical parameters were higher in the submerged *Sphagnum* moss microhabitat. In both microhabitat types the abiotic parameters spanned a wider range of values in the second section of the transects.

Non-metric multidimensional scaling (NMDS) analyses illustrated a difference in the order between benthic and periphytic samples in the plots (Fig. 2). In the case of benthic samples, we observed clear separation of samples collected in front of vs. behind the barrier. In addition, the cloud of points in the ordination plot representing samples taken from the beginning portion of the transect was more condensed in contrast to those representing samples from the second part of the transect that were expanded. This pattern was also observed on the ordination plot of moss squeezing samples from periphyton. But, in contrast to benthic samples, in the case of periphytic samples, we did not observe a clear separation between the two parts of the transect.

The ANOSIM tests confirmed statistically significant differences in species composition based on Bray–Curtis similarity index in both studied transects (benthos $r=0.392$, $p<0.0001$, periphyton $r=0.233$, $p<0.0001$). The SIMPER analyses detected species identifying individual parts of the transects (Table 2). The algal assemblages from specific sections of the transects in benthos and periphyton did not essentially differ in species composition, but rather, in the relative abundance of the species. The benthic algal assemblages (Table 2a) were composed of a higher number and diversity of species (species richness=54, Shannon diversity index=6.07), and were characterized by the occurrence of ciliates (e.g. *Euglena sp.*, *Trachelomonas abrupta*, *T. hispida*), desmids (e.g. *Closterium intermedium*, *Hyalotheca dissiliens*, *Stuarodesmus triangularis*) and diatoms (e.g. *Caloneis alpestris*, *E. bilunaris var. mucophila*, *E. exigua*, *Frustulia saxonica*). Average dissimilarity between the two portions of this transect was 31.3%. The periphyton algal assemblages (Table 2b) were principally characterized by lower species diversity (species richness=30, Shannon diversity index=3.95), and the occurrence of diatoms (*Eunotia glacialis*, *E. bilunaris var. mucophila*, *Pinnularia rupestris*) and filamentous green algae (*Microspora tumidula*, *Microthamnion kuetzingianum*, *Mougeotia sp.*). Average dissimilarity between the two divisions of the transect was 49%. Similarity in species composition of benthic assemblages was statistically significantly spatially correlated in both parts of the transect and along the entire length of the transect, even when using covariates (Table 3). I found only total phosphorus and humic acids as having an effect on the similarity in species composition. I also found correlations between pH and other abiotic parameters (conductivity, total nitrogen, total phosphorus and humic acids), as well as between total phosphorus and humic acid concentrations. The pH, total nitrogen, total phosphorus, and humic acids were spatially correlated and indicated a gradient of these factors. Similarity in species composition of periphytic assemblages was also spatially correlated in the second section of the transect behind the barrier, and along the entire length of the transect, even when using covariates (Table 3). It was determined that pH, conductivity, total nitrogen and humic acids exerted an effect on the similarity in species composition, but only on individual parts of the transect or only over its entire length. I found correlations between all pairs of abiotic parameters, and all abiotic parameters were spatially correlated on at least one part of the transect (Table 3).

In the redundancy and partial redundancy analyses performed for benthic species composition data 17.7% of the variation was explained by environmental data (including: pH, conductivity, total phosphorus, nitrogen, and humic acids), 13.5% of the variation was accounted for by spatial data (spatial distance of samples

Table 1
The environmental characteristics of the transects investigated in drainage channels on "Mlynářská slať" peat bog in the Czech Republic in September 2006.

	Benthos part 1	Benthos part 2	Periphyton part 1	Periphyton part 2
pH	5.13–5.37	5.06–5.42	3.78–3.93	3.61–3.87
Conductivity [$\mu S\ cm^{-1}$]	13–24	8–18	12–15	15–21
Total nitrogen [$\mu g\ l^{-1}$]	430.3–584.9	466.2–762.9	523.2–807.3	544.2–1325.9
Total phosphorus [$\mu g\ l^{-1}$]	81.9–91.4	77.9–98.1	79.03–110.1	82.4–140.8
Humic acids [$mg\ l^{-1}$]	15.2–19.8	14.9–21.9	22.6–29.8	24.9–50.7

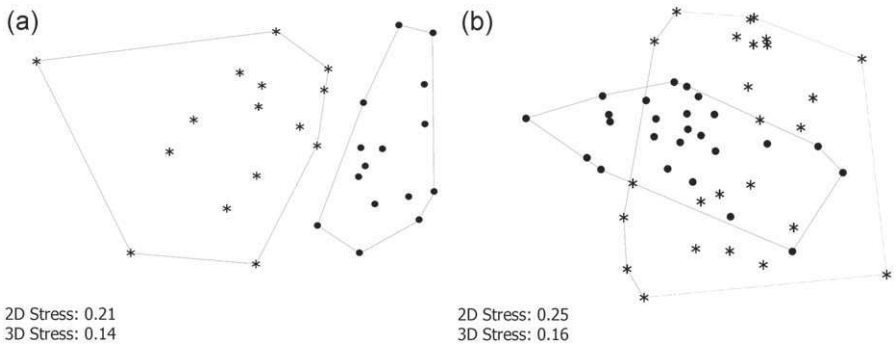

Fig. 2. Non-metric multidimensional scaling (NMDS) of data collected from two transects on the study site "Mlynářská slať" peat bog (Czech Republic) in September 2006. (a) A transect in the benthic microhabitat, (b) A transect in the periphitic microhabitat (● and ∗ indicate samples taken in front of, and behind the barrier, respectively).

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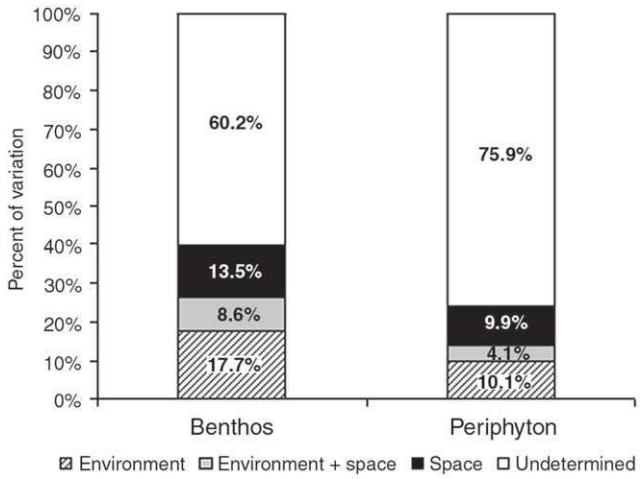

Fig. 3. Variation partitioning of the transects' data matrices.

from the beginning of the transect and location along the transect), 8.6% of the variation was explained by both environmental and spatial data, and the cause of 60.2% of the variation remained undetermined (Fig. 3). In the periphytic species composition data 10.1% of the variation was explained by environmental data, 9.9% of the variation was described by spatial data, 4.1% of the variation was accounted for by both environmental and spatial data, and the reasons for 75.9% of the variation remained undetermined (Fig. 3).

Discussion

The algal assemblages of transects investigated were characterized by species typical for acidic and oligotrophic peat bog localities (Coesel, 1982; Mataloni, 1999; Neustupa et al., 2009). The occurrence of species was principally influenced by the severe conditions of their microhabitats, especially in periphyton within moss (low pH and conductivity) that are typically characterized by low species diversity (Coesel, 1982; Mataloni, 1999). The measured values for total phosphorus, especially in the *Sphagnum*-dominated microhabitat, are comparable to those observed in Finnish boreal peatland surface waters having a similar pH (Tolonen and Hosiaisluoma, 1978). Higher amounts of total nitrogen and total phosphorus in *Sphagnum*-dominated microhabitats could be related to a lower pH that consequently affected the slower decomposition rate and reduced biomass of nutrient consumers – microbes and algae (Walbridge and Navaratnam, 2006). The pattern of differences in species composition was principally related to the division of this transect into two sections by artificial barriers, as illustrated by the NMDS plots. The differences were clearly evident in benthic samples. This pattern could be related to changes in small-scale environmental conditions of microhabitats characterized by the variations in abiotic parameters between the two portions of the investigated transect, or by a unique colonization history in each part, with a consequent dissimilarity in species composition of algal assemblages. The condensed ordination plots of samples from the initial sections of both transects, compared to the more expanded ordination plots of samples taken further along the transects, could be related to a narrower vs. wider range of abiotic parameters. Differences in species composition were associated more with the abundance of individual species than with the presence/absence of different species in specific parts of the transects. This pattern was also described previously for algal assemblages in an acidic lowland peat bog (Machová-Černá and Neustupa, 2009), and I concluded that these differences were related to the environmental conditions of the microhabitats studied (low pH and conductivity); conditions that generally support a smaller number of species (Coesel, 1982; Mataloni, 1999). I observed a strong spatial correlation of species composition in both microhabitat types even when using covariates. But I also observed that environmental parameters influenced species distribution – in benthos, total phosphorus and humic acids affected similarity in species composition; in periphyton, pH, conductivity, total nitrogen and humic acids impacted the similarity of algal assemblages. These correlations were related to gradients of environmental parameters that were revealed along the transects. My data describing spatial correlation on a scale of 10 cm are generally incongruent with results from spatial structure studies of algal assemblages in streams (Komárek, 2003), lowland peat bogs (Machová-Černá and Neustupa, 2009), or of marine algae (Coleman, 2002); in these cases, either no, or a very rare pattern of spatial autocorrelation has been revealed on this scale. On the other hand, I observed a pattern of spatial autocorrelation on a scale of 10 cm in both microhabitat types. The pattern of spatial autocorrelation in a homogenous environment without the presence of a water current could be explained either by the limited ability for motion of propagules (Underwood and Chapman, 1996), or of whole organisms (Hillebrand et al., 2001), related to their small size or the absence of flagella. Another explanation is the effect of the environment itself that influences the fitness, and thus, the occurrence of individual organisms under specific environmental conditions (niche-based approach; Soininen, 2007). I suppose that both explanations are partially accurate, as both spatial correlation and influence of environmental parameters on spatial distribution, as well as a similarity among algal assemblages were found in our study. Results of RDA and partial RDA analyses appeared to correspond well with the results of Mantel tests that established a significant effect of geographic distance and environmental parameters on species composition. Similarly, with my data, these analyses showed that a high proportion of the observed variation could be explained either by environmental descriptors or by the spatial matrix. The spatially accounted for variation could also be considered a synthetic descriptor of unmeasured underlying processes such as external causes or biotic factors (e.g. social aggregation; Borcard et al., 1992). The variation explained by environmental and spatial parameters combined made up about one fifth of the observed variation. I speculate that this accounted for variation that is related to spatial correlation of environmental parameters along observed transects in both microhabitats, and corresponds to a somewhat similar spatial structuring of both the species and the environmental data. The large amount of unexplained variation, especially in the case of the periphytic analyses, could be related to either indeterminate overlooked, or insufficiently and/or inaccurately measured factors (especially biotic factors which are difficult to measure); alternatively, it might be due to a large amount of stochastic processes. However, the exact reasons remain unclear, and the ultimate causal factors cannot be ascertained from ecological studies of natural assemblages alone, without experimental research as well.

It is doubtless that the sheet pile walls established a barrier to water and nutrient flow, and to algal migration or movement with water current. This consequently leads to variations in environmental conditions and differences in algal species composition. Conversely, I believe that these barriers did not affect processes such as colonization, migration via other means, or niche differentiation. Thus, I conclude that species composition is more influenced by environmental conditions and small-scale processes (e.g. niche differentiation, competition, grazing) than by the barrier. In benthos the impact of the barrier and the

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Table 2
SIMPER analyses: species typical in specific portions of transects on "Mlynářská slat'" peat bog (Czech Republic), and species responsible for the differentiation of these sections. S_i (%) – percentage contribution of individual species to the intra-group similarity, S_i:SD (S_i) – contribution of individual species to intra-group similarity to standard deviation of the contribution ratio, contrib% – contribution in % of each species to the total similarity or dissimilarity, av.abundance 1, 2 – average abundance of species in the first or the second section of the transect.

(*A*). *The transect in the benthic microhabitat*

Species typifying the section in front of the barrier (Average similarity 74.9%)

	S_i (%)	S_i:SD(S_i)	Contrib%
Eunotia bilunaris var. mucophila	6.3	8.21	8.4
Trachelomonas hispida var. hispida	6.3	8.47	8.4
Eunotia exigua	6.01	6.77	8.02
T. hispida var. crenulatocollis	4.83	5.68	6.45
Pinnularia subcapitata var. subcapitata	4.54	6.94	6.05
Hyalotheca dissiliens	4.47	9.58	5.96
Frustulia saxonica	4.41	14.52	5.88
Caloneis alpestris	4.41	14.52	5.88
Placoneis paraelginensis	4.18	2.77	5.57
Eunotia glacialis	2.77	2.98	3.7
Pinnularia anglica	2.26	1.34	3.02

Species typifying the section behind the barrier (Average similarity 71.4%)

	S_i (%)	S_i:SD(S_i)	Contrib%
Eunotia bilunaris var. mucophila	7.78	9.37	10.89
Trachelomonas hispida var. hispida	7.47	5.48	10.46
Eunotia exigua	7.07	6.19	9.89
Pinnularia subcapitata var. subcapitata	4.79	5.68	6.7
Frustulia saxonica	4.5	2.43	6.29
Synura sp.	4.4	2.0	6.15
Caloneis alpestris	3.26	2.9	4.56
Placoneis paraelginensis	3.26	2.95	4.56
Hyalotheca dissiliens	2.69	1.43	3.76
Pseudanabaena sp.	2.46	2.03	3.44
Eunotia glacialis	2.36	2.08	3.3
T. hispida var. crenulatocollis	2.19	0.85	3.06

Species discriminating between the two parts of the transect (Average dissimilarity 31.3%)

	Av. abund 1	Av. abund 2	Av. dissimilarity	Contrib%
T. hispida var. crenulatocollis	2.47	1.47	1.37	4.37
Stauroneis anceps	1.27	0.2	1.31	4.18
Synura sp.	1.27	2.07	1.26	4.01
T.abrupta var. minor	1.07	0.07	1.21	3.86
Cryptomonas sp.	1.0	0.93	1.17	3.72
Pseudanabaena sp.	0.87	1.27	1.09	3.49
Placoneis paraelginensis	2.27	1.53	1.07	3.4
Microspora tumidula	1.2	0.73	1.0	3.19

(*B*). *The transect in the periphytic microhabitat*

Species typifying the section in front of the barrier (Average similarity 61.3%)

	S_i (%)	S_i:SD(S_i)	Contrib%
Eunotia bilunaris var. mucophila	17.36	4.18	28.33
Mougeotia sp.	15.3	1.56	24.97
Pinnularia rupestris	7.47	1.16	12.18
Eunotia glacialis	7.19	1.28	11.72
Microspora tumidula	6.29	1.31	10.27
Cryptomonas sp.	3.06	0.59	4.99

Species typifying the section behind the barrier (Average similarity 51.5%)

	S_i (%)	S_i:SD(S_i)	Contrib%
Eunotia bilunaris var. mucophila	15.23	2.82	29.56
Pinnularia rupestris	11.83	1.62	22.96
Mougeotia sp.	7.11	1.15	13.8
Microspora tumidula	4.29	0.61	8.33
Microthamnion kuetzingianum	3.28	0.67	6.36
Eunotia exigua	3.09	0.68	5.99
Eunotia glacialis	2.33	0.63	4.53

Species discriminating between the two parts of the transect (Average dissimilarity 49%)

	Av. abund. 1	Av. abund. 2	Av. dissimilarity	Contrib%
Mougeotia sp.	2.36	1.48	5.46	11.15
Pinnularia rupestris	1.44	2.08	4.41	8.99
Eunotia glacialis	1.44	0.88	4.38	8.94
Microspora tumidula	1.28	1.12	4.35	8.88
Microthamnion kuetzingianum	0.76	1.16	4.1	8.36
Eunotia exigua	0.08	1.16	3.87	7.89
Cryptomonas sp.	0.92	0.24	3.72	7.6
Eunotia bilunaris var. mucophila	2.48	2.4	2.68	5.48
Placoneis paraelginensis	0.44	0.48	2.38	4.86

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Table 3
Results of simple and partial Mantel tests calculated through the use of different types of matrices over the entire transect in individual microhabitat type, and for the two divisions of each transect on "Mlynářská slať" peat bog (Czech Republic), *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. (Results for the entire transect are indicated in bold; results for the section in front of the barrier are indicated in italics, and results for the portion behind the barrier are indicated in standard).

	Transect in benthos	Transect in periphyton
Similarity × distance	−0.502***/−0.468***/**−0.227****	*−0.1*/−0.166*/**−0.203*****
Similarity × distance × pH	*−0.489****/−0.492***/**−0.226****	*−0.109*/−0.166*/**−0.164****
Similarity × distance × conductivity	*−0.502****/−0.467***/**−0.235*****	*0.015*/−0.167*/**−0.196*****
Similarity × distance × TN	*−0.519****/−0.465***/**−0.213****	*−0.068*/−0.167*/**−0.17****
Similarity × distance × TP	*−0.527****/−0.123/**−0.216****	*−0.116*/−0.17*/**−0.203*****
Similarity × distance × humic acids	*−0.507****/−0.409**/**−0.213****	*−0.1*/−0.244**/**−0.194*****
Similarity × pH	*−0.137*/−0.089/**−0.16**	*0.038*/0.022/**−0.147***
Similarity × conductivity	*−0.01*/−0.03/**0.139**	*−0.217**/0.046/**−0.64**
Similarity × total nitrogen	*−0.039*/0.068/**−0.163**	*−0.235*/0.017/**−0.143***
Similarity × total phosporus	*0.071*/0.55***/**−0.366****	*0.082*/0.139/**−0.21**
Similarity × humic acids	*0.128*/−0.267*/**−0.08**	*−0.018*/0.162*/**−0.072**
pH × distance	*0.34***/0.505**/**0.097**	*0.173**/0.001/**0.349*****
pH × conductivity	*0.003*/0.348**/**0.186**	*0.126*/0.316**/**0.164***
pH × TN	*0.006*/0.252*/**0.064**	*−0.056*/0.489**/**0.486*****
pH × TP	*0.41**/0.414**/**0.415*****	*0.224**/0.557***/**0.483*****
pH × humic acids	*−0.136*/0.563***/**0.294****	*0.118*/0.448**/**0.5*****
Conductivity × distance	*0.02*/0.063/**0.048**	*0.52****/0.027/**0.457*****
Conductivity × TN	*−0.094*/0.17/**−0.133**	*−0.075*/0.387**/**0.517*****
Conductivity × TP	*0.181*/0.242/**0.065**	*−0.043*/0.134/**0.252***
Conductivity × humic acids	*0.4*/0.148/**0.115**	*0.154**/0.292*/**0.42*****
TN × distance	*−0.187**/−0.08/**−0.01**	*0.148*/0.048/**0.3*****
TN × TP	*−0.18*/−0.004/**0.047**	*−0.148**/0.57**/**0.529*****
TN × humic acids	*0.089*/0.105/**−0.007**	*0.2**/0.471**/**0.505*****
TP × distance	*0.192*/0.721***/**0.07**	*0.167**/0.02/**0.201*****
TP × humic acids	*−0.005*/0.453**/**0.297****	*0.084*/0.654***/**0.647*****
Humic acids × distance	*0.009*/0.705***/**0.285*****	*0.336****/0.361***/**0.499*****

resulting small-scale alterations in environmental conditions seemed to be of greater significance and have more influence on the variation in species composition throughout the transect. Furthermore, I concluded that the two years separation of the microhabitat by the sheet pile walls represents a relatively brief time period for the formation of diverse algal assemblages.

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Paper 3

The pH-related morphological variation of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic

Kateřina Černá & Jiří Neustupa

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The pH-related morphological variations of two acidophilic species of Desmidiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic

Kateřina Černá · Jiří Neustupa

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Abstract Morphological variation related to pH was investigated in two acidophilic desmid species (*Euastrum binale* var. *gutwinskii* and *Staurastrum hirsutum*) utilizing geometric morphometric methods. Clones isolated from acidic habitats were cultured using a range of pH values from 3.5 to 6.5. The plasticity of ensuing populations was quantified and illustrated by the general Procrustes superimposition of landmarks placed along the outline of cells and subsequent statistical analyses of shape data. In both species, there was a significant effect of pH on the morphology of cells. In *Staurastrum hirsutum*, the pH-related morphological change was accompanied by a decrease in the size of cells cultured at a higher pH. However, in *Euastrum binale*, cell size did not differ in relation to pH, but cell shape was characterized by a deepening of the incisions between cell lobes at higher pH. In both species, cell complexity based on surface-to-volume ratio was positively correlated with increasing pH. We conclude that by manipulating their surface-to-volume ratios, these desmid species can respond to pH variations in their environment.

K. Černá (✉) · J. Neustupa
Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 01 Prague, Czech Republic
e-mail: kaca.cerna@gmail.com

Keywords Desmidiales · Geometric morphometrics · pH · S/V ratio · Green algae · Peat bogs

Introduction

Desmids are typically unicellular or filamentous microorganisms belonging to the green algae. Generally, they occur in phytobenthos of standing freshwater habitats with the majority of the species inhabiting oligo-to mesotrophic, slightly acidic wetlands. Members of Desmidiales also often dominate in phytobenthos of pronouncedly acidic peat bogs with pH values varying from 3.5 to 5.0. The environmental conditions of these low-pH habitats are often correlated with low diversity of benthic microorganisms (Blouin 1989; Mataloni 1999; Ceosel and Meesters 2007). Highly acidic pH can be damaging to cell walls as it weakens hydrogen bonds in the cellulose strands comprising the walls and can result in uncontrolled cell expansion (Gross 2000). In addition, cells in these conditions may be placed under stress by the need to maintain the neutral pH of their cytoplasm as the H^+ ions continuously penetrate their plasmatic membrane (Gimmler and Weis 1992). Several adaptive physiological mechanisms of microalgae living in low-pH conditions were suggested (Gimmler 2001; Gerloff-Elias et al. 2005), e.g. maintaining a positive membrane potential and a positive charge outside the

plasmatic membrane (Remis et al. 1994), decreasing permeability of protons through the plasmatic membrane or sustaining active proton pumping (Gross 2000; Gimmler 2001). Additionally, morphological adaptations of microalgae to conditions of extremely low pH were also proposed (Nixdorf et al. 2001). Generally, cells need to have low surface-to-volume ratios (S/V ratios) in order to minimize the stress posed by H^+ ions penetrating their walls and membranes. Clonal strains of protists with versatile cell shape are known to rapidly adjust their S/V ratios by expanding their cells (Weisse and Stadler 2006; Weisse et al. 2007). Similarly, the microalgae strains with cell wall may simplify their cell morphology in subsequent generations in order to adaptively manipulate the S/V ratio (Neustupa and Hodač 2005; Neustupa et al. 2008). The morphologically elaborate cells of many desmid species have high S/V ratios in compared with other microalgae (Padisák et al. 2003). This feature has been considered advantageous in oligotrophic conditions as it maximizes the surface area available for nutrients uptake (Coesel 1982). However, Coesel (1982) also mentioned that desmids inhabiting highly oligotrophic and acidic localities (e.g. elevated boreal peat bogs) tended to have lower S/V ratios than desmids from assemblages in localities with higher pH. In extremely acidic conditions, the pH may act as the critical environmental factor driving shape features of desmid cells. Species with a lower S/V ratio may have higher fitness under low-pH conditions, because of their lower surface area and reduced exposure to the acidic environment. However, pH of natural localities varies due to different biotic and abiotic processes (Rydin and Jeglum 2006). Photosynthesis and respiration of algae and other photosynthetic organisms cause diurnal changes in water pH and carbon/oxygen availability. During respiration, CO_2 is released into the water, causing the pH to decline. Conversely, during photosynthesis, CO_2 is utilized by photosynthetic organisms, and the pH increases (Odum 1956; Edwards and Owens 1965). Also, pH levels often vary within same locality. This variability is influenced by the moisture-aeration regime (Rydin and Jeglum 2006), or microhabitat types in the area (e.g. tychoplanktonic communities of the open water of peat-pits, benthic communities of the water-filled shallow hollows or emergent communities in mossy vegetation (Coesel 1982; Mataloni 1999). Interestingly, individual traditionally defined desmid species typically inhabiting acidic peat bog localities are known to occur at a range of different pHs e.g. *Closterium striolatum*—4.0 to 7.0, *Euastrum binale* var. *gutwinskii*—3.9 to 7.5, *Penium cylindrus*—4.5 to 6.8 and *Tetmemorus laevis*—3.8 to 7.0, (Růžička 1977, 1981; Negro et al. 2003; Ceosel and Meesters 2007; Neustupa et al. 2009). These very different H^+ concentrations produce quite different conditions for individual desmid populations, inducing them to manifest adaptive plastic responses.

In this study, we investigated two strains of frequently co-occurring acidophilic species of Desmidiales. The strains were isolated from acidic Central European peat bog habitats. They were cultivated at pH values ranging from 3.5 to 6.5. Our central question was whether these species exhibited a plastic response to varying the pH of their environment, and if the pH influenced their morphology and S/V ratios. We employed geometric morphometrics (e.g. Zelditch et al. 2004) to examine and quantify the morphological plasticity of experimental clonal populations cultured under various pH conditions, so that we could ascertain and separate their subsequent shape-related and size-related plastic responses.

Materials and methods

The strains of *Euastrum binale* var. *gutwinskii* (Schmidle) Homfeld (CAUP K 503) and *Staurastrum hirsutum* (CAUP K 302) used in this study were isolated as the single cell isolates from benthos of a Břehyně lowland peat bog near Doksy, Czech Republic (pH 3.8–5.5, conductivity 80–170 μS/cm) in September 2006. The isolated cells were cultured in 50-ml Erlenmeyer flasks with liquid CAUP oligotrophic medium (http://botany.natur.cuni.cz/algo/caup.html) in a series of seven different pH levels in gradations of 0.5 pH (3.5–6.5), at 17°C and day:night (14:10 hours) light regime for 30 days. The designated pH was maintained using 5-mM MES buffer. The initial inoculum was 0.5 ml of algal suspension (aprox. 100,000 cells), and there were ca. 20–45 divisions in individual pH levels. At each pH tested, 60 randomly chosen cells were photographed using Olympus BX51 light microscope and Olympus Z5060 digital photographic equipment. Thus, for each species, we photographed and analysed a total of 420 cells.

The two-dimensional morphology of cells was investigated using geometric morphometric methods, and for most of the analyses, the TPS-series software (Rohlf 2006) was used. In total, 60 landmarks depicting the frontal outline of the cells (Fig. 1), and length and width of the cells in the frontal view were digitized in TpsDig, ver. 2.05. The landmark configurations were superimposed by generalized Procrustes analysis (GPA) in TpsRelw, ver. 1.42. We used the generalized Procrustes superimposition with semilandmark registration, such that the resulting Procrustes coordinates described the outline of individual cells. The single fixed landmark was placed on the apex of an older semicell, and all the other 59 semilandmarks were placed regularly along the investigated outlines as the sliding algorithm (following e.g. Bookstein 1997 or Zelditch et al. 2004) optimizes the inter-landmark distances. The principal component analyses (PCA) were carried out using the Procrustes aligned data of all cells of each species grown at different pHs. The morphological variations related to the changing pH values were characterized by multivariate regression of shape data with pH taken as the independent variable; TpsRegr, ver. 1.31 was used for this analysis. Significance of a regression model was evaluated by permutation tests (with 1,000 permutations) on Wilks′ λ and Goodall′s F-ratio (Zelditch et al. 2004; Rohlf 2006). Scores of the objects on first 10 PC axes (spanning 98.52% of the total variation) for *E. binale* and 15 PC axes (spanning 95.84% of the total variation) for *S. hirsutum* were used for canonical variates analyses (CVA) in PAST, ver. 1.81 (Hammer et al. 2001) to test for and visualize the differences between pH values. The number of PC axes chosen was estimated, so that all the axes with eigenvalues higher than the Jolliffe cut-off value (Jolliffe 1986) were included. In addition, the scores on PC axes were also used in the two-group multivariate permutation tests (with 2,000 permutations) on Mahalanobis distance between all pairs of the populations from different pH values to measure the significance of their shape differences.

As a part of the Procrustes superimposition, landmark coordinates are scaled to a unit size, so that the resulting data describe "pure" shape properties of the investigated objects with size differences removed (Zelditch et al. 2004). Therefore, in geometric morphometrics, the variations in size and shape, i.e. allometric changes (Zelditch et al. 2004) can be investigated separately. It is often interesting to ascertain whether shape differences are allometric or are unrelated to size. To determine the effect of allometry, the Procrustes aligned data were regressed on centroid size of the cells. Then, we took the residuals from this multiple regression and tested for the effect of pH on the size-unrelated fraction of cell

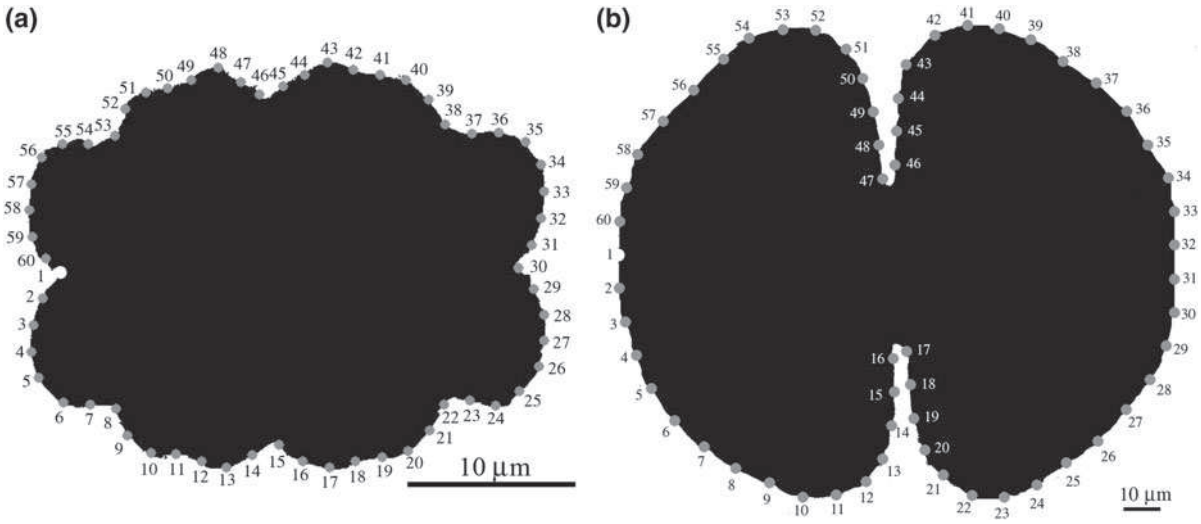

Fig. 1 Morphology of a vegetative cell of **a** *Euastrum binale* var. *gutwinskii* and **b** *Staurastrum hirsutum*, with landmark positions and numbers indicated. The single fixed landmark is shown by *white circle*. The semilandmarks are positioned counter clock-wise in regular intervals along the outlines

shape (Debat et al. 2003). The multivariate regression of size-unrelated shape data on pH (with cell size taken as a covariate) was conducted, and the canonical variates analysis (CVA) of size-unrelated shape data was made to test for the size-independent morphological separation of cells grown under specific pHs. Scores of the objects on first 10 PC axes (spanning 98.52% of the total variation) in *E.binale* and 13 PC axes (spanning 95.25% of the total variation) in *S. hirsutum* were used. Cell length, width and centroid size are illustrated as box plots.

Apart from multivariate datasets describing actual cell shape, the *complexity* of a cell's outline is also of interest. Generally, this measure indicates the deviation of a particular outline (spanned by landmark configuration) from a circular shape. We used the Procrustes aligned configurations (i.e. scaled to a unit size) and summed the Euclidean distances of all adjacent landmarks along an outline of all investigated cells. Then, we compared these values among individuals under specific pH conditions using permutation *t*-test with 10,000 permutations in PAST ver. 1.81 (Hammer et al. 2001).

Results

There was a significant effect of pH on the two-dimensional shape of both investigated strains. The multivariate regression of shape data in *Euastrum binale* populations, with pH as the independent variable, revealed a significant correlation (Wilks' $\lambda = 0.356$, permutation p-value $= 0.001$; Goodall's F-ratio $= 41.887$, permut. p-value $= 0.001$; proportion of unexplained variance: 91.1%). Thin-plate spline deformation grids depicting positive and negative deviations from the consensus (average) cell shape were calculated to visualize shape variation along the pH gradient. At lower pH, the cells were nearly cylindrical with shallow lobes; whereas, when grown at a higher pH, they had markedly deeper incisions (Fig. 2a). Specifically, at the highest pH (6.5), the apical cell incisions were ca. 78% deeper (average 1.33 μm at pH 6.5 vs. 0.75 μm at pH 3.5), and the sinuses were about 17% deeper (average 1.9 μm at pH 6.5 vs. 1.63 μm at pH 3.5) than those of cells grown at the lowest pH (3.5). The shape of the cells was also related to their size, albeit less than that with the pH.

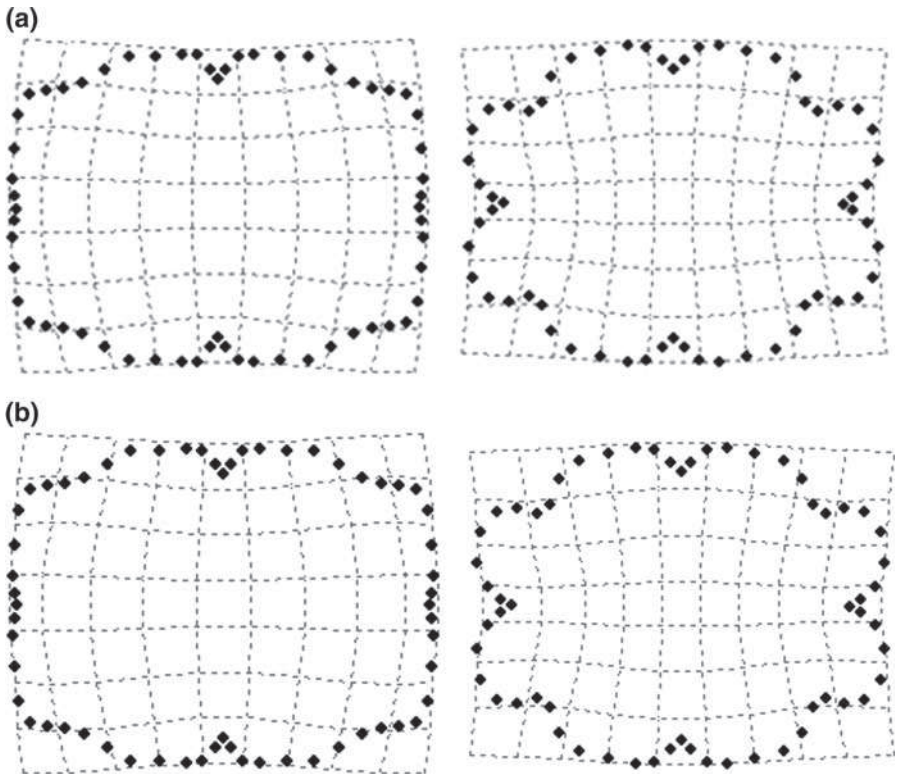

Fig. 2 The model for shape data **a** with the allometric component and **b** without the allometric component, illustrating the results of multivariate regression of cell shape on pH in *Euastrum binale*. Individual outlines illustrate the cell shapes in the lowest (*left*) and the highest (*right*) pH levels according to the regression model. Shapes are represented by thin-plate splines, and for better illustration, the deformations were extended three times

Multivariate regression of shape data based on centroid size revealed a significant effect (although with relatively little variance explained by the regression model) (Wilks′ $\lambda = 0.751$, permut. p-value $= 0.001$; Goodall′s F-ratio $= 6.925$, permut. p-value $= 0.001$; proportion of unexplained variance: 98.42%). In order to determine the significance of the size change on the shape of the cells, we asked whether there was any pH-related shape change completely independent from the size-related (allometric) change in the cells' shape. We took the residuals from multivariate regression of shape data on size and analysed their relation to pH. The relationship remained clearly significant (Wilks′ $\lambda = 0.368$, permut. p-value $= 0.01$; Goodall′s F-ratio $= 15.488$, permut. p-value $= 0.01$; proportion of unexplained variance: 96.53%), and there were no visible differences between the morphological changes associated with this regression model (Fig. 2b) and the regression of size-uncontrolled data on the pH (Fig. 2a). Thus, the allometry based on size and shape was probably not related to pH and shape relationship. We detected no correlation between pH and centroid size but a weak positive one between pH and cell length and pH and width (Fig. 3).

The significance of cell shape difference between individual populations from varying pH environments was evaluated by multivariate permutation tests on Mahalanobis distance. The cell shape of all group pairs (populations from individual pH levels) significantly differed in size effect-uncontrolled, as well as in shape data with the allometric effect removed (Table 1).

The multivariate regression of *Staurastrum hirsutum's* shape data on pH changes revealed a significant effect of pH on cell shape (Wilks′ $\lambda = 0.363$, permut. p-value $= 0.01$; Goodall′s F-ratio $= 96.11$, permut. p-value $= 0.01$; proportion of unexplained variance: 82.0%). The semicells of *S. hirsutum* were more globular under low-pH conditions; but markedly elliptical at a higher pH (Fig. 4a). The multivariate regression revealed a significant relationship between the shape and size of cells of *S. hirsutum* (Wilks′ $\lambda = 0.265$, permut. p-value $= 0.01$; Goodall′s F-ratio $= 118.868$, permut. p-value $= 0.01$; proportion of unexplained variance: 78.6%). Thus, the shape data were regressed on the pH with the allometric effect controlled, and the multivariate regression revealed a significant relation (Wilks′ $\lambda = 0.907$, permut. p-value $= 0.049$; Goodall′s F-ratio $= 5.858$, permut. p-value $= 0.003$; proportion of unexplained variance: 98.71%). The shape variation related to this regression model (Fig. 4b) was similar to those of allometric effect-uncontrolled regression, but the corresponding morphological changes were barely visible (Fig. 4b). The cell dimensions were significantly correlated with pH (Fig. 5). Irrespective of the size measures used, the cell size decreased with increasing pH. The multivariate permutation tests on Mahalanobis distance revealed significant shape differentiation among most data pairs, both with and without an allometric component. However, there were a few exceptions. No differences were detected between shapes in populations at pH of 3.5 and 4.0 or between pH 5.0 and 5.5 in size-controlled comparisons; likewise, no differences were detected between shapes in populations at pH 4.0 and 5.5 in size-uncontrolled data (Table 1).

In addition to multivariate regressions, we completed CVA analyses of shape data in both species in order to discriminate positions of populations at different pHs. We asked whether the position of groups in the ordination space would differ in size effect-uncontrolled data and in data with the allometric effect removed. The CVA ordination plot of the size

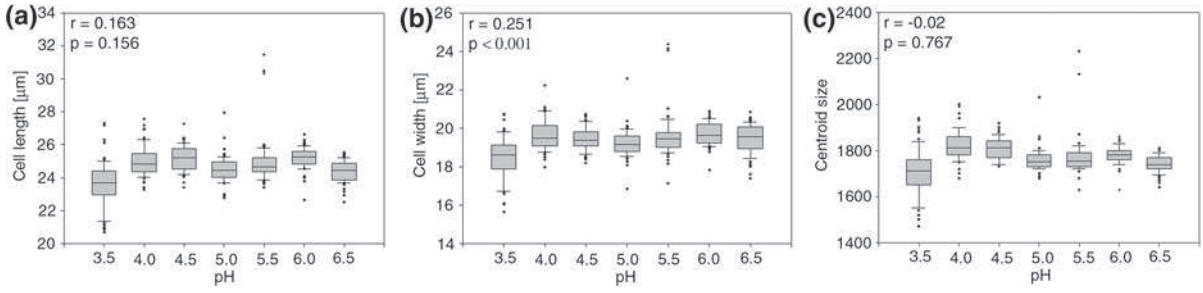

Fig. 3 Experimental pH levels correlated with **a** cell length, **b** cell width and **c** centroid size in *Euastrum binale*

Table 1 Differences between populations from pH levels expressed as Mahalanobis distance for both strains

	3.5	4.0	4.5	5.0	5.5	6.0	6.5
3.5		*0.075*/*0.217****	*0.144****/*0.239****	*0.203****/*0.142****	*0.193****/*0.156****	*0.221****/*0.094***	*0.212****/*0.09**
4.0	0.145***/0.106*		*0.154***/*0.13****	*0.188****/*0.13****	*0.179****/*0.085*	*0.216****/*0.094**	*0.443****/*0.12****
4.5	0.236***/0.216***	0.25***/0.251***		*0.39****/*0.229****	*0.341****/*0.184****	*0.511****/*0.252****	*0.443****/*0.215****
5.0	0.29***/0.304***	0.345***/0.354***	0.195***/0.209***		*0.085*/*0.097***	*0.168****/*0.128****	*0.14****/*0.014****
5.5	0.3***/0.315***	0.371***/0.373***	0.258***/0.27***	0.131***/0.144***		*0.137****/*0.11****	*0.137****/*0.118****
6.0	0.348***/0.386***	.463***/0.457***	0.346***/0.355***	0.272***/0.281***	0.152***/0.159***		*0.129****/*0.121****
6.5	0.259***/0.289***	0.371***/0.341***	0.332***/0.315***	0.323***/0.309***	0.252***/0.245***	0.182***/0.183***	

Significance was evaluated using two-group permutation tests

Values of Mahalanobis distances for shape data with allometric component and shape data without allometric component are separated by a slash

Values for Euastrum binale are written in normal, values for Staurastrum hirsutum are written in italics

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Underlined values are statistically not significant

effect-uncontrolled group's centroids in *Euastrum binale* (Fig. 6a) illustrated a clear division among those at lower pHs (3.5–4.5) from those at higher pHs (5.0–6.5) along the first CV axis. The second CV axis separated those at "extreme" pH conditions (3.5, 6.0 and 6.5) from the other groups. The CVA of size effect-controlled shape data illustrated identical positioning of this group of centroids and similar trends along the first two CV axes (Fig. 6b). Pattern for *Staurastrum hirsutum* differed: the CVA plot of centroids of populations from particular pH values confirmed their clustering into three groups (pH 3.5 and 4.0; pH 5.0, 5.5, 6.0, 6.5; and the isolated pH 4.5 group; Fig. 7a). The first CV axis primarily contrasted the low-pH groups (3.5–4.5) from the others, whereas the second, CV2 axis, was largely defined by the difference among pH 4.5 and other groups. However, after removing the allometric component from the shape data, the CVA revealed a considerably different positioning of group centroids (Fig. 7b). The first CV axis clearly separated pH 4.5, and the second CV axis essentially separated the low from the high pH groups (Fig. 7b).

The complexity of cell outlines was evaluated, as the sum of Euclidean distances between adjacent landmarks in size-standardized data. Indirectly, this measure establishes the deepness of a cell's incisions in relation to its area. In both investigated strains, there was a significant positive correlation of cell outline measure with pH (Fig. 8), thus, indicating a greater complexity of cell shapes with more pronounced incisions under conditions of higher pH.

Discussion

Morphological changes that were related to differences in environmental pH were revealed for both of the investigated desmid species. All of the two-dimensional shape and cell size changes observed in the low-pH environment clearly diminished the S/V ratio of cells. We see this pattern as a possible adaptive response of these populations that restricts the osmotic pressure exerted on their cells. These findings generally confirm the field observations of Coesel (1982), who reported a gradual change in overall average cell shape of desmids along a pH gradient in peat bogs. Under conditions of low pH, the cylindrical cells with a lower S/V ratio were

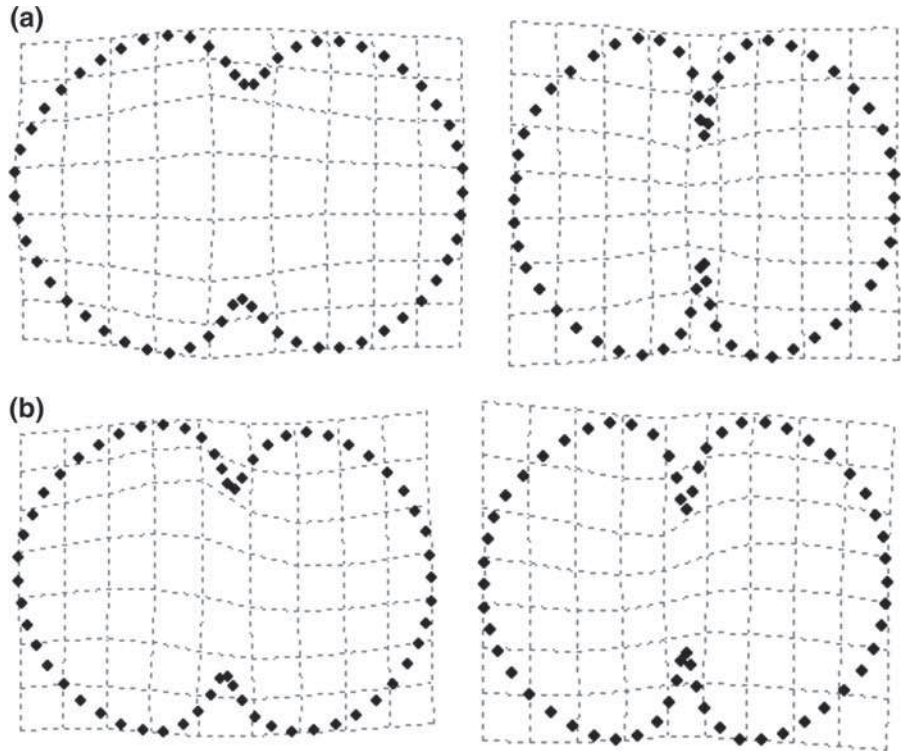

Fig. 4 The model for shape data **a** with the allometric component and **b** without the allometric component, illustrating the results of multivariate regression of cell shape on pH in *Staurastrum hirsutum*. Individual outlines illustrate the cell shapes in the lowest (*left*) and the highest (*right*) pH levels according to the regression model. *Shapes* are represented by thin-plate splines, and the deformations were extended three times for better illustration (Fig. 4b)

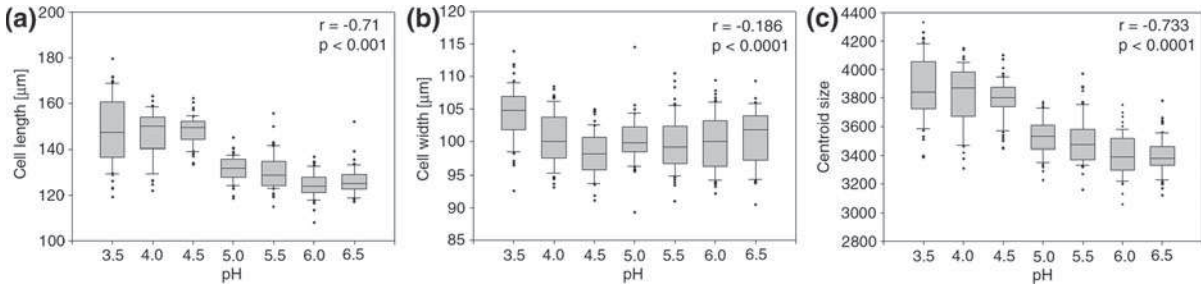

Fig. 5 Experimental pH levels correlated with **a** cell length, **b** cell width and **c** centroid size in *Staurastrum hirsutum*

abundant but were gradually replaced by cells with a higher S/V ratio when the pH of the environment increased.

In *E. binale* var. *gutwinskii*, the shallowing of the lateral incisions was generally similar to differences between this variety and several further infraspecific taxa (*E. binale* var. *binale*, *E. binale* var. *groenbladii* (Messikommer) Willi Krieger) characteristic by less-ornamented cell outline. Similarly, in *S. hirsutum*, the observed size and shape trends related to pH level resembled transition between the type variety of this species and *S. hirsutum* var. *muricatum* (Brébisson ex Ralfs) Kurt Förster, which is characteristic by larger cells similar to those observed in low-pH conditions. However, the taxonomic relevance of these observations can only be assessed by molecular comparisons of multiple strains corresponding to these traditionally defined varieties.

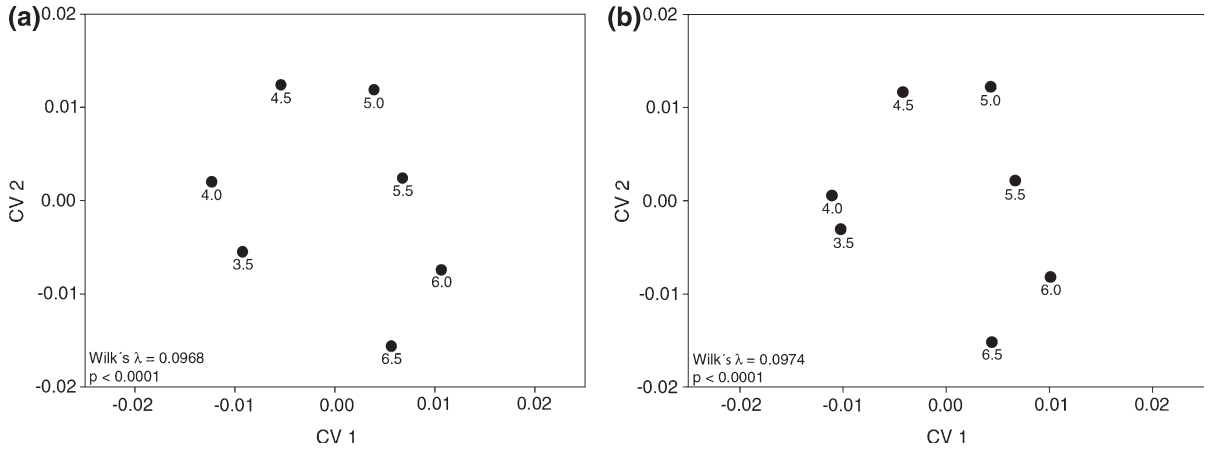

Fig. 6 Discrimination of cell shapes from different pHs in *Euastrum binale*. The CVA ordination plot of shape data **a** with allometric component (CV1 spanned 59.4% and CV2 31.8% of the variation) and **b** without the allometric component (CV1 spanned 63.2% and CV2 28.7% of the variation). Each group is represented by its centroid

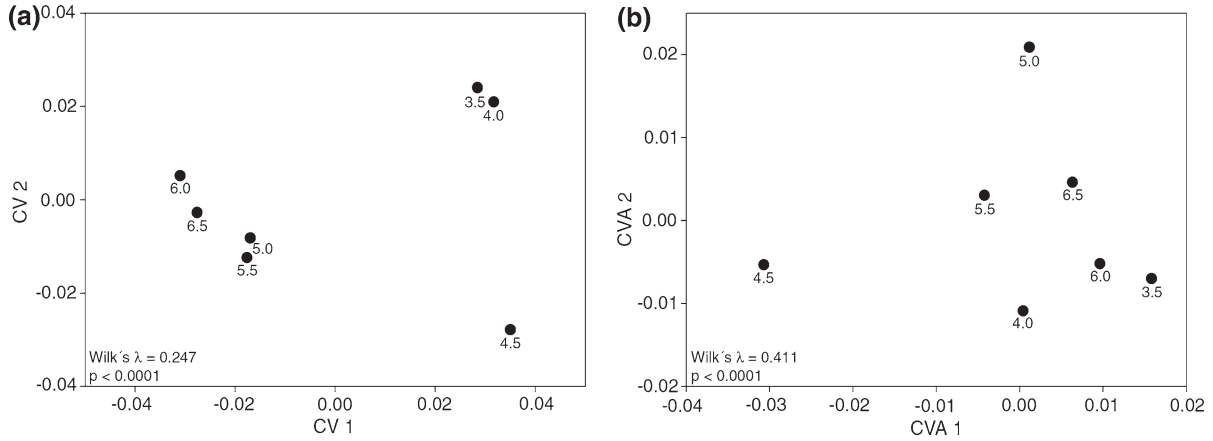

Fig. 7 Discrimination of cell shapes from different pHs in *Staurastrum hirsutum.* The CVA ordination plot of shape data **a** with allometric component (CV1 spanned 52.5% and CV2 17.8% of the variation) and **b** without the allometric component (CV1 spanned 73.1% and CV2 13.6% of the variation). Each group is represented by its centroid

A comparison of the investigated species

The two species we investigated differed notably in their pattern of pH-related response. In *Staurastrum hirsutum*, the cells' S/V ratio (indirectly estimated from complexity data and size values) at low-pH environments was clearly diminished, presumably by changes in cell size. The cells are generally larger in low pH than when in a nearer neutral environment. Although we did not specifically measure growth rates, an increase in cell size in unicellular protists is commonly known to indicate a decrease in their growth rates (Reynolds 1984; Niklas 1994; Nielsen et al. 1996). In addition to changes in size, the cells of *Staurastrum hirsutum* also alter their shape in relation to pH. The semicells were noticeably more globular like (as confirmed by complexity measure, Fig. 8b) under conditions of low pH that further decreased their S/V ratio. Our observations for the various cell size measurements in *Euastrum binale* are quite different. We find that cell length and width slightly increase with increasing pH, and this is associated with the cell lobes becoming more shallow, or conversely, deepening. Notably, the overall cell area, expressed as centroid size, does not seem to differ in relation to the pH. It is likely that the adaptive

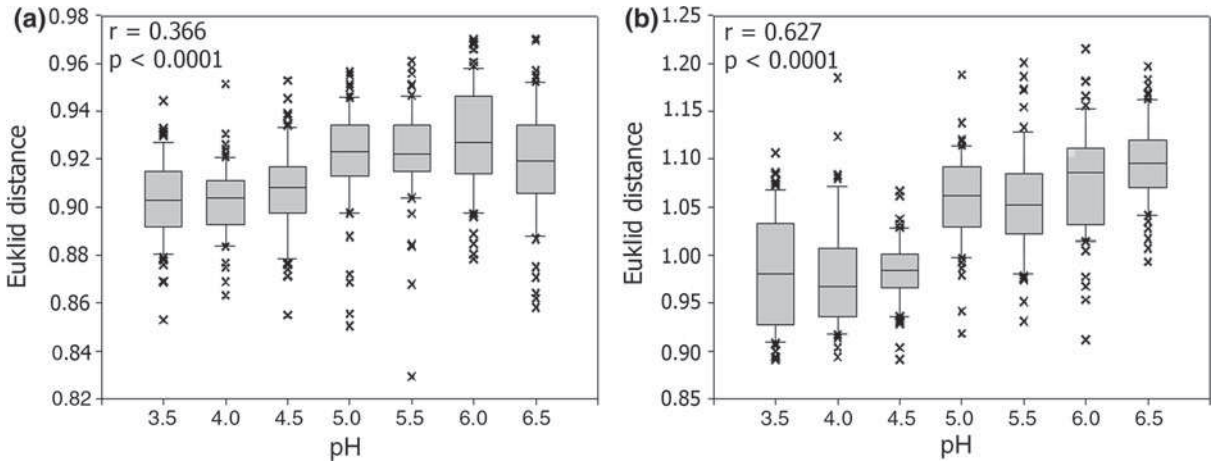

Fig. 8 The cell outlines at different pHs in **a** *Euastrum binale* and **b** *Staurastrum hirsutum*. The cell outline complexity was evaluated as a sum of Euclidean distances between landmarks in Procrustes aligned cells

decrease in S/V ratio in this species is achieved solely by changes in cell morphology. There is a clear pattern of incisions between a cell's individual lobes becoming shallower for cells grown at low pH; whereas, the cells from a higher pH environment typically indicate more pronounced incisions (Fig. 2). Consequently, the complexity of *Euastrum binale* cells increases with increasing pH. Thus, due to the intricate morphology of its cells, *E. binale* is able to manipulate its S/V ratio without increasing its cell sizes (and subsequently lowering its growth rates). A similar phenomenon was observed in *Micrasterias rotata* by Neustupa et al. (2008), who described a desmid species with extremely complex cells as its adaptive temperature-related plastic response. In this species, incisions seem to play a key role in affecting the S/V ratio of cells in response to temperature that influenced the population's growth rates.

Adaptive significance of morphological responses

The possible adaptive value of the intricate desmid morphologies was not recognized for a long time. However, the field observations desmid on communities made by Coesel (1982) and the experiments related to temperature effects (Neustupa et al. 2008) and pH-related plasticity of individual strains (this study) demonstrate the intriguing possibility that the morphological features of desmid cells may be involved in plastic response of a single species to environmental factors. Both the composition of desmid assemblages in natural wetlands and the plasticity observed by us in individual species illustrate that plastic desmid species may shape their cells in relation to environmental conditions. The desmids may thus optimize their S/V ratio, as this parameter seems to have a critical effect on the interface between the outer and inner cell environments. In oligotrophic conditions, desmid species have high S/V ratio as it maximizes the surface area available for nutrients uptake. However, in oligotrophic and acidic environment, species with a lower S/V ratio may have higher fitness, because of their lower surface area and reduced exposure to the acidic environment (Coesel 1982). Notably, in *Micrasterias rotata*, as well as in the species investigated here, plasticity is expressed at the level of individual clones. We presume that the variability between different populations of a particular species could be even more considerable. Thus, the strikingly morphologies of desmids, which have long intrigued biologists like Ralfs as early as in mid-nineteenth-century, could also be interpreted and evaluated in the context of their life strategies and reaction norms.

The future studies evaluating presented plastic responses of clonal strains should involve not only the morphometric analyses of the natural populations from which the strains were derived, but also the evaluation of their taxonomic homogeneity using molecular methods to provide further insight into the ecology of desmid species and clarify the relevance of our findings with the "real world". The investigated Břehyně lowland peat bog provides varying pH

conditions, resembling those used by us in experiments (with pH $> 4.5 < 6.0$). Thus, the evolutionary and ecological relevance of the phenotypic adaptation in the investigated species could be tested in respect to the range of morphologies exhibited in the natural environment.

In conclusion, we revealed the significant effect of pH level on cell shape in two investigated desmid species. In *Euastrum binale*, the allometric effect on shape was not correlated with shape change related to pH level. On the other hand, in *Staurastrum hirsutum*, the cell size-related shape change correlated with the pH-related shape change. However, both species demonstrated similar morphological response to changing pH level of the environment. In low-pH conditions, the cells tended minimize the surface-to-volume ratio. In *Euastrum binale*, this effect was primarily connected with shallowing the incisions. In *Staurastrum hirsutum*, a species without any incisions on semicells, this response was mediated through more globular shape and higher size of the semicells. We conclude that with manipulating their surface-to-volume ratio (either by shallowing the incisions—in *E. binale* or by changes in cell size and globularity—in *S. hirsutum*) these desmid species can respond to pH conditions of the environment.

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Paper 4

Diversity and morphological disparity of desmid assemblages in Central European peatlands

Jiří Neustupa, Kateřina Černá & Jan Šťastný

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PRIMARY RESEARCH PAPER

Diversity and morphological disparity of desmid assemblages in Central European peatlands

Jiří Neustupa · Kateřina Černá · Jan Št'astný

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Abstract Morphological disparity has increasingly been used as an alternative measure of biological diversity based on the shape features of organisms. In this study, we investigated the species diversity and morphological disparity of benthic Desmidiales in Central European peatland pools. The shape features of cells were determined using the 3-D elliptical Fourier analysis of their frontal and lateral views. The resulting morphospace was used to calculate the contributions of localities and species to the morphological variation. In addition, the disparity of samples and their average cell complexity (indicating intricacy of cell shapes) was evaluated. These data were related to species diversity data and to the abiotic factors. Species diversity was positively correlated with pH and conductivity. The low-pH localities generally supported a more variable species composition than did slightly acidic to neutral localities. Conversely, the total nitrogen concentrations of these areas negatively correlated with species diversity. Interestingly, partial morphological disparity (measuring the contribution of a sample to the overall morphological variation) did not correlate with species diversity. On the contrary, several mountain peat bog localities had high disparity values, irrespective of their rather low species diversity. In addition, several samples from minerotrophic fens with high diversity had average or low values of partial morphological disparity. These results indicate the relative importance of mountain peat bogs for the total morphological diversity of *Desmidiales* within the region that could not be ascertained solely from species diversity data. The inner morphological disparity of samples was highly correlated with their species diversity. Species of the genus *Micrasterias*, *Hyalotheca dissiliens* and *Desmidium* species had the highest partial morphological disparity, thus indicating their marginal position within the morphospace. *Micrasterias* and *Euastrum* species had the highest complexity values. The average cell complexity of individual samples did not correlate with their diversity or disparity; however, it was positively correlated with the levels of total nitrogen and phosphorus, and illustrates a pattern different from that arrived at by species diversity data.

Keywords Desmidiales · Elliptic Fourier analysis · Geometric morphometrics · Peat bogs · Morphological disparity

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J. Neustupa (✉) · K. Černá · J. Št'astný
Faculty of Science, Department of Botany, Charles University of Prague, Benátská 2, 128 01 Praha, Czech Republic
e-mail: neustupa@natur.cuni.cz

Introduction

Biological diversity may be quantified by different methods based on various aspects of organisms (Magurran, 2004). Species (or taxonomic) richness has traditionally been the dominating approach in studies of algal biodiversity, including that of the Desmidiales. However, new biodiversity concepts, including molecular phylogenetic diversity (Purvis & Hector, 2000) and morphological disparity (Roy & Foote, 1997), provide intriguing ways of investigating different aspects of temporal and spatial variation in nature. In general, phylogenetic diversity reflects the sum of phylogenetic distances between individual members of an assemblage (Purvis & Hector, 2000). Morphological diversity (or disparity) is based on a morphometric distance that quantifies the shape differences of assemblage members (Roy & Foote, 1997). The multivariate space of a complete investigated set of organisms (spanned e.g. by axes of PCA of morphometric data) is called morphospace (Foote, 1993; Roy et al., 2001). Distances in morphospace (e.g. Euclidean distances between the overall mean and individual objects) may be used for calculation of morphological disparity—either of a set as a whole, or of its individual members or subgroups. Several studies have demonstrated that morphological disparity may not be correlated with taxonomic or phylogenetic diversity (Roy et al., 2001; Neige, 2003a). However, comparison of taxonomic (species) richness with morphological disparity provides a unique way of evaluating diversity across different localities or habitat types. One particular feature of morphological disparity is that individual species contribute differently to the morphological disparity of a community or sample. The contribution of individual specimens is weighted by their position in morphospace: the more morphologically eccentric, the greater its contribution to sample disparity. The concept of morphological disparity has recently been applied in an analysis of spatial or temporal patterns in communities of different organisms (e.g. Roy et al., 2001; Neige, 2003a, b, 2006—cuttlefishes; McClain et al., 2004—gastropods; Clabaut et al., 2007; Hoagstrom & Berry, 2008—freshwater fishes). Neige (2003a, 2006) reported differences in regional values of disparity versus species richness in cuttlefish. The highly diverse West Pacific region, considered a centre of origin for this group, was accompanied by relatively low disparity. This was interpreted as an example of low morphological diversity in a region considered as the primary centre of diversity containing a high proportion of species with morphologies close to the centroid of the group morphospace. Similarly, Roy et al. (2001) demonstrated low regional species richness of marine strombid gastropods that, however, had high or above-average morphological disparity. They stressed the conservational importance of regions that, notwithstanding their low species diversity, contain a significant proportion of the morphospace variation of the group. McClain et al. (2004) reported comparable levels of gastropod disparity in lower bathyal and abyssal marine habitats, even though species richness was lower in the latter communities. On the other hand, Hoagstrom & Berry (2008) detected similar trends in species diversity and disparity in communities of river fishes, and interpreted this as higher niche heterogeneity of habitats with a high species number.

The present study represents the first disparity analysis of natural protist assemblages. The Desmidiales were chosen as a species-rich monophyletic group of green algae (Gontcharov, 2008; Hall et al., 2008), typically inhabiting benthic freshwater environments (Coesel & Meesters, 2007). Natural assemblages of desmids are known to subtly reflect the environmental conditions of localities (e.g. Lenzenweger, 2003). Coesel (2001, 2003) developed an intriguing system of conservational evaluation of peatland localities on the basis of their desmid species composition. Mature cells of desmids have fixed shapes as they are surrounded by cell walls. At the same time, the Desmidiales are one of the most conspicuous protists. With their elaborate cell shapes, they have long attracted the attention of morphologists (Ralfs, 1848). Even the traditional names of desmids in several languages reflect the shape complexity or ornamentation of their cells (‘krásivky’ in Czech is little beauties, ‘Zieralgen’ in German is beautiful algae). The quantitative shape information of desmid cells has recently been used in several geometric morphometric studies that concentrated on taxonomy (Neustupa & Šťastný, 2006; Neustupa & Škaloud, 2007), and the temperature-related plasticity of the genus *Micrasterias* (Neustupa et al., 2008). At the same time, the morphological variation of desmids was also studied by means of traditional

morphometric methods utilizing measurements of distances and angles of cells (e.g. Bicudo & Gil-Gil, 2003). However, the disparity of species-rich natural desmid assemblages and the relation of disparity to their taxonomic diversity have not, thus far, been investigated. The Desmidiales often dominate in phytobenthos of peatland localities (Borics et al., 2003; Coesel & Meesters, 2007). Růžička (1977) and Coesel (2001) suggested that species richness optima of desmid assemblages can usually be found in slightly acidic wetlands with a pH of approximately 5.5–6.5. According to Vitt (2006), these values correspond to the upper pH ranges of most temperate and boreal peatland habitats. The relation of species richness and the pH of the water environment have often been investigated. Mataloni (1999) revealed the pH-related increase in species diversity of desmids in peat bogs of Tierra del Fuego in temperate South America. Coesel et al. (1978) demonstrated a decrease in species richness of the Desmidiales related to anthropogenic acidification of peatland localities in the Netherlands. Meanwhile, Tomaszewicz (1994), Nováková (2002) and Štěpánková et al. (2008) reported a positive relation of desmid diversity and pH in temperate European peat bogs. In addition, Štěpánková et al. (2008) also illustrated the significant positive relation of conductivity and diversity of desmids in mountain peat bogs. Coesel et al. (1978), Gilbert et al. (1998) and Wayda (2004) observed a decrease of peatland desmid species diversity as a result of eutrophication of previously mesotrophic, slightly acidic wetland habitats. In a unique study involving analyses of morphology-to-ecology relations in desmid assemblages, Coesel (1982) investigated an extensive set of samples from Dutch fens and marshes. He suggested that, apart from decreased species richness, low pH conditions (<4.0) in mire habitats generally correlated with a higher frequency of species with simple, cylindrical shapes; although, most of them were, however, the unicellular Mesotaeniaceae that do not actually belong to Desmidiales. Desmid species with more complex, ornamented cells typically occurred in localities with a pH higher than 5.0. However, Coesel's (1982) observations did not involve explicit quantitative analyses of shape variation in relation to abiotic factors.

Recently, the geometric morphometric methods (including outline-based analyses) have increasingly been applied in phycology (e.g. Pappas et al., 2001; Beszteri et al., 2005, Potapova & Hamilton, 2007), including studies of desmids (Neustupa & Škaloud, 2007; Neustupa et al., 2008). This methodological advancement now allows one to investigate the morphospace structure of natural desmid assemblages in phytobenthos. In this study, we selected 30 Central European peatlands, including ombrotrophic peat bogs and minerotrophic fens, spanning a pH range from 3.9 to 7.0, for investigation of diversity versus disparity patterns of their benthic desmid assemblages. We asked the following questions:

(a) What is the relation between species diversity of benthic assemblages of the Desmidiales and abiotic parameters of localities?
(b) What is the morphospace structure of Central European peatland desmid assemblages and what are the individual species contributions to the overall morphological disparity of an entire set of investigated localities?
(c) Is there any relation between local species diversity and morphological disparity measures?
(d) Is there any relation between local disparity values of desmid assemblages and abiotic factors of localities?

Materials and methods

Localities and processing of samples

The 30 investigated localities (Fig. 1; Table 1—Electronic supplementary material to this article) were sampled in July and August 2008. By selection of these localities, we aimed to span the principal environmental gradients that structure peatland freshwater habitats of the region (minerotrophy versus ombrotrophy, strongly acidic bogs versus mesotrophic peatlands). The majority of the sampled localities were located in the Czech Republic, where most of the important existing peatland habitats were included. In addition, we sampled several interesting localities in adjacent countries. The minerotrophic mountain bogs of the Vihorlat Mts., Slovakia, and Rhön Mts. Germany, were included as the examples of otherwise rare habitat type. In addition, four samples from Pohorje Mts., Slovenia, which represent the acidic mountain bog habitats were also

included. At each locality, we sampled 0.25 m^2 quadrates of a phytobenthic community. The pH and conductivity values were measured in the field using the combined pH/conductometer WTW 340i. The inorganic forms of nitrogen and phosphorus were measured by the Hach colorimeter DR/890. The NH_4^+ concentrations were evaluated using the salicylate method (Hach ammonia kit, method no. 10023). The nitrates (NO_3^-) were evaluated using the cadmium reduction method (Hach nitrate kit, method no. 8171). The total nitrogen concentrations were measured using the persulfate digestion method, which consists of a conversion of all forms of nitrogen to nitrate in an alkaline persulfate digestion and subsequent reaction of nitrate with chromotropic acid under strongly acidic conditions (Hach total nitrogen kit, method no. 10071). The reactive phosphorus (orthophosphate) was estimated using the ascorbic acid method (Hach reactive phosphorus kit, method no. 8048), where the orthophosphates react with molybdate in an acid medium to produce a phosphomolybdate complex; ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Finally, the total phosphorus concentrations were evaluated using the acid persulfate digestion method (Hach total phosphorus kit, method no. 8190). In this method, all the organic and condensed inorganic forms of phosphates in the sample are converted to orthophosphates by heating with acid and persulfate. The subsequent reactions are identical to those described above for estimation of orthophosphates. The samples for species identification were immediately fixed and desmids were determined by examination under an Olympus BX51 light microscope using Nomarski differential contrast. We randomly photographed 100 desmid cells in each sample that were also used for disparity analyses. The occasional long filaments of several trichal desmid species (e.g. *Hyalotheca dissiliens* or *Desmidium aptogonum*) were counted up to 10 cells.

Morphometric methods

In total, 3,000 objects were used for the disparity analysis. In each species, a complete cell with two mature semicells was chosen, and its shape was registered by the following procedure. In total, 56 two-dimensional points (landmarks) were placed regularly along the outline of mature cells using

Fig. 1 The map of the investigated localities

TpsDig, ver 2.12. (Rohlf, 2008) to capture their frontal view. The z coordinates of these landmarks were set to zero. The 3-D shape dimension (lateral view outlines) of cells with circular or elliptical apical view outlines was approximated using the published data on width-to-thickness ratios of individual species (extracted from Coesel & Meesters, 2007; Prescott et al., 1977; Růžička, 1977, 1981). This was then used to calculate 54 points approximating the lateral view outline of cells from the x and y positions of landmarks registered along the frontal view outline (see above). The x and y coordinates of lateral view landmarks were obtained as averages of the respective 2-D frontal view landmarks. The z coordinates of lateral view landmarks were calculated from data on width-to-thickness ratios of individual species. The R, ver. 2.3.1. software for statistical computing (R Core Development Team, 2006) was used to compute lateral view landmarks. In triradiate (or rare tetraradiate species), the lateral view was approximated by calculating the position of landmarks in third (fourth) lobe using the position of points along the outline in a frontal view.

The total 110 points spanning the frontal and lateral views of cell outlines were subjected to 3-D elliptic Fourier analysis (Lestrel, 2000; Rohlf, 2003) for calculation of coefficients describing the shape properties of individual species. One major advantage of the elliptic Fourier analysis (EFA) is that it does not rely on the homology or geometric correspondence of individual landmarks (Lestrel, 1997), as is e.g. the case in the general Procrustes analysis (Zelditch et al., 2004). Instead, the EFA calculates the coefficients of

harmonic functions describing shape properties of the entire outline. In our dataset, consisting of widely dissimilar shapes from elongate *Closterium* and *Pleurotaenium* cells to complex, deeply lobed species of *Micrasterias*, the homology (or geometric correspondence) criterion would probably be questionable. In addition, when using the general Procrustes analysis, the extremely high amount of variation, presumed in this species-rich dataset of benthic Desmidiales, could result in distortion of the tangent space projected from the original shape space of Procrustes coordinates (Dryden & Mardia, 1998). However, the EFA demands a single, homologous starting point for all the analyzed objects (cells). In Desmidiales, the choice of such a point was obvious: the apical tip of a semicell that was unambiguously evident in all the investigated species. The cells of desmids are bilaterally symmetrical, but in this study, we concentrated on disparity analyses, and symmetry/asymmetry issues were not investigated.

The original configurations of points describing outlines were replaced so that the starting point at the centre of the polar lobe of a semicell had zero coordinates. Second, the configurations were optimally rotated and scaled to a unit centroid size, the square root of the sum of squared distances from the landmarks to their centroid (Dryden & Mardia, 1998) in PAST, ver. 1.88 (Hammer et al., 2001). Third, the 3-D elliptic Fourier analysis was conducted using EFA3D, ver. 1.0 (Rohlf, 2003). In total, the 25 harmonic functions sufficiently spanning the shape of the cells (Fig. 2) were used for the subsequent analysis.

Disparity analyses

The principal component analysis (PCA) based on the variance–covariance matrix of the coefficients of harmonic functions from the EFA was used to simplify the multivariate set. For subsequent analyses, we used the first 10 PC axes spanning 98.1% of the total variation that was found significant by the Joliffe cut-off value (Jolliffe, 1986). The multivariate set of 3,000 objects characterized by their scores on these 10 PC axes constituted the morphospace for the disparity analysis.

The disparity was analyzed in several different ways. Initially, the partial morphological disparity of localities (Foote, 1993; Zelditch et al., 2004), indicating the contribution of a sample to the overall morphospace of the investigated set, was calculated. The morphological disparity of the entire set was calculated following the Footes (1993) index:

$$\mathrm{MD} = \frac{\sum_{i=1}^{N} (D_i^2)}{(N-1)}$$

where D_i is Procrustes distance of an individual object to reference form and N is number of objects. The contribution of each object to the overall morphological disparity of the set can be expressed as partial morphological disparity:

$$\mathrm{PD} = \frac{D_i^2}{N-1}$$

We see that the sum of PD values of all objects equals the value of morphological disparity index of the whole set. First, the partial morphological disparity of a particular locality was evaluated as sum of PD values of its 100 cells. This measure gives the contribution of a locality assemblage to the overall morphological disparity of desmids in investigated peatlands. At the same time, the species partial morphological disparity was evaluated as sum of PD values of cells identified as a particular species in an investigated set of 3,000 cells. Second, we also evaluated the partial morphological disparity based just on the presence/absence of species at the localities. The partial morphological disparity

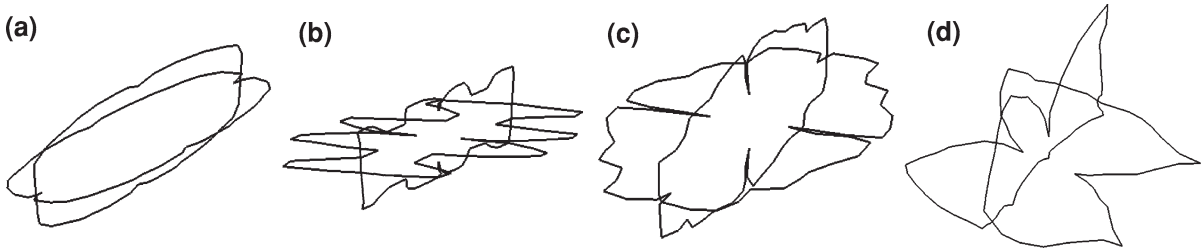

Fig. 2 The outlines of frontal and lateral views reconstructed from the 3-D elliptic Fourier analysis. **a** *Tetmemorus laevis*; **b** *Micrasterias pinnatifida*; **c** *Euastrum humerosum*; **d** *Staurastrum furcatum* var. *aciculiferum*

of localities based on this presence/absence matrix was calculated by the same equations as in the previous case.

Apart from the partial morphological disparity of individual localities that accounted for their contribution to the overall disparity of the complete set, we also calculated the disparity of a sample, or the inner morphological disparity (ID). This measure evaluated the morphological dissimilarity of individual localities. It was calculated as the sum of Euclidean distances between all the 100 objects (cells) of a particular locality in the overall morphospace of 3,000 objects. As another morphological measure, we also evaluated the *C* value that indicated complexity of a cell shape. For each species, this measure was calculated as the sum of Euclidean distances between adjacent landmarks along the outline in the size-standardized configurations of 110 landmarks along frontal and lateral views of cells:

$$C = \sum_{i=1}^{N} \sqrt{(p_{xi} - p_{xi+1})^2 + (p_{yi} - p_{yi+1})^2 + (p_{zi} - p_{zi+1})^2}$$

where p_i is the *i*th landmark of the configuration with p_{xi}, p_{yi} and p_{zi} indicating its *x*, *y* and *z* coordinates, respectively. An ideal sphere would have minimal *C* value, and its increase reflects deviation of cell shape from circularity.

Species data analyses

The patterns of species composition among localities were illustrated using the non-metric dimensional scaling (NMDS) with Euclidean distance measure in PAST, ver. 1.88 (Hammer et al., 2001). The Kruskal stress value was used as the measure of goodness of fit in representation of actual multivariate distances between localities in resulting NMDS ordination diagram (Kruskal, 1964). The effects of abiotic factors on species composition of localities were evaluated by Mantel tests of matrix correlations (Mantel, 1967; Fortin & Gurevitch, 1993). The similarity in species composition was evaluated using matrix of Euclidean distances between localities. Matrix *X* of differences in values of abiotic factors was evaluated as follows:

$$X = [|a_i - b_i|]_{m \times m}$$

where a_i and b_i are individual values of compared abiotic factors of *m* localities (Fortin & Gurevitch, 1993). Significance of matrix correlations was evaluated by 10,000 permutations in PAST, ver. 1.88. Species diversity was evaluated by Shannon–Wiener index (Magurran, 2004) and by species richness. Relation of species diversity, morphological disparity measures and abiotic factors were evaluated by linear correlation analyses with the permutation *P*-value based on 10,000 randomizations.

Results

Of the 3,000 desmid cells from 30 investigated samples, we identified 155 taxa. The descriptions and abiotic data of localities and the complete list of species in localities and their abbreviations are accessible in the Electronic supplementary material (Table 1—Electronic supplementary material). The maximum species richness was found in slightly acidic samples from the 'Břehyně' and 'Swamp' wetlands (samples no. 16—32 species; no. 13—21 species) and from 'Novohradské hory' Mts. (sample no. 22—26 species). On the other hand, several bog localities clearly had low species richness and alfa-diversity (see Table 2 as Electronic supplementary material) with just three species in 'Novodomské rašeliniště' peat bog (sample no. 8) and in 'Hybkaňa' peat bog, Vihorlat Mts., Slovakia (sample no. 30).

The NMDS ordination of samples by their species composition illustrated that the low-pH localities had quite variable species composition that positioned them typically on the margins of the ordination space (Fig. 3). Interestingly, most higher-pH localities appeared to have a more similar species composition. In order to test this observation, we separately evaluated the Euclidean distances between pairs of localities, based on their species composition, in three groups defined by their pH-level (pH < 5.0, pH 5.0–6.0 and pH > 6.0). The permutation *t*-tests (10,000 permutations) on differences in means of these three sets revealed that the low-pH localities had significantly higher variation of species composition than localities with a pH between 5.0 and 6.0 (*P*-value $= 0.0001$, low-pH group mean $= 89.7$, slightly acidic group mean $= 61.3$). In addition, the low-pH localities were more variable in their species composition than those with pH higher than 6.0 (*P*-value $= 0.0001$, high-pH group mean $= 61.2$). On the other hand, the localities with slightly acidic pH (5.0–6.0), and those with a pH

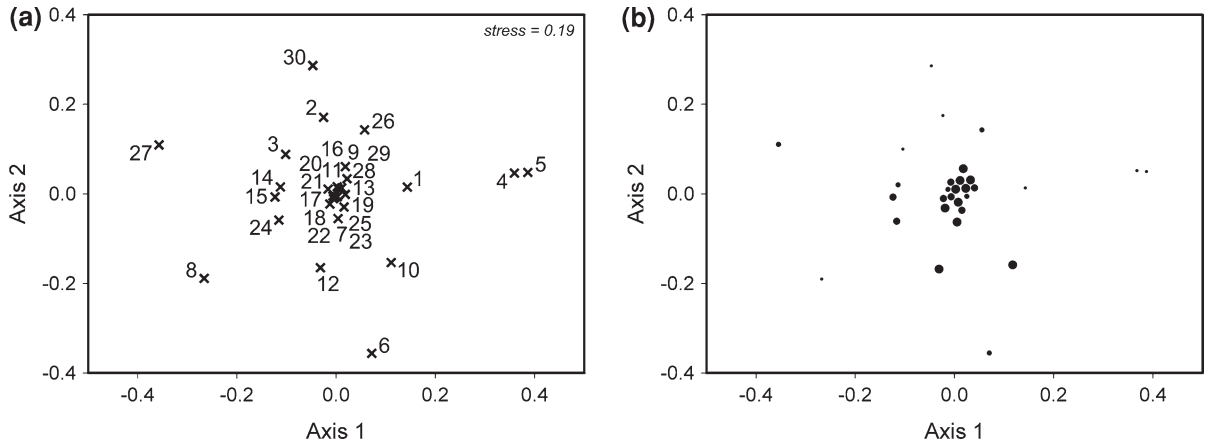

Fig. 3 The NMDS ordination plot of localities based on their species composition (**a**) and the same plot with the symbol sizes reflecting the pH values of the localities (**b**)

higher than 6.0, had similar levels of variation in their species composition (P-value > 0.05).

The Mantel tests revealed that pH ($r = 0.33$, $P = 0.0001$) and total nitrogen ($r = 0.27$, $P = 0.0071$) were the only significant abiotic factors correlating with species composition of the localities. The pH of the localities was positively correlated with the conductivity ($r = 0.61$, $P = 0.0003$), the total nitrogen content was positively correlated with total phosphorus ($r = 0.49$, $P = 0.005$) and the area of the localities was positively correlated with their depth ($r = 0.44$, $P = 0.013$). Other abiotic factors were not significantly correlated. Species diversity (evaluated by the Shannon-Wiener index) positively correlated with pH of the localities ($r = 0.73$, $P < 0.0001$), and with conductivity ($r = 0.40$, $P = 0.026$) (Fig. 4a, b), whereas there was a weakly significant negative correlation of species diversity with total nitrogen concentrations ($r = -0.38$, $P = 0.037$) (Fig. 4c). Relations of species diversity and other abiotic factors were insignificant.

The PCA of 3-D elliptic Fourier coefficients in the entire set illustrated that the elongated species (e.g. members of the genera *Closterium*, *Penium* or *Pleurotaenium*) were separated from other species along the first PC axis (Fig. 5). Species of *Tetmemorus* and *Actinotaenium* were generally in an intermediate position between elongated and compressed forms. The triradiate cells of *Staurastrum* and *Desmidium* species were separated from species with the elliptical apical view outline along the second PC axis. The third and fourth PC axes mainly separated *Micrasterias* species from the others, and on the opposite extremity of the third PC axis, *Hyalotheca dissiliens*, *Desmidium grevillei* and *Staurastrum controversum* were clustered. A group of triradiate species was separated from the others by positive values of the fourth PC axis. Species of individual traditional genera often had similar values, either of partial morphological disparity (PD) or complexity (C) (Fig. 6). While we know that most of these genera are, in fact, polyphyletic or paraphyletic (Gontcharov, 2008; Hall et al. 2008), the similarity of their disparity or complexity is not surprising, given the fact that they have been defined almost entirely on the basis of morphological data. Members of the genus *Micrasterias* generally had the highest partial morphological disparities (PD), indicating their large contribution to the total morphological disparity of the entire set (Fig. 6a; Electronic supplementary Material). *Staurastrum pseudotetracerum*, *S. minimum*, *Desmidium grevillei*, *D. aptogonum* and *Hyalotheca dissiliens* were the other species with high partial morphological disparities, while most *Cosmarium* and *Closterium* species had average PD values. Species of *Tetmemorus* and *Euastrum* typically had low partial morphological disparities, indicating their overall central position within the morphospace.

The measure of morphological disparity of localities (PD) determined their contribution to the total morphological disparity (MD) of the entire set, and revealed a pattern uncorrelated with species diversity (Fig. 4d). Neither the partial morphological disparity of localities based on the quantitative counts, nor the PD values of localities based on the presence/absence

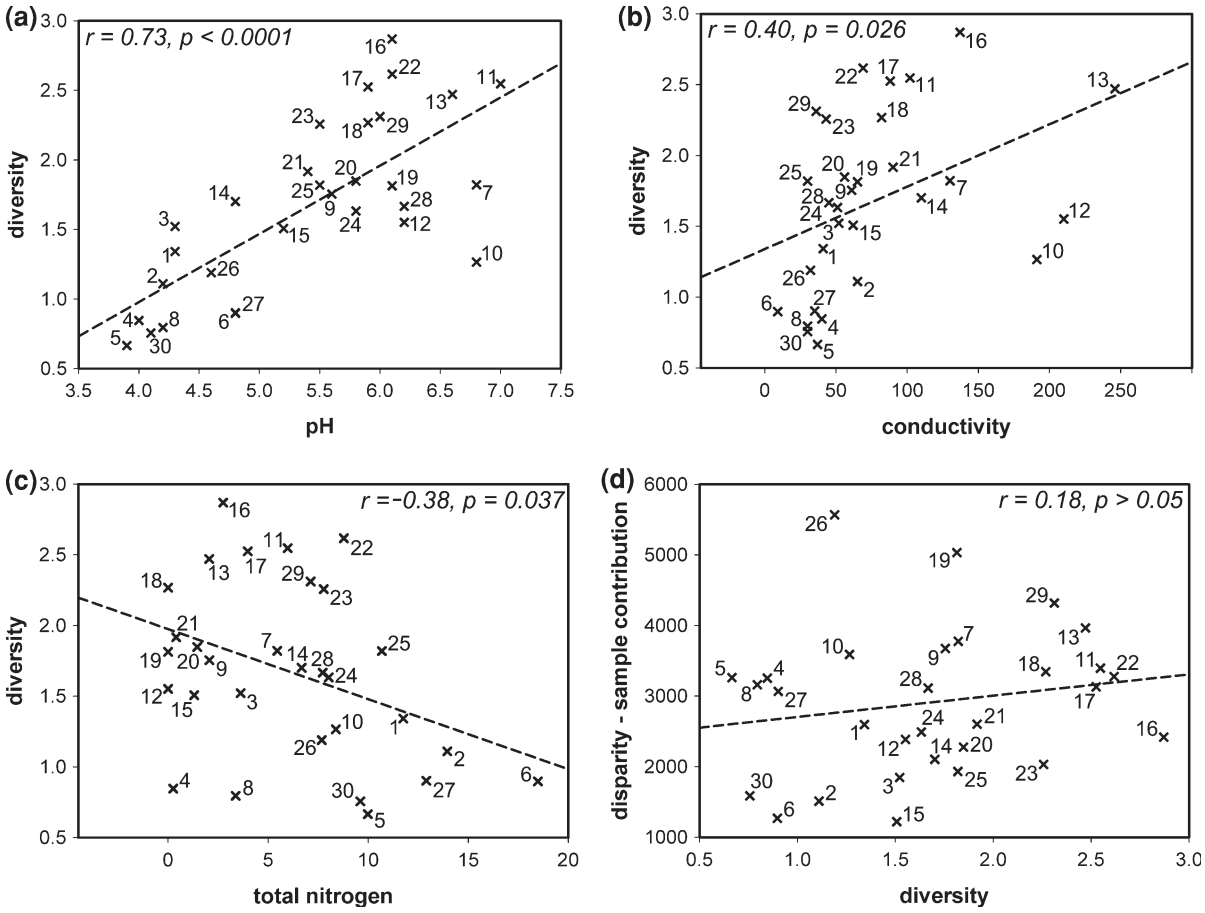

Fig. 4 The linear correlation analyses of the Shannon–Wiener index of species diversity with the pH-value of the localities (**a**), the S–W index of species diversity with conductivity (μS cm^{-2}) (**b**), the S–W index of species diversity with the total nitrogen concentrations (mg l^{-1}) (**c**) and the partial morphological disparity (based on the species counts) with the S–W index of species diversity (**d**)

matrix were correlated with species diversity or richness. The normalized values of species diversity and partial morphological disparity (PD) of localities (based on the species counts) illustrated that locality no. 16 (a pool in Břehyně wetland, Czech Republic) had the highest diversity, and locality no. 26 (Črno jezero bog in Pohorje, Slovenia) had the highest contribution to the overall morphological disparity (Fig. 7). We observed that many minerotrophic localities with higher pH and relatively high diversity had comparatively lower disparity (e.g. localities no. 11, 13–18, 22–23). On the other hand, most mountain peat bog localities had higher relative disparity in comparison to their species diversity (e.g. no. 4, 5, 8, 9, 26–29). The partial morphological disparity of the localities based on species counts was not correlated with the measured abiotic factors. Conversely, the PD values based on the presence/absence of species matrix was weakly positively correlated with the pH of the localities ($r = 0.41$, $P < 0.027$), and negatively correlated with the total nitrogen ($r = 0.44$, $P < 0.012$).

The inner morphological disparity (*ID*) of the localities measuring the morphological dissimilarity in members of a single sample (i.e. disparity of a sample) was highly correlated with species diversity ($r = 0.75$, $P < 0.0001$) (Fig. 8a). The inner morphological disparity was also correlated with pH ($r = 0.60$, $P = 0.0008$) (Fig. 8b), but it was not correlated with the other abiotic factors. The average cell complexity (*C*) of the desmid assemblages in individual localities did not correlate with species

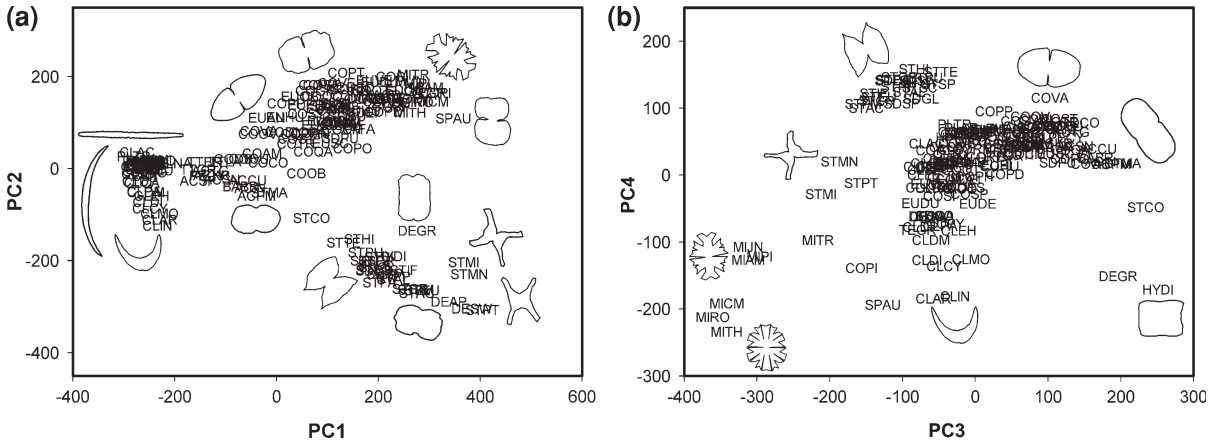

Fig. 5 The ordination plot of the first and second axes (**a**) and the third and fourth axes (**b**) of the PCA. The first axis (PC1) described 51.0%, PC2 17.8%, PC3 11.3% and PC4 8.1% of the total variation in data. The species abbreviations correspond to the Electronic supplementary material. The frontal outlines of selected cells with marginal positions on individual axes are illustrated

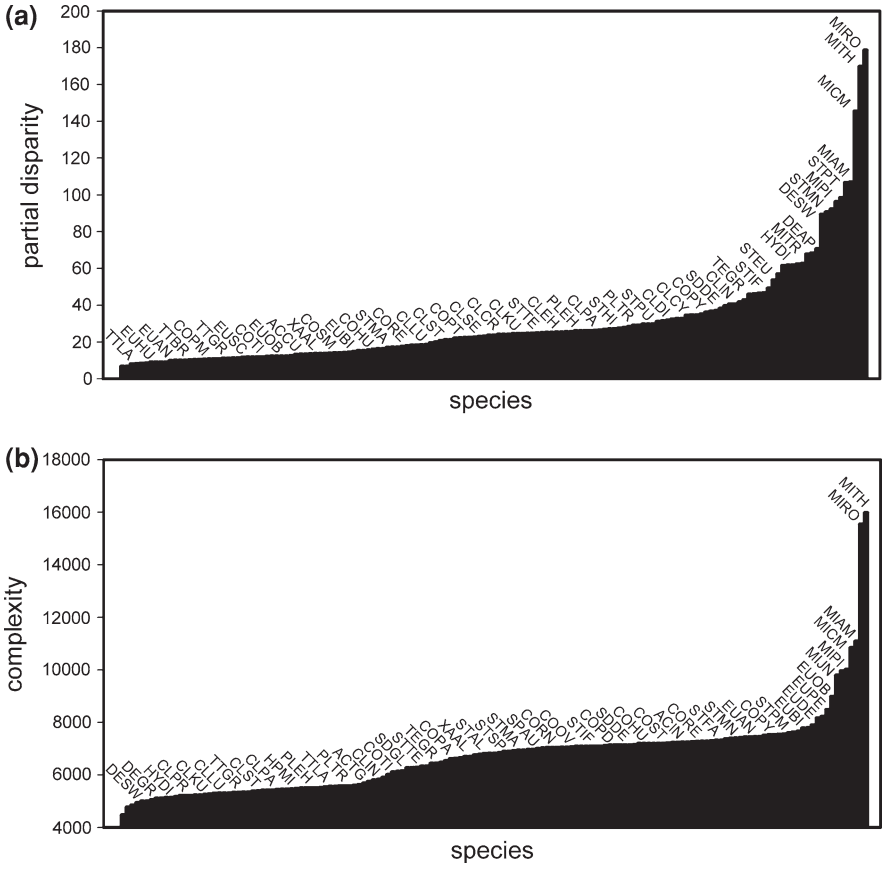

Fig. 6 The graphs of partial morphological disparity (**a**) measuring the contribution of individual species to the total variation within the morphospace and the complexity values (**b**) of individual species indicating the intricacy of their cells. The abbreviations of selected species are indicated and correspond to the Electronic supplementary material

diversity or partial and inner morphological disparities. On the other hand, complexity positively correlated with the measures of total nutrient concentrations (total nitrogen: $r = 0.47$, $P = 0.0071$; total phosphorus: $r = 0.40$, $P = 0.0243$) (Fig. 8c, d). In general, the members of the genus *Micrasterias* had clearly the

Fig. 7 The map of the investigated localities with their normalized Shannon–Wiener diversity indices in *left columns*, and the partial morphological disparity values (based on the quantitative species counts) in *right columns*

most complex cells (Fig. 6b). The *Euastrum* species, *Staurastrum polymorphum* and *S. minimum*, were the other species with relatively high cell complexity. Conversely, filamentous species, *Desmidium swartzii*, *D. grevillei* and *Hyalotheca dissiliens*, had the least complex cells. The cells of *Closterium*, *Pleurotaenium* and *Haplotaenium* species usually had low values for complexity, and most *Cosmarium*, *Xanthidium*, *Actinotaenium* and *Staurastrum* species had cells of average complexity.

Discussion

Species diversity of desmids in the investigated peatland benthic assemblages was positively correlated with pH of the localities. This phenomenon, also illustrated in other studies of benthic desmids (e.g. Coesel et al., 1978; Mataloni, 1999; Štěpánková et al., 2008), has usually been attributed to stress conditions of extremely low pH (<5.0) in many peat bog habitats that limits the occurrence of most species. In this study, the low pH localities typically had low richness with just a few species composing the actual assemblage. Certainly, by counting 100 cells, we did not record every desmid species occurring at a locality. This study was designed to evaluate the diversity versus disparity patterns based on the actual quantitative composition of assemblages, rather than to enumerate their total species richness, including very rare species. However, given the fact that our observed diversity patterns corresponded to previous published studies, we suppose that our data represented the overall differences in diversity of investigated localities. Coesel (2001) reported a unimodal response of species diversity of desmids to the pH, with optima in slightly acidic to neutral conditions. As our study concentrated on peat bogs and fens, we did not sample more alkaline environments, and pH optima corresponded to the highest pH-levels included within the investigated set. The species composition of low-pH localities showing low diversity values was more variable in comparison to higher-pH localities. The species-poor assemblages of acidic habitats differed considerably, and were much less predictable in their dominant desmid species. This phenomenon may be explained by the fluctuating and unstable environmental conditions of many acidified bog habitats (Turetsky & St Louis, 2006), which led to the varying composition of their actual assemblages. Meanwhile, slightly acidic to neutral localities generally shared more species, and this was evident in the higher similarity of their species composition.

The pH and diversity gradients did not indicate the classic distinction between acidic ombrotrophic peat bogs and minerotrophic fens with higher pH values that was reported from boreal peatland ecosystems (Wheeler & Proctor, 2000; Vitt, 2006). However, Hájek et al. (2006) illustrated different ecological dynamics of Central European mires that often reflected the nutrient status, rather than purely the pH gradient. In our study, several clearly minerotrophic localities had rather low pH (e.g. samples no. 14, 15) and low amounts of available nutrients. However, the total nitrogen and, to a lesser extent, the total phosphorus concentrations significantly influenced the species composition, whereas the concentrations of soluble nutrient ions were not correlated with species data. This suggests the ability of many desmids to utilize the organic sources of nutrients in the generally oligotrophic conditions of peatlands (Spijkerman & Coesel, 1998).Therefore, the total nitrogen and phosphorus concentrations may better correspond to actual species composition, than to the highly fluctuating and spatially variable concentrations of available ions (Walbridge & Navaratnam, 2006). The total nitrogen concentrations better corresponded to both species composition and diversity. The weak, albeit still significant, negative correlation of total N

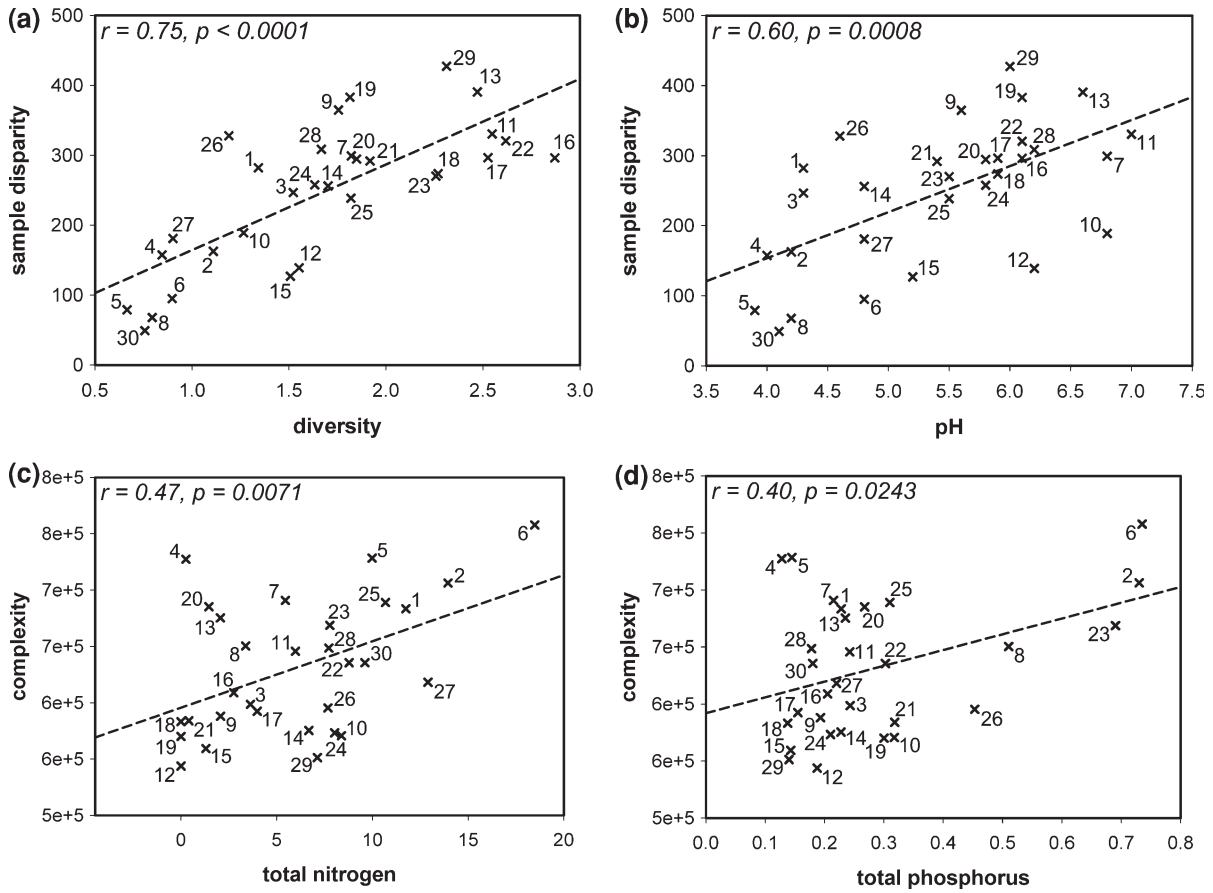

Fig. 8 The linear correlation analyses of the sample disparity levels with the Shannon–Wiener index of species diversity (**a**) the sample disparity with the pH values (**b**) the average cells complexity values of the localities with their total nitrogen concentrations (mg l^{-1}) (**c**) and the average cells complexity values of the localities with their total phosphorus concentrations (mg l^{-1}) (**d**)

with species diversity are consistent with previous studies of Coesel et al. (1978) and Gilbert et al. (1998) that illustrated a general decrease of desmid diversity in relation to eutrophication of habitats.

The inner morphological disparity (i.e. disparity of a sample) of desmids from individual samples was correlated with their species diversity. This is predictable given the fact that most desmid taxa are defined by morphological characters. However, a much more intriguing result was the lack of any correlation between species diversity and partial morphological disparity of localities. The number of species in a given locality did not predict its disparity level. On the other hand, in some samples with low species diversity (presumably from mountain bogs), the disparity values were relatively high and, comparatively, localities with the highest diversity had average or lower disparity. Similar differences in disparity versus species diversity were previously demonstrated by several different groups (Roy et al., 2001; Neige, 2003a, 2006). Regions with high disparity values that often had relatively low species diversity are considered valuable from the conservational point of view as they harbor a significant portion of the group's morphospace. In this study, we observed high disparity in several mountain bog samples (e.g. the sample no. 26 from Črno Jezero peat bog in Pohorje Mts., or the sample no. 29 from Podstavka peat bog, Vihorlat Mts.). In addition, most mountain bog samples from the Sudeten mountains (e.g. the samples no. 4-5, 8-10) had comparatively high disparity in relation to their low species diversity. In these localities (mostly ombrotrophic mountain peat bogs), the desmid assemblages were relatively less diversified, but their disparity makes them important as

they comprise a high proportion of the total morphospace of Desmidiales within the region. Obviously, in these low-diversity localities, this phenomenon was often caused by just a few or even a single dominant species with a high contribution to the total desmid disparity (e.g. *Hyalotheca dissiliens*). Thus, it is not unexpected that the partial morphological disparity values based on the presence/absence matrix (not taking into account the quantities of individual species) resulted rather in average PD values for these mountain peat bog localities (see Electronic supplementary material). However, together with their variability of species composition, the average or high contribution of the mountain bog localities to the overall morphological disparity of peatland Desmidiales demonstrates the importance of these habitats for Desmidiales in the region as a whole.

The presence/absence matrix-based measure of partial disparity also did not correlate with species richness data. The highest disparity based on presence/absence species data was found in minerotrophic localities no. 19 and 7. In both these cases, it coincided with the occurrence of *Micrasterias* species with high partial disparity values. There were some important differences in partial morphological disparity of individual species (measuring their eccentricity within the morphospace) and their cell complexity. While the species of *Micrasterias* had consistently the highest partial disparity, as well as cell complexity, most *Euastrum* species had low partial disparity (i.e. they were positioned close to the overall mean of the morphospace), but their cells had high complexity. Similarly, the cells of filamentous species, *Hyalotheca dissiliens* and *Desmidium grevillei* had high partial disparity, as they had quite eccentric cell shapes compared to other analyzed species, but their cell complexity was low. The lack of a correlation between average cell complexities of individual samples and their pH values did not correspond with the conclusions of Coesel (1982), who assumed higher incidence of more complex cell forms in localities with higher pH. However, his study also incorporated the mesotaeniacean species that, as we now know from molecular data (e.g. Gontcharov, 2008), neither belong to, nor form a monophyletic group with, Desmidiales. While the 'flagship' species with the highest complexity values (as *Micrasterias* species), occurred typically in localities with average or higher pH, some reasonably complex taxa (as e.g. *Euastrum binale* var. *gutwinski* and *Cosmarium pygmaeum*) were abundant in low pH localities. We should note that complexity of desmid cells is strictly a disparity measure, not an approximation of their surface-to-volume ratio (*S*/*V* ratio). Complexity is based on analysis of size-standardized shapes, whereas the *S*/*V* ratios critically depend on size of the cells, in addition to their morphology. The evaluation of *S*/*V* ratios for the complex cells of *Desmidiales* is extremely difficult, especially in taxa with the most complicated shapes, as e.g. *Micrasterias*, *Euastrum* and some *Staurastrum* species. In these taxa, application of classical geometric formulas approximating surfaces and volumes of cells as simple geometric objects (Hillebrand et al., 1999; Sun & Liu, 2003) may not be appropriate. While the geometric formulas for calculation of *S*/*V* ratios and biovolumes have often been successfully applied in phytoplankton studies (e.g. Salmaso & Padisák, 2007; Crossetti & Bicudo, 2008), the *S*/*V* ratios of benthic desmids should probably be evaluated directly from the morphometric data. The original landmark configurations spanning outlines of cells may be used for approximation of their volumes and surfaces, possibly for all existing desmid shapes, but such procedures have yet to be developed. Clearly, the complexity measure used in this study may be related to the form resistance factor used for evaluation of sinking stress in phytoplankton (Padisák et al., 2003). Especially, in planktonic desmids may the changes in average cell complexity correlate with their ability to survive in the epilimnion of stratified water bodies. The morphometric evaluation of complexities in different planktonic natural assemblages could then be of much use for understanding the shape- and form-related life strategies of phytoplankton (Naselli-Flores et al., 2007).

In this study, the average complexity of sample cells positively correlated with the nutrient concentration of their habitat. This pattern was rather surprising, as we know that increasing trophic status decreased diversity of assemblages (Coesel et al., 1978; Wayda, 2004, this study). This phenomenon certainly warrants further study. We propose an intriguing hypothesis that the complexity of cells, possibly reflecting complicatedness of their morphogenetic processes, increases in peatland environments with less nutrient-related stress. However, average complexity of assemblages certainly was also influenced by other critical factors,

including ecological stability of individual localities. In this respect, the biomonitoring method of Coesel (2001, 2003), based on desmid species composition, which is related to ecological stability of habitats, could certainly be tested by quantitative disparity measures, such as the cell complexity value. Coesel (2001) included three separate series differing in their acidity, and each one constituted a gradient from a disturbed to stable habitat. The potential correlation of these series of Coesel's biomonitoring index with different disparity measures will be of interest in quantitative morphological studies to further elucidate the ecology of desmids. Clearly, definition of an overall 'desmid morphospace' containing all the species occurring within a particular region (e.g. temperate Central Europe) and/or habitat type would be of much interest for such studies. Comparison of such data based on an overall species list from a region (with known pH- or nutrients level affinity) with our data that were based on investigation of a limited number of localities may confirm, modify or disprove the conclusions of this study.

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4 Conclusions

The pattern of spatial distribution of algal assemblages was studied in various habitats, but data from freshwater homogenous acidic habitats are scarce. In this thesis, I present the results of studies concerning spatial distribution of algal assemblages in temperate peat bogs on various spatial scales, and the influence of environmental condition (pH-level) on morphological variation of algae occurring in the environment of acidic peat bogs.

We observed the important influence of seasonal changes including a cold winter on algal assemblages in the environment of peat bogs of a temperate zone. The winter season acts as a disturbance that influenced every year's succession, colonization and subsequent niche differentiation in habitats. This pattern was expressed as changes in species composition during the year, and exhibited as the decrease in dominance and increase in evenness of species in the course of the year. The small-scale processes (colonization and niche differentiation) accompanied by spatial distance, microhabitat type and conductivity were described as the most important factors influencing spatial distribution of algal assemblages.

In a homogenous environment on a small scale, the spatial distribution of algae is affected by both the environmental conditions of the microhabitats and their dispersal limitations. Moreover, an artificial barrier constituted an obstruction for water and nutrient flow, as well as algal migration, and had an impact on species composition of assemblages.

Even if the role of pH on spatial distribution of algal assemblages was not demonstrated in our studies we observed an interesting morphological variation related to this key environmental factor in an acidic environment. We observed significant changes in cell morphology of studied algal strains related to pH. These desmid species are able to minimize their surface-to-volume ratio in a low pH environment as a defense against hydrogen ions. We conclude that by manipulating their surface-to-volume ratios desmid species can respond to pH variations in their environment.

Not only traditionally used species diversity, but characteristics derived from geometric morphometrics (e.g. morphological disparity, cell complexity) could be used as alternative measures of biological diversity. We used the shape characteristics to evaluate the importance of a studied locality in relation to total morphological variation. An intriguing finding that we observed was that in some samples with low species diversity (presumably from mountain bogs), the disparity values were relatively high and, comparatively, localities with the highest diversity had average or lower disparity. In these localities (mostly ombrotrophic mountain peat bogs), the desmid assemblages were relatively less diversified, but their disparity makes

them important as they comprise a high proportion of the total morphospace of *Desmidiales* within the region. Such regions with high disparity values that often had relatively low species diversity are considered valuable from a conservational point of view, as they harbor a significant portion of the group's morphospace.

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6 Curriculum vitae

Kateřina Černá (born Machová)
born 6th November 1980 in Vimperk, Czech Republic

Charles University in Prague, Faculty of Science
Department of Botany
Benátská 2
Prague 2
CZ-128 01
e-mail: kaca.cerna@gmail.com

Study and practice:

- since July 2006 – position of a research worker at the Department of Botany, Faculty of Science, Charles University in Prague
- since 2004 - PhD. study in botany, Faculty of Science, Charles University in Prague
- 1999 – 2004 – undergraduate study in biology, specialisation: diversity of periphytic algal assemblages, ecology and ecophysiology of psychrophilous algae, Faculty of Biological Sciences, University of South Bohemia, České Budějovice

Publications in SCI journals:

- **ČERNÁ K** (2010) Small-scale spatial variation of benthic algal assemblages in a peat bog. Limnologica, DOI 10.1016/j.limno.2009.11.015 (in press)
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- **ČERNÁ K**, NEUSTUPA J (2009) Geometric morphometrics analyses of pH-related morphogenetic dynamics in unicellular Desmidiales (Viridiplantae). – SMEF 6, Montpellier, France
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