Charles University in Prague

Faculty of Science

Study program: Biology

Study branch: Botany – Phycology and algal ecology

Bc. Petra Mutinová

Substrate specificity of epiphytic communities of diatoms (Bacillariophyceae) and desmids (Desmidiales)

Substrátová specificita epifytických společenstev rozsivek (Bacillariophyceae) a krásivek (Desmidiales)

Master´s thesis

Supervisor: doc. RNDr. Jiří Neustupa, Ph.D.

Prague, 2015

Statement

I hereby state that I have completed this thesis by myself and that I have properly cited all literature and other information sources I have used. Neither this thesis nor its parts have been submitted to achieve any other academic title(s).

Prohlášení

Prohlašuji, že jsem svou závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

In Prague / V Praze, 7.4.2015

Acknowledgements

It was 7th April 2010 when I became a student of the Prague phycological research group. It is 7th April 2015 and I am about to finish my Master´s thesis. And there is another adventure waiting for me abroad...

Firstly, I would like to thank a lot my supervisor Jiří Neustupa for all the enthusiasm, support and great leadership during my studies, as well as to Jana Kulichová and Jan Šťastný for their help with the species determination and tips of sampling sites. Secondly, my thanks go to Karl Inne Ugland for his never-ending ideas and support. Also, I highly appreciate advices and comments of Yvonne Němcová, Pavel Škaloud, Antonio Terlizzi and Stanislao Bevilacqua that led to the improvement of the thesis.

I would like to thank Martin Pusztai, one of my best friends, for that he came to the Prague Phycology Research Group before me, made me really angry, and thus persuaded me to start studying algae so soon. I am also grateful to my other amazing friends in Prague and Oslo, especially to Annie Evankow, Christopher Hinchcliffe, Erik Gilmore, Katie Dean and Shane Walker, as well as Abigail Cooksey and Megan Lee for the corrections of my English writing.

A hlavně děkuji své rodině za to, že teď „všichni mají kliku“.

Abstract

It has been always assumed, and frequently reported, that host plants, as biologically active substrates, should have a direct influence on associated epiphyton. However, some studies favoured the neutral substrate hypothesis. Thus the relationship between host plant and epiphytic community remained unresolved. This Master´s thesis focused on the basal question that numerous previous studies overlooked. Is there any significant influence of host plant on freshwater algal epiphyton in comparison to the influence of other factors, e.g. site and environmental conditions? In addition, substrate specificity of individual algal taxa was investigated. The research concerned several types of natural plant substrates at several water bodies in the Czech Republic, which provided a more accurate and general insight in the ecology of microphytobenthos.

The results have demonstrated that site was the main factor affecting epiphytic community structure, followed by mild, but still noticeable, effect of environmental conditions (pH and conductivity). In contrary, host plant had almost no influence and very few algal species were found to be host specific. Therefore, the neutral substrate hypothesis is considerably supported, suggesting that epiphyton can be used in biomonitoring regardless of substrate type. Moreover, the research concerned diatoms (Bacillariophyceae) and desmids (Desmidiales), two groups of microscopic algae that are monophyletic, unrelated and ecologically very important. All analyses were done in parallel for both algal groups, and finally, the direct comparison of community structures of both algal groups was performed. Apparently, the group strategies were mostly identical, and therefore they could be generalized for the entire microphytobentic community.

Key words: community structure, desmids, diatoms, epiphyton, host plant, substrate specificity

Abstrakt

Vždy se předpokládalo a často bylo pozorováno, že hostitelská rostlina, jakožto biologicky aktivní typ substrátu, má přímý vliv na epifyton žijící na jejím povrchu. Avšak některé studie spíše upřednostňují hypotézu neutrality substrátu, tudíž vztah mezi hostitelskou rostlinou a epifytonem je stále diskutabilní. Tato diplomová práce se zaměřila na základní otázku, kterou mnoho předchozích prací opomnělo. Má hostitelská rostlina signifikantní vliv na sladkovodní epifytické společenstvo řas v porovnání s dalšími faktory, např. lokalitou a podmínkami prostředí? Navíc byla zkoumána i substrátová specificita jednotlivých taxonů řas. Práce se zabývala epifytonem na několika typech přirozených rostlinných substrátů, odebíraných v několika vodních plochách v České republice. Poskytuje tak přesnější a obecnější pohled na ekologii mikrofytobentosu.

V rámci této práce se ukázalo, že lokalita byla hlavním faktorem ovlivňující epifytické společenstvo, následována slabším, ale stále zaznamenatelným, vlivem podmínek prostředí (pH a konduktivitou). Naproti tomu hostitelská rostlina nehrála skoro žádnou roli a jen pár druhů řas vykazovalo substrátovou specificitu. Tyto výsledky tak významně podpořily hypotézu neutrality substrátu, což vedlo k závěru, že epiphyton může být využit v biomonitoringu nezávisle na typu substrátu. Práce navíc zkoumala dvě skupiny řas zároveň - rozsivky (Bacillariophyceae) a krásivky (Desmidiales). Obě skupiny řas jsou monofyletické, nepříbuzné a ekologicky klíčové. Všechny analýzy byly provedeny paralelně pro obě skupiny řas a v poslední fázi byla jejich společenstva porovnána analýzami přímo. Je zřejmé, že skupinové strategie byly stejné, a tak mohou být výsledky této studie zobecněny pro celý mikrofytobentos.

Klíčová slova: struktura společenstva, krásivky, rozsivky, epifyton, hostitelská rostlina, substrátová specificita

Content

1. Introduction

1.1 Epiphyton and factors influencing its community

Epiphytic community[1] of microscopic algae and cyanobacteria is an important component of aquatic ecosystems. Epiphytic community is one of the basic parts of food webs in ecosystems (Kitting et al., 1984; James et al., 2000; Hart & Lovvorn, 2003) and the productivity of epiphytic algae may equalize or even exceed the productivity of their host plants and phytoplankton (Brock, 1970; Allen, 1971; Cattaneo & Kalff, 1980; Wetzel, 1993). Moreover, the complex interactions between phytoplankton, benthic microalgae (particularly epiphyton) and macrophytes determine the whole ecosystem character and the ecosystem responses to changing environmental conditions (Sand-Jensen & Borum, 1991; Havens et al., 2001; Liboriussen & Jeppesen, 2003).

Freshwater epiphyton, as well as other benthic communities, are influenced by many different factors. The environmental conditions seem to belong to the main ones and they are easy to study, so there are many studies published on this topic. They concentrate mainly on the effects of pH, conductivity, nutrients (e.g. Coesel, 1982; Poulíčková et al., 2004; Soininen et al., 2004; Charles et al., 2006; Fránková et al., 2009; Machová-Černá & Neustupa, 2009; Neustupa et al., 2013), and light conditions (e.g. (Gons, 1982; Müller, 1999; Albay & Akcaalan, 2003; Asaeda et al., 2004; Hillebrand, 2005). Further, factors such as space and, to a much lesser extent, time can play an important role in determining the community structure of benthic microalgae (Messyasz & Kuczyńska-Kippen, 2006; Machová-Černá & Neustupa, 2009; Krivograd Klemenčič et al., 2010; Neustupa et al., 2012; Svoboda et al., 2014). Epiphytic organisms can also be influenced by biotic interactions, including intraspecific competition (Jones et al., 2000) and predation (Cattaneo, 1983; Dudley, 1992; Jones et al., 2000; Hillebrand, 2005; Kuczyńska-Kippen et al., 2005). Predation can greatly reduce the abundance of epiphytic organisms and even change the whole character of epiphyton, for example predation pressure may lead to the reduction of filamentous or loosely attached epiphytic algae. It is also noteworthy that cyanobacteria and algae, especially diatoms and desmids, represent part of microbial biofilms (Ács et al., 2003; Domozych & Domozych, 2008). Thus, algal epiphyton may be influenced by interactions between microorganisms

[1] *Epiphytic community*, or alternatively *epiphyton*, is a type of benthic community that includes organisms living on the surface of macrophytes. The summary of all types of benthic communities was published in Poulíčková et al. (2008). The term *macrophytes* usually represents the group of larger aquatic photosynthetic organisms, from larger algae to vascular plants. For more details about aquatic macrophytes see e.g. Chambers et al. (2008).

within these biofilms and by biofilm chemical composition and succession (Sekar et al., 2002; Barranguet et al., 2004). It was far beyond the reach of this thesis to cover this aspect though.

The question whether epiphytic community is influenced by substrate, i.e. host plant, appeared a long time ago. For instance, Prowse (1959) belongs to the first ones that reported the existence of specific macrophyte-epiphyton associations. To the best of my knowledge, the influence of the host plant still remains very debatable because of methodological discrepancies and the existence of just a few studies that proposed good comparison of the influence of substrate and other factors, e.g. environmental conditions and space (more in Chapter 1.5). Despite the difficulties associated with this question, there are more studies that assumed right from the right beginning that host plants, as biologically active substrates, affected associated epiphyton and subsequently the researchers wanted to explain how. The possible effects of host plant on associated epiphytic community, i.e. positive, negative or neutral, are summarized in the following three chapters. At this point, it is important to note that this thesis only focuses on freshwater epiphyton, excluding the tropics.

1.2 Positive effect of host plant on associated epiphyton

The first hypothesis is that host plant positively affects associated epiphyton. It has been already known that some higher plants can release the part of inorganic nutrients through their surface (Riber et al., 1983). The released nutrients become available for epiphytic organisms and may enhance epiphytic growth, mainly in oligotrophic waters (Eminson & Moss, 1980; Burkholder et al., 1990). This could be especially important for adnate algae forming a firm biofilm that are relatively isolated from nutrient supplies in overlying water (Burkholder et al., 1990). However, a study by Kahlert & Pettersson (2002) emphasized the importance of substrate as a source of nutrients even in the lakes with increased trophy. Such a direct nutrient input may also support the early stages of epiphyton development (Albay & Akcaalan, 2003).

Nutrients released through the surface of macrophytes may even lead to mutualism between macrophytes and epiphytic algae. For instance, host plants might well be in turn supplied by carbon dioxide and some organic micronutrients (Allen, 1971). Host plants can also be protected from predation by the layer of epiphyton, simply because grazers would prefer to feed on microscopic epiphytic organisms over plant tissues (Hutchinson, 1975; Thomas et al., 1985; Hart & Lovvorn, 2003). A very illustrative example of mutualism is the

model of positive feedback that may appear in oligotrophic waters between the genus of carnivorous plants *Utricularia* and its epiphytic community (Ulanowicz, 1995). *Utricularia* provides nutrients for the epiphytic community, and thus enhances the growth of epiphyton and indirectly increases the attraction of zooplankton predators. As soon as zooplankton predator approaches the higher plant, it is caught in *Utricularia´*s trap and then digested. Finally, nutrients are returned to the macrophyte. Nevertheless, *Utricularia* cannot catch all zooplankton, otherwise it would be overgrown by epiphyton, which would not be limited by the predation pressure.

Nutrient exchange through the macrophyte surface is just one way of positive influence on associated epiphyton. Furthermore, some macrophytes have the ability to alter the surrounding physicochemical environment (Morin & Kimb, 1983; Wilcock et al., 1999; Joniak et al., 2007; Soudzilovskaia et al., 2010). The genus *Sphagmun* is a well-known example of this because it can acidify its surroundings through cation exchange[2] (Clymo, 1964; reviewed in Andrus, 1986). As a result, a higher occurrence and abundance of acidophilic algae can be expected in the immediate vicinity of *Sphagnum* and plants with similar ability.

1.3 Negative effect of host plant on associated epiphyton

In contrast, host plant may negatively affect the associated epiphytic community. It is probable that macrophyte and epiphytic organisms compete for nutrients or light (Fitzgerald, 1969; Phillips et al., 1978; Sand-Jensen, 1990; Roberts et al., 2003; Köhler et al., 2010). The shading effect of the epiphytic layer may even cause damage to leaf structures and chloroplasts (Asaeda et al., 2004), as well as some morphological changes of host plants, specifically the allocation of greater biomass to roots rather than stems and leaves (Sultana et al., 2010). Therefore in these cases, the reduction or total removal of epiphytes might be favorable.

The first way for a host plant to inhibit the growth of an undesirable epiphytic community is to produce allelopathic substances. It is important to note that Molisch (1937, referred after van Donk & van de Bund, 2002) classically defined allelopathy as any biochemical interaction, including both stimulation and inhibition, among higher plants and

[2] *Sphagmun* is able to release carbon cations from its cells in exchange for e.g. calcic, magnesium or potassium cations from the surroundings (Clymo, 1964; reviewed in Andrus, 1986).

between higher plants and microorganisms. Presently, allelopathy is usually mentioned in the negative context. There are many studies on the subject of allelopathy (reviewed in van Donk & van de Bund, 2002; Gross, 2003; and Hilt, 2006). However, they typically focus on a particular plant taxon (e.g. genus *Chara*, *Myriophyllum*, *Ceratophyllum*, *Elodea*) and how it influences the growth of particular taxon of epiphytic or planktonic organism. Interestingly, Hilt (2006) suggested that allelopathic substances cause rather the inhibition of planktonic organisms, because the epiphytic community lives in the immediate vicinity of host plant and is therefore better adapted to any excreted allelopathic substances. In other words, co-evolution between host plants and epiphytes is facilitated by tolerance of the epiphytic community to allelopathic substances produced by host plants.

Another way how host plants can negatively affect epiphytic growth is by attracting of predators that selectively remove epiphytes. Brönmark (1985) claimed that host plant excreted dissolved organic matter to attract predators directly, meaning that it did not contribute to epiphytic growth at all. Furthermore, indirect predator attraction can also reduce the growth of epiphyton, like in the already mentioned model of macrophyte-epiphyton-zooplankton interaction (Ulanowicz, 1995) or in the similar model of macrophyte-epiphyton-snails interaction (Thomas et al., 1985). In these cases of indirect attraction, the host plant would improve the nutritional value of epiphyton, increasing predator attraction. In turn, the predators reduces epiphytic biomass. However, the results of Jones et al. (2000) and Mormul et al. (2010) showed that even though predators grazed on the layer of epiphyton, host plant did not probably contribute to their attraction.

1.4 Host plant as a neutral substrate

The neutral substrate hypothesis (Shelford, 1918; referred after Cattaneo & Kalff, 1979) offers a totally different view on the relationship between host plant and associated epiphytic organisms. This is often tested by the comparison of epiphytes on natural and artificial substrates[3] that are the same size and shape. If there are no differences between the epiphyton on both types of substrates within a single water body, the host plant is regarded as neutral, meaning that host plant does not interact biologically nor chemically with epiphyton.

[3] Some may argue that using of artificial substrates provides misleading results. Therefore they must be used with more caution, but natural diatom community, that often represents the epiphytic dominant, seems to be well simulated on artificial substrate (Cattaneo & Amireault, 1992).

The only possible influence is indirect (Cattaneo & Kalff, 1979, 1980; Cattaneo et al., 1998; Kuczyńska-Kippen et al., 2005; Laugaste & Reunanen, 2005; Messyasz & Kuczyńska-Kippen, 2006). The neutral substrate hypothesis was also supported by Siver (1977) and Cejudo-Figueiras et al. (2010), who concluded that in this case epiphyton can be used for biomonitoring regardless of substrate type. Based on these studies, one of the relevant indirect effects is plant morphology (in other words plant architecture or substrate complexity; Fig. 1), as it is known that diversity and abundance of microorganisms increases with habitat complexity at smaller scales (Taniguchi & Tokeshi, 2004). Further, host plant can indirectly affect epiphyton for example through density of vegetation and position in the water column (light and shading effect), or movement in the water (income of new nutrients).

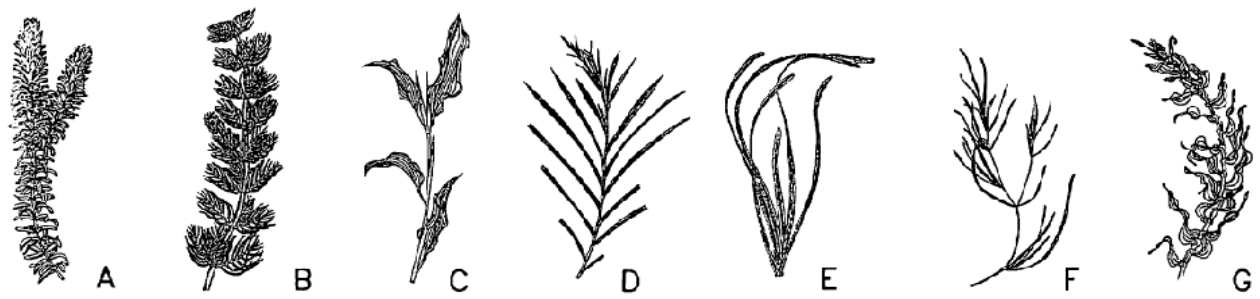

Fig. 1 Illustration of host plants with different architecture: (A) *Elodea canadensis*, (B) *Myriophyllum spicatum*, (C) *Potamogeton amplifolius*, (D) *Potamogeton robbinsii*, (E) *Vallisneria americana*, (F) *Potamogeton* sp., (G) *Potamogeton richardsonii*. The figure is from Lalonde & Downing (1991).

There are several opponents of the neutral substrate hypothesis, like the already mentioned studies on positive and negative substrate interactions in Chapter 1.2 and Chapter 1.3. Then, commentary by Gough & Gough (1981) can also be taken into account. The authors objected to the results in Cattaneo & Kalff (1979) that showed species composition, biomass and production of epiphyton did not differ on *Potamogeton* and its plastic model. According to Gough & Gough (1981), the results of Cattaneo & Kalff (1979) were based on the comparison of just a few macrophytes and subsequently too generalized. This disagreement was also supported by other studies (Gough & Woelkerling, 1976; Blindow, 1987), which reported significant differences in algal epiphyton on different natural plant substrates with similar morphology, even within one site. Therefore, it seemed likely that some macrophytes were a neutral substrate for epiphytic microorganisms, while others actively influenced associated epiphytic community.

1.5 Comparative effects of host plant and other factors on epiphyton

There has always been a question which factor has the greatest influence on freshwater algal epiphyton. Does substrate even matter in comparison to other environmental and spatial factors? This basic question is highly underestimated regarding to the high number of studies that concerns only the influence of host plant. The publications that provided more accurate insight into ecology of epiphyton, by investigating the effects of several factors on epiphyton at once, are relatively uncommon.

Eminson & Moss (1980) studied the communities of epiphytic algae at two sites. The results of this study showed that macrophytes had a greater influence on associated epiphyton in oligotrophic waters, in other words that environmental conditions had a greater impact on epiphyton. Lalonde & Downing (1991) showed that epiphytic biomass was dependent on lake trophic status, depth, and to a lesser extent on plant architecture. However, the influence of environmental variables, differing between lakes, was greater than the influence of host plant. Cejudo-Figueiras et al. (2010) found significant differences in diatom communities again between sites with different trophy, but not between host plants. Additionally, Pals et al. (2006) observed some significant differences in epiphytic communities of desmids between several types of substrates within oligotrophic and mesotrophic lakes. The authors could not explain these dissimilarities with plant morphology, nor with the chemical influence of host plant on the immediate vicinity. They concluded that the differences were determined by local environmental factors, which were possibly closely associated with the substrate. Nevertheless, they always found much greater differences between epiphyton from different sites (Pals et al., 2006). A similar conclusion was provided in Millie & Lowe (1983), which emphasized that variation of diatom communities within replicate samples from a particular macrophyte was as great as, or even greater than, variation between macrophytes. Gough & Woelkerling (1976) and Woelkerling (1976) also reported that there were significant differences between algal epiphyton on various macrophytes both within and among several water bodies. However, the differences of epiphyton between sites could not be explained by environmental factors. The differences of epiphyton within sites led to the conclusion that the substrate itself was able to affect associated epiphytic communities, in contrast to the explanation in Pals et al. (2006).

To summarize these papers, space and environmental variables are likely more important for epiphytic algae than the substrate itself, which favors the neutral substrate hypothesis once again.

1.6 Aims of this Master´s thesis

The aim of this Master´s thesis was to study which factors have the greatest influence freshwater algal epiphyton, thus to provide more general and accurate insight into ecology of freshwater microphytobenthos. Diatoms (Bacillariophyceae, Stramenopila, SAR) and desmids (Desmidiales, Viridiplantae, Archaeplastida) were chosen as the model algal groups for this research, because both diatoms and desmids are monophyletic and unrelated (see the supergroups according to Adl et al. (2012) in Fig. 2). Moreover, both selected algal groups are very important ecologically as they often dominant in given microhabitats and ecosystems (particularly for freshwater epiphyton see e.g. Lazarek, 1982; Kuczyńska-Kippen et al., 2005; Domozych & Domozych, 2008; Krivograd Klemenčič et al., 2010), and they are frequently used as model organisms for biomonitoring (Dixit et al., 1992; Coesel, 2001, 2003; Charles et al., 2006; Blanco et al., 2014).

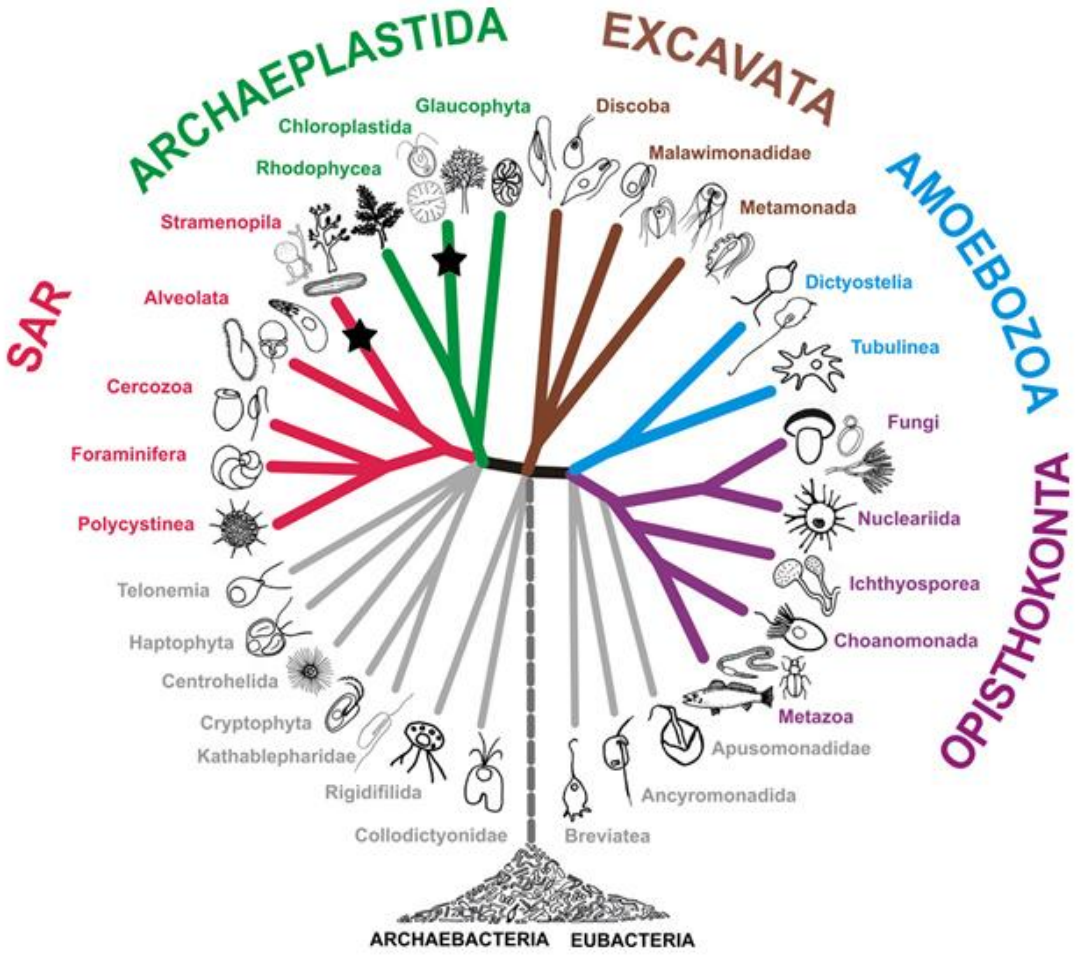

Fig. 2 Tree of Eukaryotes (Adl et al., 2012). The positions of diatoms and desmids are marked by black stars.

The central questions of the Master´s thesis were: (1) Is there a significant influence of host plant on associated algal epiphyton? (2) Do particular algal taxa show substrate specificity? (3) Are the strategies of diatoms and desmids parallel or contrast? To what extent can discovered trends be generalized for the entire microphytobentic community?

I suggested that the effects of different factors (i.e. host plant, site and environmental variables) on the community structure of epiphytic algae should have been investigated simultaneously. Such an approach would show the relative influence of host plant on associated epiphyton and if that influence was significant or negligible. Furthermore, I would conclude that if both algal groups, diatoms and desmids, showed similar strategies, then these patterns could be generalized for the entire microphytobentic community.

2. Materials and methods

2.1 Study sites and sampling

This thesis focused on a comparison of the algal epiphytic communities associated with different types of natural plant substrates. To achieve this, sampling of epiphyton was done at 15 isolated water bodies (further the term *site* is used), but strictly in stagnant waters. The sites were selected within eight areas in the Czech Republic (Fig.3): PR Rybníčky u Podbořánek, Horní Kracle, PP Rybníček u Studeného, PP Ďáblík, NPP Swamp and adjacent peatlands (including the site called tůň u Klůčku), Borkovická blata, PR Kozohlůdky and pískovny Cep[4]. All 15 sites were characterized as oligothophic or mesotrophic. For the list of the sites see Table 2 and for the complete overview of the sites with additional information see Appendix 1. The eutrophic sites were excluded right from the beginning of the study, because of the possible discrepancy between the epiphyte-response pattern in oligotrophic and eutrophic waters, suggested by Eminson & Moss (1980). The eutrophic sites represent ecosystems where multiple different factors may play an important role in the determining the community structure. For example, increased nutrient loading and dominance of plankton make benthic communities heavily influenced by turbidity and consequential shade, leading

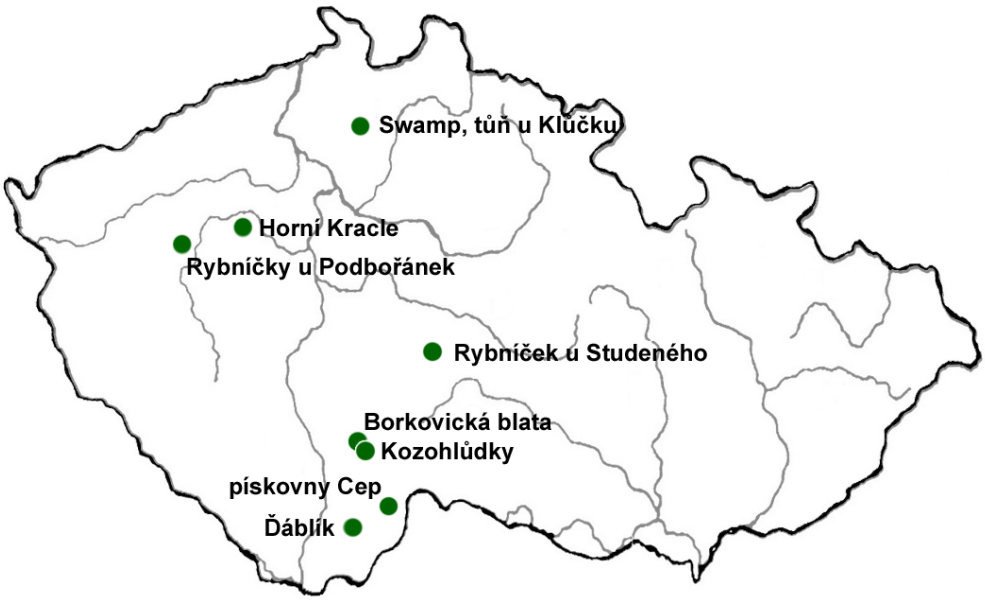

Fig. 3 Map of the Czech Republic with marked study areas.

[4] I decided to maintain all names of areas and study sites in Czech to make it easier to find those sites afterwards, if interested.

to an overall reduction of benthic production (Sand-Jensen & Borum, 1991; Havens et al., 2001; Liboriussen & Jeppesen, 2003). Besides, the criterion of low trophy allowed finding the host plants that were chosen for the study.

There were eight types (genera) of host plants (macrophytes) sampled: *Sphagnum* spp., *Utricularia* spp., *Nymphaea* spp., *Potamogeton natans*, *Calla palustris*, *Chara* spp., *Typha* spp. and *Equisetum fluviatile* (Table 1). They were chosen with regard to their common occurrence and required overlap between selected sites. In most cases, these host plants and the associated epiphyton have already been studied and there are published data concerning their possible interactions. The host plants could be divided into three groups, according to the plant architecture (i.e. substrate complexity), which might also affect the associated epiphyton community structure. These groups were the following: (1) complex plant architecture characterized by a dense branching and numerous smaller leaves, (2) simple plant architecture characterized by a smooth, relatively unbranched stem, and (3) simple plant architecture with smooth stem and floating leaves.

At least three host genera and three replicates (i.e. the samples from the same type of substrate, the distance between the replicated samples was at least 5 m) were collected at each site, if possible. A similar sampling approach was used e.g. in Millie & Lowe (1983) and Townsend & Gell (2005). Sometimes, however, there were only two host types present, or fewer than three replicates were taken. These data were included in the analyses anyway.

To sum this methodological part up, firstly, the epiphyton variation among the sites within one substrate type could be investigated, thanks to the overlap of host plant types between individual sites. Secondly, the epiphyton variation within the sites, both within and among substrate types, could be also examined thanks to the sampling of several substrate types and collected replications. The complete lists of study sites and macrophytes, as well as used abbreviations, are included in Table 1 and Table 2.

The sampling was held in 2011 (as a pre-study, 7 sites, 39 samples) and in 2012 (14 sites, 132 samples). In total, 171 samples were collected (see Appendix 2). Although it was done so, it was not the main aim of this research to reveal the inter-annual variation in epiphytic community, as reported in e.g. Laugaste & Reunanen (2005) and dos Santos et al. (2013). The datasets from 2011 and 2012 are unequal concerning the number of samples, but most of the samples from 2011 were collected again in 2012. However, the sampling in 2012 was far more complex, and thus provided much better data.

Table 1 Overview of selected host plants, regardless of sampling year. In total, 171 samples were collected from 15 sites and 8 types (genera) of host plants. More details are included in the appendix.

host plant	abbr.	plant architecture	no. of sites	no. of samples
Sphagnum spp.	SP	complex	9	39
Utricularia spp.	UT	complex	11	42
Nymphaea spp.	NY	simple, floating leaves	6	20
Potamogeton natans	PO	simple, floating leaves	7	21
Calla palustris	CA	simple	4	18
Chara spp.	CH	simple	4	10
Equisetum fluviatile	EQ	simple	2	9
Typha spp.	TY	simple	5	12

Table 2 Overview of the sites and sampled host plants (the grey fields), regardless of sampling year. More details are included in the appendix. (x) The samples that were included in *the reduced datasets* which were used in majority of analyses. ([a]) The sites that were not included to the analysis of desmid communities due to low abundances in the samples. ([b]) The site sampled just in 2011.

site	abbr.	no. of samples	SP	UT	NY	PO	CA	CH	EQ	TY
Swamp 1	S1	12								
Swamp 2	S2	6								
Swamp 3	S3	12	x	x	x					
tůň u Klůčku	TK	15	x	x	x	x				
Kozohlůdky	KO	15								
Borkovická blata	BB	6								
pískovny Cep 1	C1	9								
pískovny Cep 2	C2	5								
pískovny Cep 3	C3	9	x		x	x				
Rybníčky u Podbořánek 1	P1	30	x	x	x	x				
Rybníčky u Podbořánek 2	P2	12								
Horní Kracle	HK	9								
Ďáblík 1	D1	18	x	x		x				
Ďáblík 2 [a]	D2	7								
Rybníček u Studeného [ab]	RS	6								

All the samples were collected in late summer and autumn. In general, host plants may have different growth rates which is supposed to affect the epiphytic colonisation rates (Millie & Lowe, 1983). As the shoots of some macrophyte genera, e.g. *Utricularia* and *Nymphaea*, have to grow up every vegetative season, the late summer and autumn was possibly the best time for sampling, as the macrophytes have already grown up and been covered by relatively

well developed epiphyton. Also, there were no disturbances related to winter temperature decrease, freezing and significant light limitation that may affect epiphyton (Machová-Černá & Neustupa, 2009; Neustupa et al., 2012). Thus, the pre-study sampling in 2011 was done within few weeks from the end of September to mid October. Afterwards for the main sampling in 2012, it was decided to done it one month earlier, from the end of August to mid September. The reason was that even though the algal epiphytic communities were well developed in autumn, it was too late to find some host plants at the sites. For the sampling dates see Appendix 1. The pre-study also ensured that all chosen genera of macrophytes were suitable for the epiphyton sampling as most samples contained enough cells to be included in the statistical analysis.

The samples of epiphyton were obtained by plant squeezing or careful brushing of plant surface. Both these techniques are commonly used and highly efficient ways to sample attached epiphytic communities of microorganisms (e.g. Asaeda et al., 2004; Pals et al., 2006; Neustupa et al., 2011). Only the top submerged part of the host plant (max. down to 10 cm in depth) were sampled to avoid any variability caused by different positions of macrophytes in the water column (Morin & Kimb, 1983) and by often reported vertical zonation of epiphytic community (Gons, 1982; Lalonde & Downing, 1991; Müller, 1995, 1999). Further, Lugol´s solution was used to fix the samples right in the field. Therefore, any changes in the species ratios in the epiphytic communities, which might have been caused by a sudden change of ambient conditions, were prevented. However, Lugol´s solution may eventually break down, so more drops of the solution were subsequently added into the samples, whenever it was necessary.

The actual environmental variables (pH and conductivity; Appendix 2) were measured immediately in the field, using a combined pH/conductivity meter WTW 340i (WTW GmbH, Weilheim, Germany). These environmental variables were chosen because they have been shown to explain significant part of variation in the benthic microalgal communities (e.g. Fránková et al., 2009; Neustupa et al., 2013).

2.2 Sample processing

The current investigation is based on community structure data. Epiphytic diatoms (Bacillariophyceae) and desmids (Desmidiales) were chosen as the model groups (Chapter 1.6). In the laboratory, relative abundances of algal species in their community were counted

directly in the light microscope Olympus CX 31. From every sample, precisely 200 randomly encountered diatom cells and 200 randomly encountered desmid cells were identified to the species level. The colonies were counted up to 10 cells. Determination of 200 desmids per sample has widely been used in other studies (e.g. Pals et al., 2006; Neustupa et al., 2012; Svoboda et al., 2014). It was decided to do the same for diatoms (as in Neustupa et al., 2013), although it is more common to count 300 or even more diatom cells, or alternatively valves[5], per sample (Eminson & Moss, 1980; Millie & Lowe, 1983; Gaiser & Johansen, 2000; Poulíčková et al., 2004; Charles et al., 2006; Fránková et al., 2009). The purpose of the unification of the cell counts was to obtain comparable datasets.

In order to check whether or not the number of determined cells significantly affects the recorded community structure, a simple investigation was performed. There were two samples chosen, the first one (2-S1-UT1) as the representative of peatlands and the other one (2-P1-UT1) as the representative of mesotrophic ponds. Cumulatively from every sample, 200 cells, 400 cells and 1000 cells of diatoms were identified to the species level. To examine if the datasets differ, the Kolmogorov-Smirnov test was done in the software PAST, ver. 2.17c (Hammer et al., 2001). The Kolmogorov-Smirnov test is a nonparametric test which determines whether two datasets come from the same, respectively identical distributions (Young, 1977; Legendre & Legendre, 1998). Thus, three pairwise tests were run separately for each of the chosen samples. The first analysis compared the datasets of 200 cells and 400 cells, the second one compared the datasets of 200 cells and 1000 cells, and the third one compared the datasets of 400 cells and 1000 cells. The same tests were performed for the desmid community. The Kolmogorov-Smirnov tests were non-significant in all cases (P values not shown), indicating that the distributions within the sample did not differ, regardless of number of counted cells. Therefore, counting of 200 cells of particular algal group per sample should be sufficient to give the relevant community structure.

For the illustration of similar distributions within the sample, histograms are shown in Fig. 4. The graphs also reveal that there were always just few species that dominated the community. As it could be noted from species lists (not included in the thesis), these dominant species remained the same within all numbers of counted cells. Secondly, lots of rare species were present in the samples. This is considered to be true in general and detected cumulative

[5] The diatom frustule (i.e. silicate cell wall) consists of two separate valves. Therefore, if for example Millie & Lowe (1983) or Gaiser & Johansen (2000) identified 500 diatom valves, theoretically**,** they might have recorded 250 complete diatom cells. The advantage of this technique is that it takes into account the presence of dead cells that already fell apart, and the cells that were crushed during a slide preparation. Nevertheless, the majority of published studies uses the cell as the count unit. The same was done within this thesis.

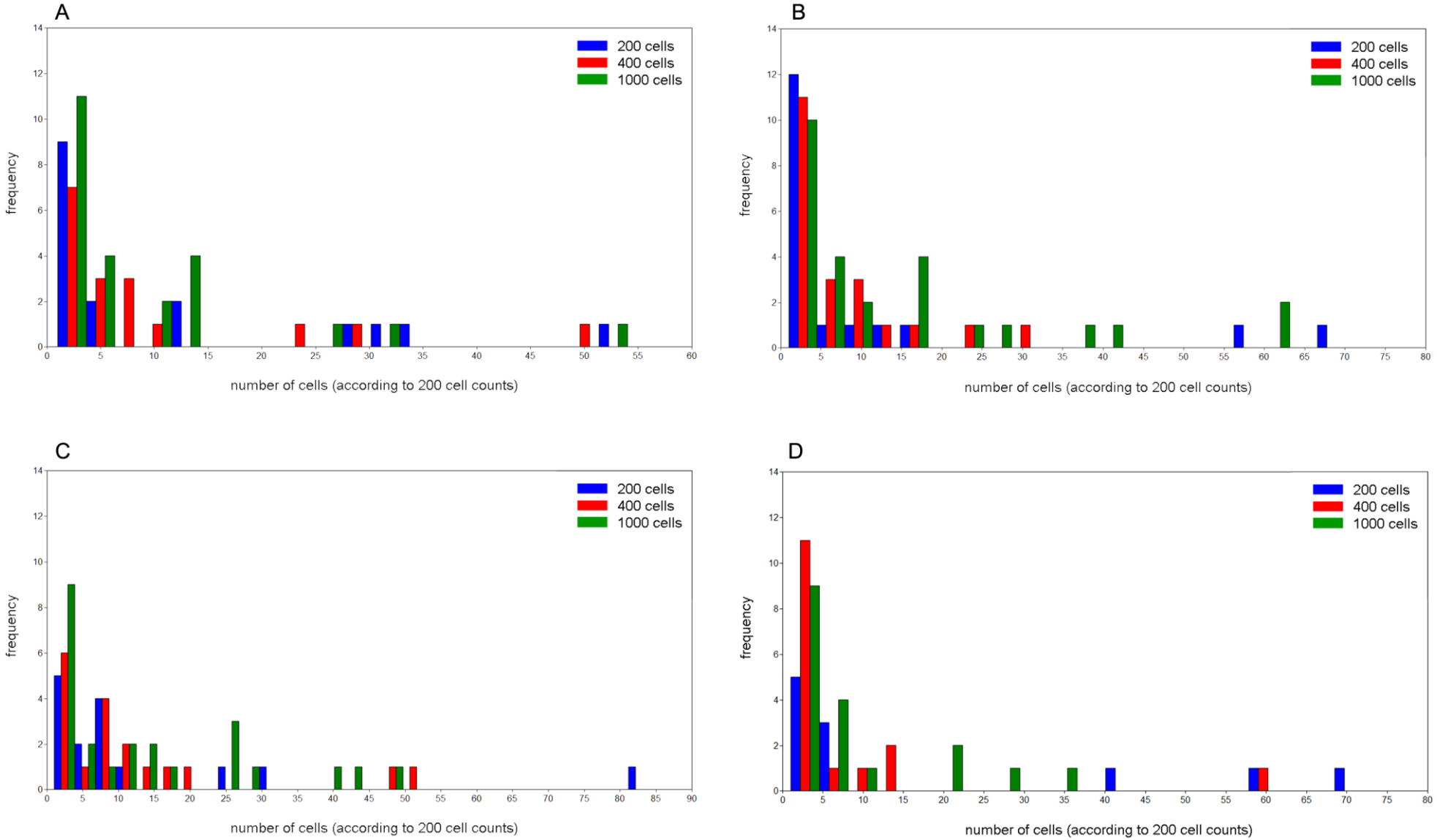

Fig. 4 The histograms of (A) diatoms recorded in the sample 2-S1-UT1, (B) diatoms recorded in the sample 2-P1-UT1, (C) desmids recorded in the sample 2-S1-UT1, and (D) desmids recorded in the sample 2-P1-UT1. The number of bins (i.e. intervals) was set as 20. The x-axis corresponds to the absolute number of cells determined within 200 cell counts. Other cell counts were automatically converted to match the 200 cell counts relatively. The Kolmogorov-Smirnov test, which determines whether two datasets come from the same, respectively identical distributions, was non-significant in all cases.

increase in species richness with higher number of counted cells is not surprising at all (see e.g. Finlay & Clarke, 1999; Fontaneto et al., 2006). The other scarce changes in the species distribution might have been just stochastic, for instance connected to the underestimation of bigger species as reported in Snoeijs et al. (2002). In conclusion, since the rare species may not be so crucial for revealing ecological patterns (Heino & Soininen, 2010), there was no point of counting more cells than 200 per sample for each algal group. It would be very time consuming due to a high number of samples (171 in total), and the most abundant species would be recorded anyway, even within such relatively low cell counts.

The identification of 200 diatom cells and 200 desmids cells per sample was done separately, due to slightly different methodological approaches in the species determination. Desmid species were identified in the light microscope 400× magnification, right from the samples preserved by Lugol´s solution. To identify diatom species, the morphology of diatom frustule (i.e. silicate cell wall) is crucial. Therefore, diatom species were always determined at 1000× magnification, from permanent slides which were made by the method of annealing over a gas burner flame (Battarbee et al., 2001). This method leads to removal of all cell organic material, and thus it makes the structure of diatom frustule better visible. The method of annealing over a gas burner flame does not provide such clear permanent slides in comparison to the method of oxidation using hydrogen peroxide or other acids, but the resulting slides were still good enough for species identification for the purpose of this study. Moreover, while using the method of annealing over a gas burner flame, the frustules are usually not destroyed and whole colonies do not fall apart, in comparison with the use of the chemicals. After the protoplast removal, the prepared cover slip with empty diatom frustules was put into the synthetic resin Naphrax (Brunel Microscopes Ltd. Wiltshire, UK). Naphrax is a mounting medium that increases refractive index (Flemming, 1954), so the structural details of diatom frustule are even more highlighted. Unfortunately, the identification of diatom species from the permanent slides makes it impossible to distinguish between living and dead diatoms (meaning at the moment of sampling). However, in this study, the short-term temporal variability was not explored at all. Thus, it did not really matter if some dead cells were occasionally counted within 200 determined cells. All species had most probably lived during the investigated year. The dead desmid cells, which rarely occurred in the samples, were also counted.

The identification of diatom species was done using the standard taxonomic monographs: Kramer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Krammer (2000, 2002,

2003), Lange-Bertalot & Metzeltin (1996), Lange-Bertalot (2001), Lange-Bertalot et al. (2011). The identification of desmid species was done using these standard taxonomic monographs: Růžička (1977, 1981), Lenzenweger (1996, 1997, 1999, 2003), Coesel & Meesters (2007).

2.3 Statistical analyses

2.3.1 Datasets

As it has been already mentioned, the statistical analyses of epiphyton associated with different types of natural plant substrates concern mainly the diatom and desmid community structure. A total of 200 diatom cells and 200 desmid cells per sample were examined. The resulting datasets were prepared separately for diatoms and desmids and comprised of all determined cells including the rare species. Therefore no standardization of species data was necessary. Identical analyses were done separately for each dataset in the majority of cases, allowing the indirect comparison of the discovered trends of both algal communities. The only exception was the Procrustes statistic (Chapter 2.3.6) where both datasets were used at once, for the purpose of the direct comparison. The analyses were conducted in the software PAST - ver.2.17c (Hammer et al., 2001) and R - ver. 2.15.1 (R Core Team, 2012) using the *vegan* package (Oksanen et al., 2012).

Basically, the statistical analyses were done using *the complete datasets* and *the reduced datasets* (Table 3, more details in Appendix 3). In total, there were 171 samples collected, but afterwards the reduction of the number of samples in the datasets was appropriate to obtain the relevant results. Firstly, all samples with very low algal abundances (i.e. samples where less than 200 diatom cells, or less than 200 desmids cells, were found on five slides) were excluded from further analyses. Thus, the complete datasets comprised of all samples that could be eventually used. Out from 171 samples, the complete diatom dataset contained 170 samples from 15 sites and 8 genera of host plants. The complete desmid dataset contained 141 samples from 13 sites and 8 genera of host plants. However, as it can be seen from Table 2, the complete datasets were very fragmented due to the absence of some macrophyte taxa at the sites. If a dataset with many host plants missing is used e.g. for PERMANOVA (one of the most important analyses done within this theses; Chapter 2.3.2), it might consequently provide some misleading results. Thus, the best way to perform the analyses was to extract a near complete subset from the data. It was important to use the sites

Table 3 List of datasets with species richness recorded within the determination of 200 cells per sample. Note that 50 samples in the reduced datasets are not the same for both algal groups, whereas 140 samples in the Procrustes complete dataset and 49 samples in the Procrustes reduced datasets are exactly the same.

datasets		no. of samples	no. of sites	no. of hosts	no. of species
all collected samples		171	15	8	-
complete	diatoms	170	15	8	171
	desmids	141	13	8	161
reduced	diatoms	50	5	4	106
	desmids	50	5	4	103
Procrustes complete	diatoms	140	13	8	152
	desmids	140	13	8	161
Procrustes reduced	diatoms	49	5	4	102
	desmids	49	5	4	102

and the host plants with the highest possible overlap, which in this case included the sites (Swamp 3, tůň u Klůčku, pískovny Cep 3, Rybníčky u Podbořánek 1, Ďáblík 1) where three or more of *Sphagnum*, *Utricularia*, *Nymphaea* and *Potamogeton* were sampled (Table 3). Only the samples from the year 2012 were considered because the data from 2011 were limited. These so called the reduced datasets contained only 50 samples from 5 sites and 4 genera of host plant. Note that the reduced datasets of diatoms and desmids were numerically equal, but they differed in one sample (2-D1-SP3 was included only for diatoms, 2-TK-UT2 only for desmids; see Appendix 3).

It was decided that the reduced datasets were the most appropriate for the analysis done within this thesis. The only exception was again the Procrustes statistics. This test was carried out twice, firstly using the reduced datasets, and secondly using the complete datasets to aid any potential generalisation of algal community trends. The datasets for the Procrustes statistic needed some additional reductions as the analysis generally requires that datasets being compared contain exactly the same objects. Therefore only the samples where both 200 diatom cells and 200 desmid cells were found were included. The complete datasets were pruned to 140 samples from 13 sites and 8 genera of host plant and the reduced datasets were pruned to 49 samples from 5 sites and 4 genera of host plant.

2.3.2 Effects of individual factors on epiphyton

The first part of the statistical evaluation was done to estimate the effects of individual factors on the algal epiphytic community. This was tested using a permutational multivariate

analysis of variance (the permutational MANOVA or PERMANOVA, formerly called nonparametric MANOVA; Anderson, 2001, 2005; McArdle & Anderson, 2001). The analysis is run by the function *adonis* which is implemented in the *vegan* package in R software. PERMANOVA is distribution free and it is a robust alternative to parametric MANOVA and redundancy analysis (Legendre & Anderson, 1999). PERMANOVA works with two matrices. Firstly, the distance matrix is calculated from the original species data matrix according to selected distance measure. The second matrix contains the tested factors. PERMANOVA partitions the variation attributed to the factors sequentially, meaning that the factors and their mutual effects are tested in the same order as they are stated in the model (i.e. *adonis* formula). By creating several models with different order of factors, this approach leads to the determination of a pure effect for an individual factor (always the factor that is quoted as the last one in the model). Its significance is assessed by the permutation test with pseudo F ratios.

In this study, PERMANOVA considering the factors *site*, *host*, *pH* and *conductivity* was done to quantifying patterns of variation in the epiphytic community structure and species richness. The analysis was run separately for diatoms and desmids. For each PERMANOVA, two matrices were prepared based on the reduced datasets (50 samples from 5 sites and 4 genera of host plant). The first matrix contained the community structure of particular algal group, respectively the data of species richness, and the second one was the matrix with coded factors (site and host plant) and numerical environmental parameters (pH and conductivity). No data transformation was needed. The analyses were conducted using Bray-Curtis similarity index for the community structure and Euclidean distance for the species richness. Bray-Curtis index (Bray & Curtis, 1957) belongs to the most widely used index in ecological analysis (Clarke, 1993). It takes into account both species and their abundance, moreover it can cope with the prevalence of rare species or zeros in the data. The Euclidean distance is suitable for the univariate analysis (Anderson, 2005). All tests were done using 999 permutations.

As previously mentioned, the PERMANOVA results assess the proportion of explained variation, so called coefficient of determination (R^2). However, R^2 is dependent on the degrees of freedom that every factor has. If the degrees of freedom differ between the factors, as in the case of this research, R^2 values are not comparable. Therefore, it is appropriate to recount the R^2 values to the adjusted R^2 that take account of the number of samples and degrees of freedom (Peres-Neto et al., 2006). The calculation was done using the

function *RsquareAdj* which is again implemented in the *vegan* package in R software. The adjusted R^2 are given in the thesis in addition to the R^2 values.

To support and indirectly illustrate the PERMANOVA results, non-metric multidimensional scaling (NMDS) was performed in PAST software. NMDS is a widely used ordination analysis to show the species composition patterns in the dataset (Kruskal, 1964; Clarke, 1993; Legendre & Legendre, 1998). NMDS basically transforms multidimensional space, in which the data are, to the three-dimensional or two-dimensional space. Simultaneously NMDS preserves the distance relationships among the data based on chosen similarity index. It can be easily plotted in the diagram where each point represents one sample from the dataset. The distance between the points exposes the dissimilarity between them. In other words, the closer points are, the more similar the community structures of given samples are. The analysis also allows grouping the samples according to the selected criterion. The groups are then illustrated by different symbols and colours.

In this study, since the Kruskal´s stress values representing reliability of NMDS (Borg & Groenen, 2005) were not very high, the two-dimensional diagrams were done using Bray-Curtis similarity index. For completeness, the coefficients of determination (R^2) for each axis are given in the graphs. The analyses were run always separately for diatoms and desmids. The 50 samples from the reduced datasets, which were used for PERMANOVA, were divided into the groups reflecting the factor *site*, *host plant*, *plant architecture* or *pH*. The plant architecture was included as a factor to verify whether or not to use it in PERMANOVA. The factor *conductivity* was not used in the end. The reason was that two out of three set categories of conductivity mostly contained the samples just from one site and thus the NMDS ordination diagram did not provide any contribution. For example the third category, in which conductivity were higher than 200 μS/cm, contained 10 samples only from the site Rybníčky u Podbořánek 1.

2.3.3 Analyses of epiphytic species diversity

The community structure of algal epiphyton was also explored by using diversity indices, in order to support the results obtained from PERMANOVA and to provide more information about the epiphytic community. In addition to species richness, which is equal to the number of taxa found in the sample, Shannon diversity index (Shannon & Weaver, 1949) was used. This index takes into account both species richness and relative abundances of

particular species (i.e. number of individuals of particular species), and thus express the evenness of community. Shannon diversity index varies from 0, indicating a complete dominance by a single species, to higher values, meaning that there are more species in the community and that their relative abundances are lower (Legendre & Legendre, 1998). In this study, the diversity indices were calculated using all 200 determined cells per sample (either diatoms or desmids), and they were calculated for every sample from the reduced datasets. The analyses, including calculation of indices, following formal tests and graph generation, were carried out in PAST software, always separately for diatoms and desmids.

First of all, the datasets of species richness and the datasets of Shannon diversity indices were tested for normality by the Shapiro-Wilk test (Shapiro & Wilk, 1965), which is so far the most powerful test to investigate the normality of data (Razali & Wah, 2011). Since three out of four datasets were not normally distributed (species richness of diatom samples, $P = 0.04$; species richness of desmid samples, $P = 0.04$; and Shannon diversity indices of desmid samples, $P = 0.004$), further analyses were performed using nonparametric tests. It could be claimed only about one dataset that it came from the normal distribution (Shannon diversity indices of diatom samples, $P = 0.1$), nevertheless the nonparametric tests were done in all cases to reach the comparability of the results. The nonparametric tests are common alternatives to parametric tests such as t-test or ANOVA, but they do not assume any data distribution. The nonparametric tests are based on ranks of observations, and thus work with *median* and *range* instead of *mean* and *variance*, and they remain valid even for very small datasets (Legendre & Legendre, 1998).

The particular indices were divided into the groups identically to the approach of NMDS. The groups reflected the factor *site, host plant, plant architecture* or *pH*, and the factor *conductivity* was not used. As described earlier, the differences in diversity indices between particular groups were examined by nonparametric tests. The Mann-Whitney test (Mann & Whitney, 1947) was used to compare two groups, e.g. complex and simple plant architecture. To compare more than two groups, e.g. different host plants, the Kruskal-Wallis test (Kruskal, 1964) with post-hoc Mann-Whitney pairwise comparisons using Bonferroni correction (Rice, 1989; Cabin & Mitchell, 2000) was performed. Both tests have a similar null hypothesis, saying that the medians of the groups are the same. Additionally, boxplots were created to illustrate the differences in the diversity indices of individual groups, since boxplots can easily show the mean, upper and lower quartiles, minimum and maximum, alternatively outliers (Williamson et al., 1989).

The formation of separate categories based on *pH* or *conductivity* (highly problematic in case of this thesis) is generally unnatural because these parameters are continuous. Thus, it was appropriate to test the relationship between diversity indices and one of environmental variables by using simple linear regression (Legendre & Legendre, 1998). The model is performed if it is known which of the variables is explanatory (independent) and which is explained (dependent). The relationship between selected variables is given by the correlation coefficient (r), coefficient of determination (R^2) and in this case the straight regression line, which is based on the method of least squares (i.e. the shortest possible distances between data and regression line that represents the model). The null hypothesis of linear regression is that there is no relationship between selected variables, in other words that the slope of regression line is equal to zero. The method of linear regression, however, cannot show the pure effects of individual factors like PERMANOVA, therefore the explanatory variable itself could be correlated with other parameters and the interpretation of the results may not be so straightforward.

2.3.4 Substrate specificity of epiphytic species

The second question of this thesis was whether particular algal taxa show the substrate specificity. The correct interpretation of the results of such an analysis partly depends on the PERMANOVA outcomes. To be in good agreement with PERMANOVA, the same reduced datasets (50 diatom samples, alternatively 50 desmid samples) were used again. In contrary, the substrate specificity analysis based onthe complete datasets (171 diatom samples, alternatively 140 desmids samples) would be rather questionable. The reason is that in general there would be no report of the macrophyte influence on the complete datasets. Therefore those results are not included in the thesis.

In this work, only the 25 % most abundant species from each dataset were considered as relevant for the ecological analysis (Heino & Soininen, 2010). In addition species had to occur at least at two sites in order to exclude species unique to a particular sample or site. Such criteria were considered to be important for finding species suitable for the substrate specificity analysis. The substrate preferences of chosen 25 diatom species and 18 desmid species were examined by the correlation using the Kendall rank correlation coefficient (Kendall´s tau; Kendall 1938). Kendall´s tau has confident intervals which are more reliable than the alternative nonparametric coefficient called Spearman´s (Newson, 2002). The

correlation measures the degree of association between two variables, in this case between the species abundance and host plant. The correlation coefficient (r) varies between +1 and -1 and reflecting positive and negative, respectively, dependence. Values closer to +1 or -1 indicate stronger correlations. If the value is around 0, there is no or very weak correlation. Finally, it is recommended that the significant substrate specificity, if any, must be compared with the findings of already published studies, as this may reveal a species to be ubiquitous if it also occurs in higher abundances on other substrates (Townsend & Gell, 2005).

2.3.5 Comparison of algal group strategies

The last remaining analysis is the direct comparison of diatom and desmid epiphytic communities. To this point, all analyses were performed separately for diatoms and desmids, so the comparison of discovered trends could have been drawn only indirectly. The direct one was performed by the permutation test based on the Procrustes statistic (PROTEST; Legendre & Legendre, 1998; Peres-Neto & Jackson, 2001). The function *procrustes* is implemented in the *vegan* package in R software. PROTEST compares multivariate datasets by measuring the degree of their concordance. For this analysis, it is crucial to work with the datasets concerning exactly the same objects. The Procrustean superimposition approach (Gower, 1971) is a method which utilises the raw data matrices, or alternatively similarity or distances matrices (provided by e.g. NMDS), which are scaled and rotated in order to minimize the sum of squared distances between corresponding objects of the two matrices, and thus to maximize their fit. The correlation coefficient (r) and the significance of the non-randomness of the evaluated congruence are assessed by permutation tests. Additionally, the plot shows the differences between the two original matrices. Each object is visualized twice in the diagram and the distance between them represents the extent of their congruence.

In this study, the distance matrices based on a Bray-Curtis similarity index were taken from the two-dimensional NMDS. The Procrustes analysis using 999 permutations was done for the reduced datasets pruned to 49 samples, so that it worked with the datasets concerning exactly the same objects. In those samples, both 200 diatom cells and 200 desmid cells were found. Such an analysis was meant to support all indirect comparisons of both algal groups that could be made based on previous statistical analysis. In the same way, the complete datasets were pruned to 140 samples. By performing PROTEST for much greater datasets, the potential generalisation of algal community strategies can be reinforced even more.

3. Results

3.1 General description of datasets and epiphytic species composition

For the purpose of this thesis, epiphytic communities of diatoms and desmids associated with different types of natural plant substrates were sampled at 15 sites in the Czech Republic. A total of 171 samples were collected in 2011 and 2012. After that, algal community structure was examined and the datasets containing applicable samples were prepared separately for diatoms and desmids. Typically, identical analyses were done separately for each of the algal datasets, allowing the indirect comparison of the discovered trends for both algal groups. The only exception was the Procrustes statistic, where both datasets were used at once, in order to get direct comparison and confirmation of similarity or distinctness of algal group strategies.

This study was based on algal morphospecies identified in the light microscope. Species lists from the complete datasets are included in Appendix 4 and 5. Basic information about all datasets, including recorded species richness, are provided in Table 3. The majority of analyses, which were done in this research, worked with the reduced datasets within which host plants were evenly represented at every site (Table 2). All samples in the reduced datasets came from the sampling in 2012. The reduced datasets of diatoms contained 50 samples from 5 sites and 4 types of host plants. There were 106 recorded diatom species. The reduced dataset of desmids contained 50 samples from the same 5 sites and the same 4 types of host plants. There were 103 desmid species recorded. Note that 49 samples were the same for diatoms and desmids and thus could be used in the Procrustes analysis, but the diatom and desmid reduced datasets differed in the last sample (2-D1-SP3 was included only for diatoms, 2-TK-UT2 only for desmids; see Appendix 3).

The species composition and most common genera of the complete datasets are summarised in following paragraphs. Within 200 cell counts of particular algal group per sample, a total of 172 diatom species belonging to 40 genera (Appendix 4) and a total of 161 desmid species belonging to 18 genera were identified (Appendix 5). Diatom species richness per sample ranged from 5 to 33 within in the samples collected in 2011, and from 2 to 36 in 2012. Desmid species richness per sample ranged from 4 to 18 in 2011, and from 5 to 28 in 2012. The reported species richness is in good agreement with other records of freshwater benthic diatoms and desmids from similar types of localities (Millie & Lowe, 1983; Poulíčková et al., 2004; Kuczyńska-Kippen et al., 2005; Neustupa et al., 2012; Svoboda et al.,

2014). As given in Table 3, diatom species richness and desmid species richness were virtually the same. However, this finding is not general. For instance, two times as many desmid species as diatom species were identified in the study of Neustupa et al. (2013), in contrast to Krivograd Klemenčič et al. (2010) where exactly opposite results was mentioned, even though both studies were done in peatlands. Concerning lakes, Eminson & Moss (1980) registered much greater desmid species richness than diatom species richness in the sample from Otis Lake in Michigan, whereas they found more diatom species and no desmid species in the samples from Hickling Broad in Norfolk. Of course any reported species richness is not absolute. It is hardly achievable to know the absolute number of species in a community, but for ecological analysis it is not necessary at all. In any case, the abundant species are surely recorded and as such they are the most important for analyses (Heino & Soininen, 2010).

In this study, the most frequent diatom genera in the complete dataset were *Pinnularia* (40 recorded species) and *Eunotia* (27 species), followed by *Gomphonema* (12 species) and *Nitzschia* (11 species). These genera contain many species and are common in freshwater benthic microhabitats. On the other hand, the genera *Amphora, Caloneis, Cymbopleura, Denticula, Epithemia, Fallacia, Hippodonta, Chamaepinnularia, Lemnicola, Luticola, Placoneis, Planothidium, Pseudostaurosira, Rhopalodia, Staurosira* and *Staurosirella* were represented by a single species in the complete dataset. It is also important to note that large genera that have been split, such as *Achnanthes* (Bukhtiyarova, 2007, 2008; Guiry, 2015), *Fragilaria* (Williams & Round, 1987; Guiry, 2015), and *Navicula* (Guiry, 2015), and their related genera, were quite common in the samples of epiphyton. For instance, there were 4 species identified as cf. *Achnanthes*. Furthermore, this group included *Achanthidium* (3 species), *Rossithidium* (3 species) and rarely present genera *Lemnicola* and *Planothidium*. Other acidophilic genera, e.g. *Brachysira*, *Frustulia* and *Tabellaria,* were also present in the samples.

The most frequent desmid genera in the complete dataset were *Cosmarium* (50 recorded species), *Closterium* (27 species), *Staurastrum* (26 species), followed by *Euastrum* (14 species) and *Staurodesmus* (10 species). These genera belong to the most diversified ones and are very common in freshwater benthic microhabitats. The acidic sites also provided a good condition for the occurrence of several species of *Actinotaenium*, *Micrasterias* and *Tetmemorus*. Rarely, the genera *Bambusina*, *Haplotaenium*, *Hyalotheca*, *Netrium*, *Penium*, *Spondylosium* and *Teilingia* appeared, but these genera are known for relatively lower species diversity in comparison to those previously listed. Thus, such a result could have been

predicted. Although taxonomical revision of desmids based on a combined approach is required as suggested by e.g. Gontcharov & Melkonian (2008, 2011) and Nemjová et al. (2011), traditional morphospecies and genera remain used in ecological studies and biomonitoring to date.

3.2 Effects of individual factors on epiphyton

As discussed in the introduction, there are many reported cases of the macrophyte influence on associated epiphyton in fresh waters. These studies usually assumed right from the beginning that host plants, as biologically active substrates, affected associated epiphyton, and subsequently the researchers wanted to explain how. But is there actually any significant influence of host plant compared to other factors which apparently affect benthic microorganisms? To answer this core question, the comparison of influence of substrate (host plant), space (site) and environmental parameters (pH and conductivity) on epiphytic community structure and species richness was carried out. The analyses used the reduced datasets, always separately for diatoms and desmids.

The partitioning of variation in algal communities was performed by PERMANOVA. The results of individual tests are summarized in Table 4-7. Only the pure effects of particular factors and residuals (i.e. remaining unexplained variation) are noted. In addition to the R^2 values that are highly dependent on the degrees of freedom that every factor has, the adjusted R^2 values, which are comparable to each other, are given. As evident from Table 4-7, site was always the factor explaining the greatest part of the variation in the data. It explained as much as 28 % (from adjusted R^2) of the variation in diatom community structure ($P < 0.001$), 49 % of the variation in diatom species richness ($P < 0.001$), 39 % of the variation in desmid community structure ($P < 0.001$), and 27 % of the variation in desmid species richness ($P < 0.001$). Other factors, including host plant, have to be reported as negligible. Even though they sometimes significantly explained the variation in the samples, the adjusted R^2 was strikingly low or even had negative values, which are interpreted as equal to 0. The only noteworthy exception was conductivity, which explained just about 5 % of variation in diatom species richness, but the significance was obviously lower ($P < 0.05$) than the significance of the effect of site. On the other hand, the influence of conductivity was not significant within the diatom community structure analysis. It is unclear why conductivity only affected the diatom species richness. In any case, the mild effect of the environmental parameters is not

Table 4 Results of individual PERMANOVA tests that partitioned the variation in diatom community structure. For each factor, only the pure effect is given. The analysis was conducted using the Bray-Curtis similarity index and 999 permutations. *** P < 0.001, ** P < 0.01, * P < 0.05

factor	df	sums of squares	F ratio	R^2	adjusted R^2	P value
site	4	6.17	10.98	0.34	0.28	***
host plant	3	1.28	3.03	0.07	0.01	***
pH	1	0.39	2.74	0.02	0.00	***
conductivity	1	0.17	1.24	0.01	-0.01	
residuals	40	5.62	-	0.31	-	-

Table 5 Results of individual PERMANOVA tests that partitioned the variation in diatom species richness. For each factor, only the pure effect is given. The analysis was conducted using the Euclidean distance and 999 permutations. *** P < 0.001, ** P < 0.01, * P < 0.05

factor	df	sums of squares	F ratio	R^2	adjusted R^2	P value
site	4	1288.21	13.72	0.53	0.49	***
host plant	3	104.13	1.48	0.04	-0.02	
pH	1	42.91	1.83	0.02	0.00	
conductivity	1	178.69	7.61	0.07	0.05	*
residuals	40	938.8	-	0.39	-	-

Table 6 Results of individual PERMANOVA tests that partitioned the variation in desmid community structure. For each factor, only the pure effect is given. The analysis was conducted using the Bray-Curtis similarity index and 999 permutations. *** P < 0.001, ** P < 0.01, * P < 0.05

factor	df	sums of squares	F ratio	R^2	adjusted R^2	P value
site	4	8.26	18.40	0.44	0.39	***
host plant	3	0.96	2.84	0.05	-0.01	***
pH	1	0.33	2.92	0.02	0.00	**
conductivity	1	0.33	2.96	0.02	0.00	**
residuals	40	4.49	-	0.24	-	-

Table 7 Results of individual PERMANOVA tests that partitioned the variation in desmid species richness. For each factor, only the pure effect is given. The analysis was conducted using the Euclidean distance index and 999 permutations. *** P < 0.001, ** P < 0.01, * P < 0.05

factor	df	sums of squares	F ratio	R^2	adjusted R^2	P value
site	4	549.6	10.06	0.33	0.27	***
host plant	3	89.47	2.18	0.05	-0.01	
pH	1	1.26	0.09	0.00	-0.02	
conductivity	1	2.32	0.17	0.00	-0.02	
residuals	40	546.13	-	0.33	-	-

that unexpected since the sampling sites were chosen to be as similar as possible, in order to fall under the category of oligotrophic and mesotrophic sites.

In order to support and graphically illustrate the results from PERMANOVA, as well as to further explore the epiphytic community patterns, other analyses followed. Firstly, two-dimensional NMDS ordination plots were made. The results reflected the factors site, host plant, plant architecture and pH. The factor conductivity was not examined by NMDS as it was not really possible to create suitable categories. Regarding the factor plant architecture (i.e. substrate complexity), PERMANOVA did not reveal any sufficient effect of host plant to investigate whether or not plant architecture affects the associated epiphytic community. Nevertheless, the factor plant architecture was added to NMDS and other following analyses to support the decision not to include this factor in PERMANOVA.

Secondly, the differences in species richness and Shannon diversity indices between particular groups of samples were investigated using nonparametric tests (Mann-Whitney test or Kruskal-Wallis test). The division of diversity indices to the groups was made according to each of the factors site, host plant, plant architecture or pH, identically to the division for NMDS. Species richness has already been investigated in terms of variation partitioning by PERMANOVA, whereas this time the analyses of chosen diversity indices show exact numbers belonging to a particular group of samples (e.g. particular site). Additionally, the relationship between diversity indices and numerical environmental variables (pH and conductivity) were tested using linear regression. Each time the reduced datasets were included in the analyses, as in PERMANOVA. The analyses were performed again separately for diatoms and desmids. The results of these supportive analyses are summarized in following chapters.

3.3 Effect of site

As mentioned previously, the factor site explained the greatest part of the variation in the data. The comparison of NMDS plots in Fig. 5 with the others (Fig. 8, 9, 12) clearly reinforced the outcome of PERMANOVA. The groups representing particular sites in Fig. 5 are well separated. Such a distinct spatial pattern was also visualized in other studies concerning microphytobentos (Eminson & Moss, 1980; Svoboda et al., 2014).

Moreover, it is again evident from the investigation of diversity indices that the factor site plays a crucial role in determining epiphytic community. Regarding diatoms, there was

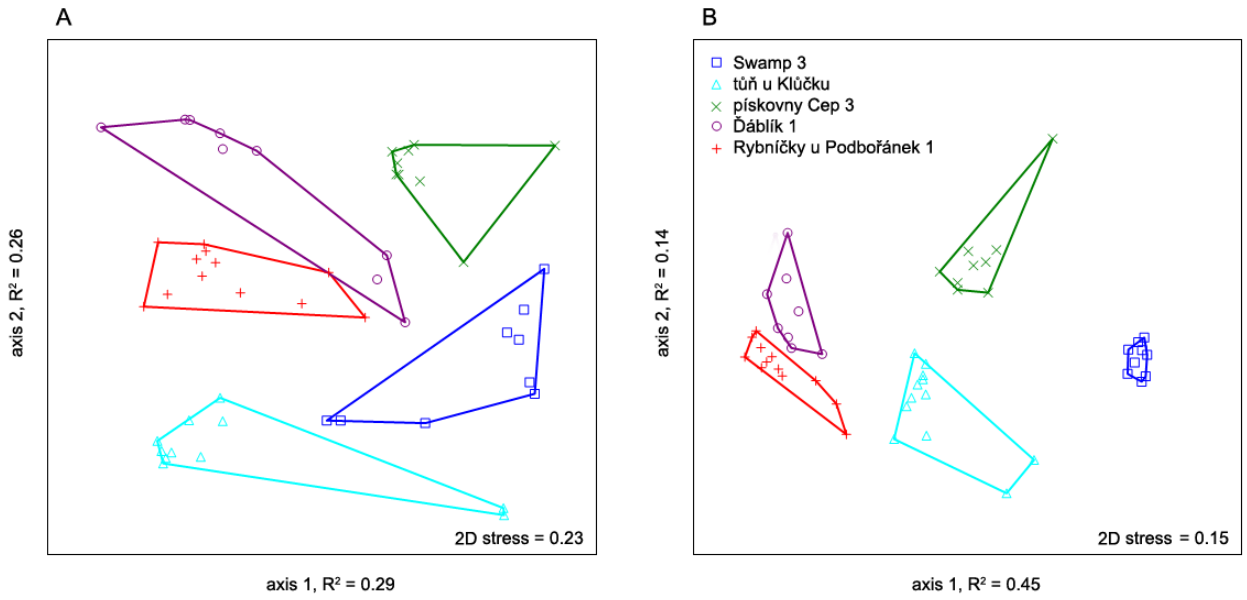

Fig. 5 NMDS ordination plots of samples collected at different sites. The Bray-Curtis similarity index was used. The analysis was performed separately for (A) diatoms and (B) desmids. The graph legend applies for both plots.

significant difference among sites (Fig. 6) in species richness (Kruskal-Wallis test, $P < 0.001$), as well as in Shannon diversity indices (Kruskal-Wallis tests, $P < 0.001$). The post-hoc Mann-Whitney pairwise comparisons using Bonferroni correction revealed that sites were split into two groups that differed in species richness. The first group was represented by the sites Rybníčky u Podbořánek 1 (P1) and Ďáblík 1 (D1), which are characterized as oligo-mesotrophic ponds with higher values of species richness. The second distinct group was made by Swamp 3 (S3), tůň u Klůčku (TK) and pískovny Cep 3 (C3), which are all peatland pools and have lower species richness probably due to more acidic conditions. But the analyses of Shannon diversity indices did not separate the groups according to the site characteristic. The overall significance of Shannon diversity indices analysis was basically determined by just a couple of differences (both with $P = 0.02$), i.e. between pískovny Cep 3 (C3) and Rybníčky u Podbořánek 1 (P1), and then between pískovny Cep 3 (C3) and Ďáblík 1 (D1), as displayed in Fig. 6.

The analyses of desmid datasets did not show a distinct division between peatlands and lakes either. The difference in desmid species richness and Shannon diversity indices among sites (Fig. 7) were overall significant (Kruskal-Wallis test, both $P < 0.001$). The pairwise comparisons indicated that Rybníčky u Podbořánek 1 (P1) and Swamp 3 (S3) were significantly different in both diversity indices from all the other sites except for the site

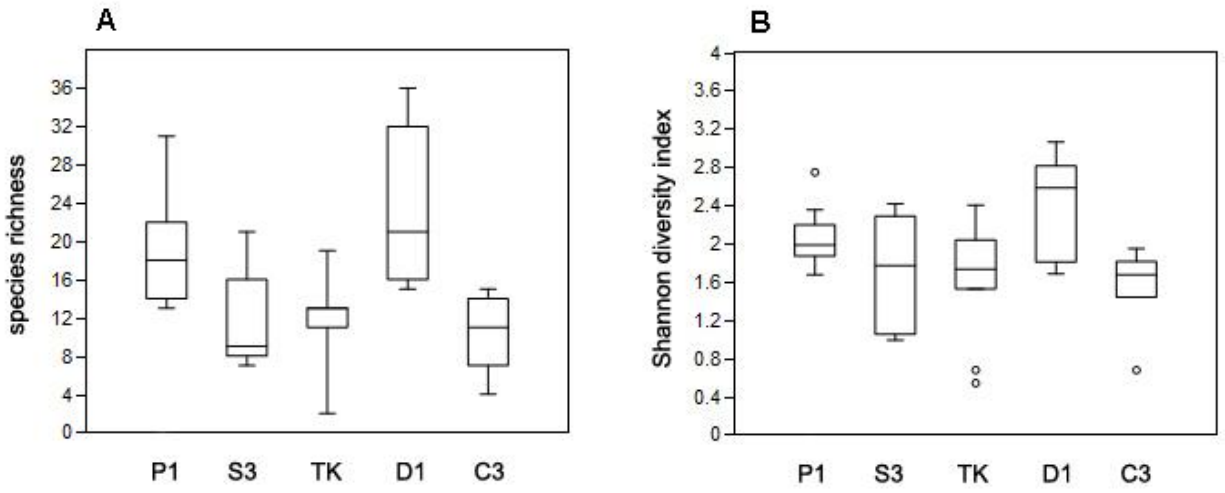

Fig. 6 Illustrated comparison of (A) species richness and (B) Shannon diversity indices of diatoms among individual sites. The differences among sites were significant in both cases (Kruskal-Wallis tests, $P < 0.001$).

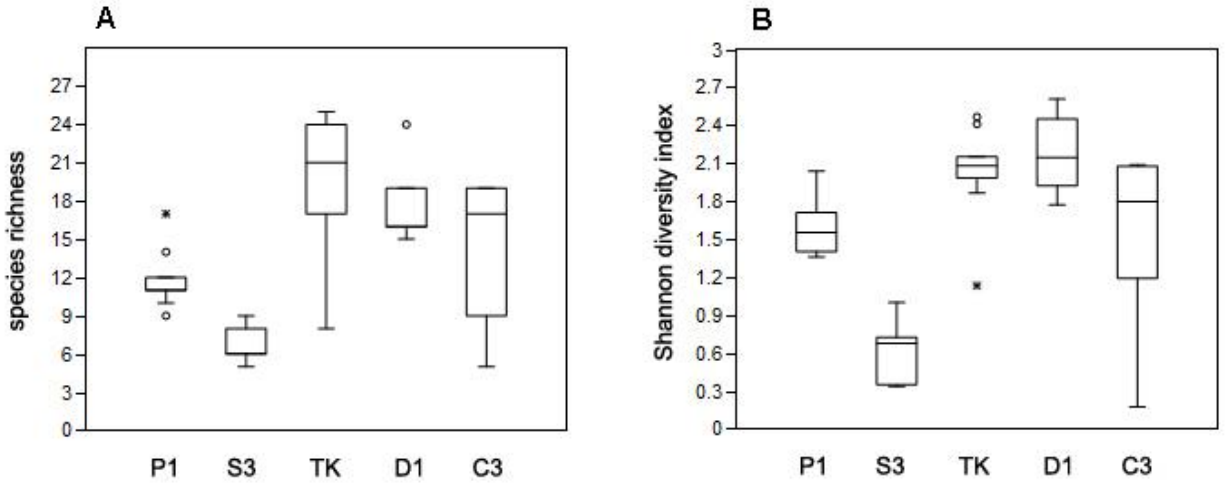

Fig. 7 Illustrated comparison of (A) species richness and (B) Shannon diversity indices of desmids among individual sites. The differences among sites were significant in both cases (Kruskal-Wallis tests, $P < 0.001$).

pískovny Cep 3 (C3). The site Swamp 3 (S3) was characterized by the lowest values of species richness and Shannon diversity indices, probably due to severe acidic and oligotrophic conditions which favoured the occurrence of few adapted species that fully dominated the desmid community (as in Mataloni, 1999). The narrow range of boxplot quartiles of Rybníčky u Podbořánek 1 (P1) refer to the finding that the samples from this site had very similar species richness in majority, even though all host plants were present and pH values ranged from more acidic to neutral conditions. Therefore, some other undetected factor may determine the specific pattern of species richness of the desmid community at Rybníčky u Podbořánek 1 (P1).

3.4 Effect of host plant

In contrast to Fig. 5 where NMDS shows distinct spatial pattern of epiphyton, the groups representing host plants in Fig. 8 substantially overlap. Also, no compelling differences in algal species richness and Shannon diversity indices were detected among different types of host plants (Kruskal-Wallis tests, all non-significant; Fig. 10 and Fig. 11). Therefore, this outcome strongly corroborated the results of PERMANOVA. It does not matter from which type of macrophyte growing within one site the epiphyton is sampled, but where (i.e. from which of the sites) the sample is taken.

Since PERMANOVA did not reveal any remarkable effect of host plant on associated epiphyton, it was not worth exploring from where the influence comes (e.g. substrate complexity, biological and chemical interactions). Although it has been suggested that *Sphagnum* and *Utricularia*, as more complex substrates, should support more diverse epiphytic communities, the investigating of the influence of plant architecture by NMDS and other supportive analyses confirmed the outcome of PERMANOVA. For better visualisation, the NMDS diagrams reflecting different types of plant architecture are presented in Fig. 9. It may appear that there was some influence of degree of substrate complexity, however, the convex hulls largely overlap and the greater range of the samples from complex substrates is mostly caused by single outlying samples, i.e. the samples with a very distinct community structure. See for example the single sample located at the bottom left of the desmid graph (Fig. 9, plot B). This sample alone adds approximately one third to the resulting convex hull of complex plant architecture and so it makes the convex hull optically bigger. Without such exceptions, the convex hulls are more or less similar in size.

With regard to diversity indices, although it may appear from Fig. 10 that *Sphagnum* (SP) and *Utricularia* (UT) had wider ranges of diatom species richness values and that *Sphagnum* (SP) had a wider range of Shannon diversity indices than other macrophytes, the Mann-Whitney tests did not reveal any significant dissimilarity between complex (*Sphagnum* and *Utricularia*) and simple (*Potamogeton* and *Nymphaea*) substrates. As can be observed in Fig. 11, the diversity indices of the desmid community should not differ between complex and simple substrates. The non-significant Mann-Whitney tests again confirmed this prediction (box plots not presented).

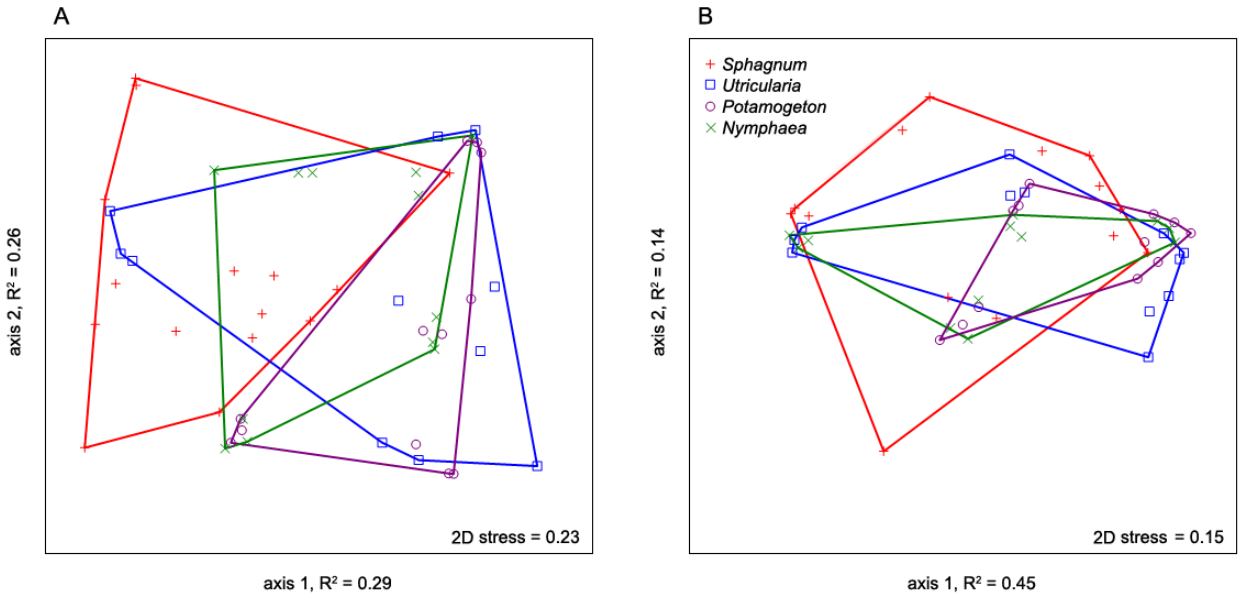

Fig. 8 NMDS ordination plots of samples collected from different host plants. The Bray-Curtis similarity index was used. The analysis was performed separately for (A) diatoms and (B) desmids. The graph legend applies for both plots.

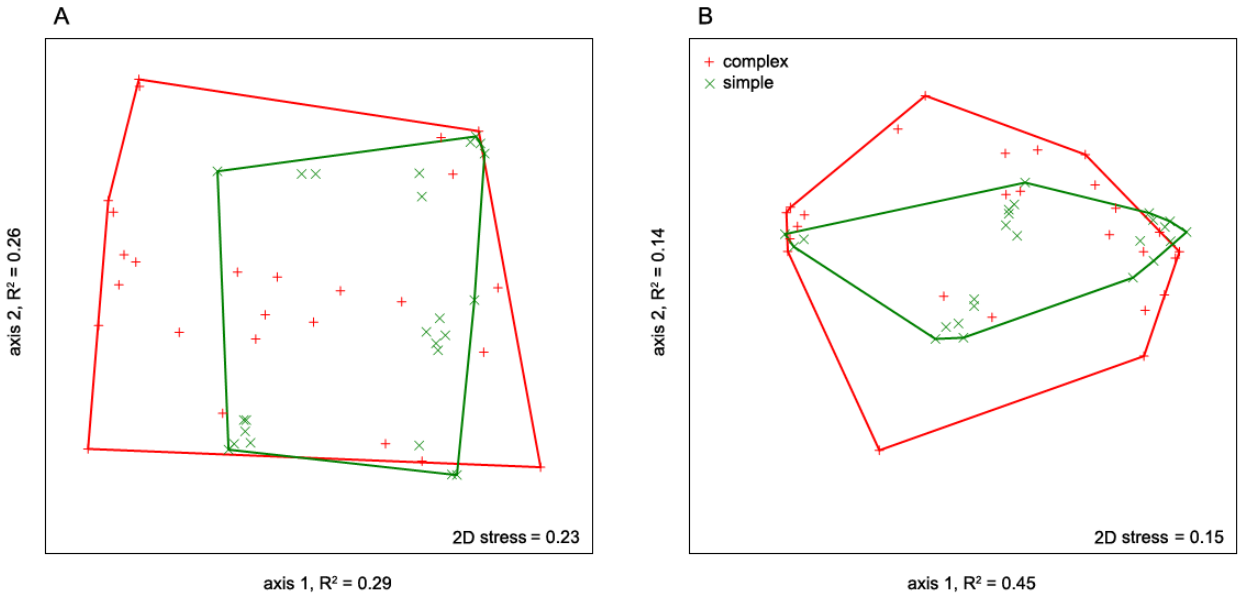

Fig. 9 NMDS ordination plots of samples reflecting the host plant architecture (complex or simple). The Bray-Curtis similarity index was used. The analysis was performed separately for (A) diatoms and (B) desmids. The graph legend applies for both plots.

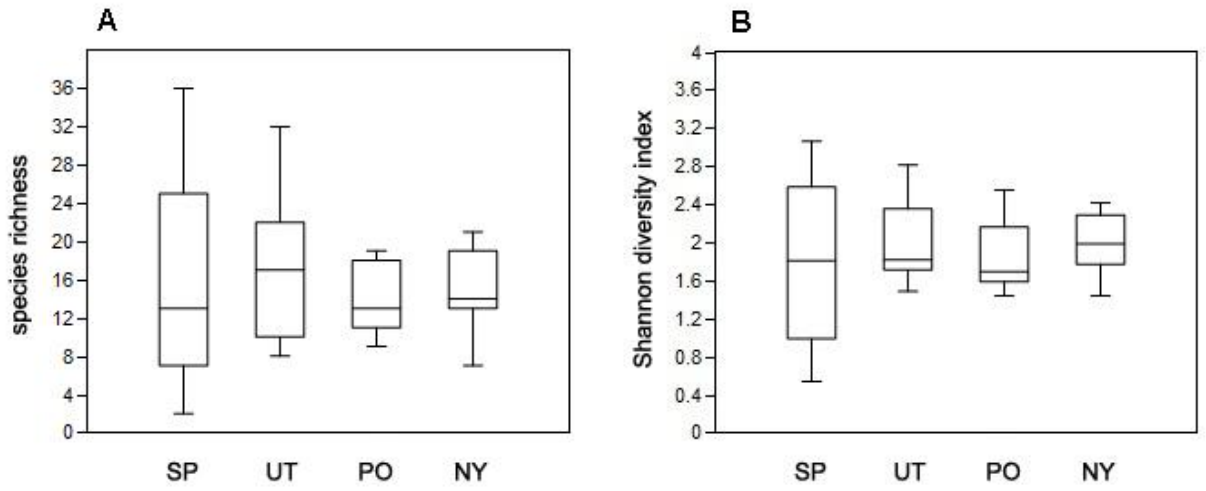

Fig. 10 Illustrated comparison of (A) species richness and (B) Shannon diversity indices of diatoms among the types of host plant. The Kruskal-Wallis tests were non-significant.

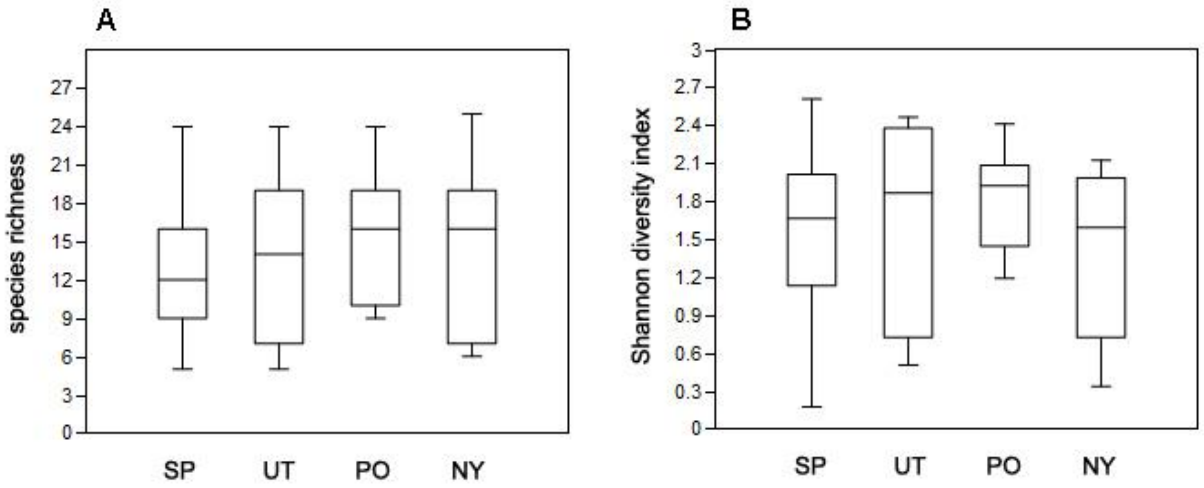

Fig. 11 Illustrated comparison of (A) species richness and (B) Shannon diversity indices of desmids among the types of host plant. The Kruskal-Wallis tests were non-significant.

3.5 Effect of environmental parameters

According to many published studies, which were outlined in the introduction (Chapter 1.1), pH and conductivity often influence the microalgal benthos. However, PERMANOVA done within this work revealed that environmental parameters had the effect of almost no significance. The only exception was conductivity, which explained barely 5 % of variation in the diatom species richness ($P < 0.05$). Nevertheless, following analyses of the effects of pH and conductivity provided some interesting results, mainly with regard to the diversity indices characterizing epiphytic communities.

As can be seen from the NMDS plots dividing samples according to the pH categories (Fig. 12), it is likely that the samples with pH lower or equal to 5 contained less diverse diatom and desmid communities, probably due to the more acidic conditions leading to the presence of specialized algal communities where few species dominate the entire community (as in Mataloni, 1999). Desmids in the samples with pH lower or equal to 5 formed a remote group on the left, and just a few samples felt in the range of other pH categories (Fig. 12, plot B). In case of diatoms, the samples with pH lower or equal to 5 followed mildly similar trend like desmids, however the distance between the group on the left and other samples is not that big (Fig.12, plot A).

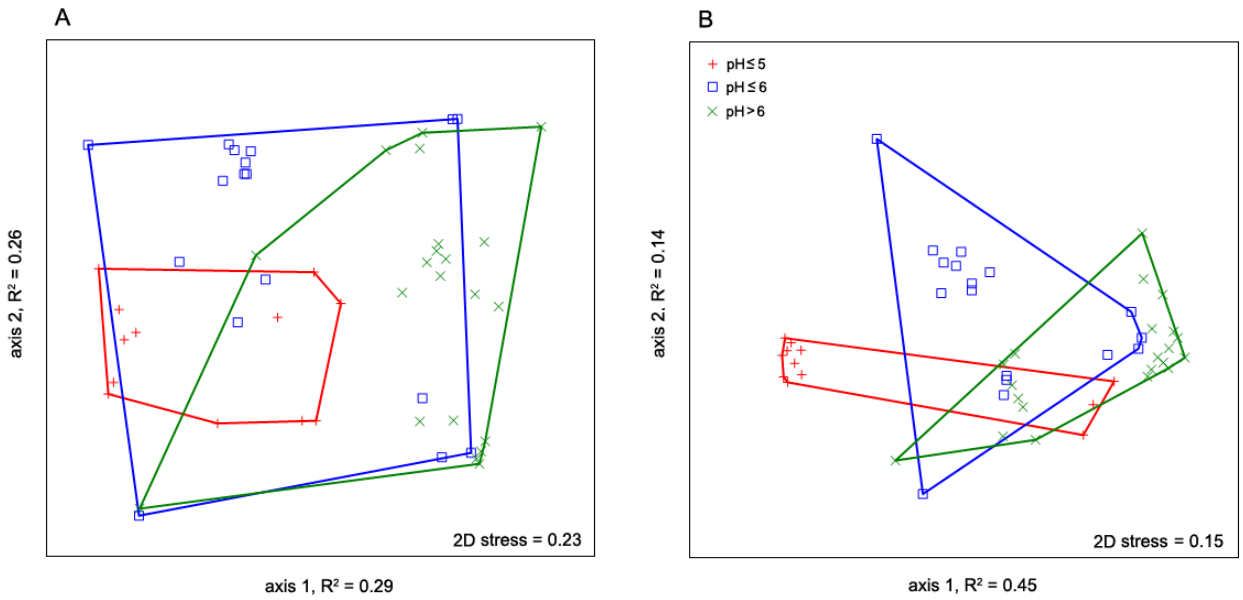

Fig. 12 NMDS ordination plots of samples divided in the groups reflecting pH. The Bray-Curtis similarity index was used. The analysis was performed separately for (A) diatoms and (B) desmids. The graph legend applies for both plots.

The identical results, conforming the presence of a distinct desmid community in more acidic conditions, were provided by the analyses of species richness (Kruskal-Wallis test, $P < 0.001$) and Shannon diversity indices (Kruskal-Wallis test, $P < 0.001$), illustrated in Fig 14. The overall significance was indeed defined by the difference between the category of pH less or equal to 5 and the other categories, in cases of both diversity indices (the post-hoc Mann-Whitney comparison, both $P < 0.001$). Yet the Kruskal-Wallis tests, ascertaining the differences in diatom species richness and Shannon diversity indices among pH categories, ended up showing no significant influence by pH (see also Fig. 13).

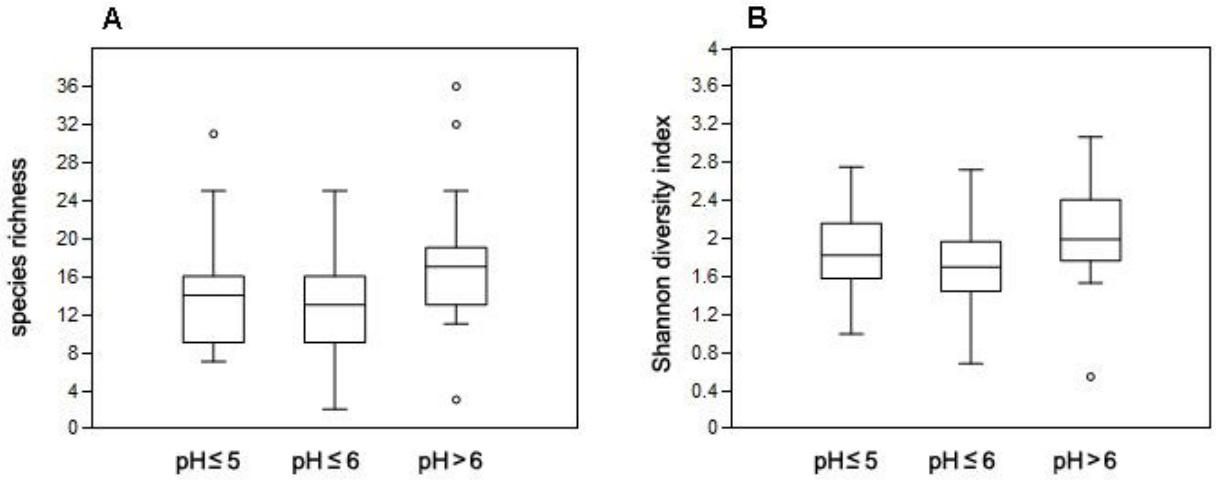

Fig. 13 Illustrated comparison of (A) species richness and (B) Shannon diversity indices of diatoms among the categories of pH. The Kruskal-Wallis tests were non-significant in both cases.

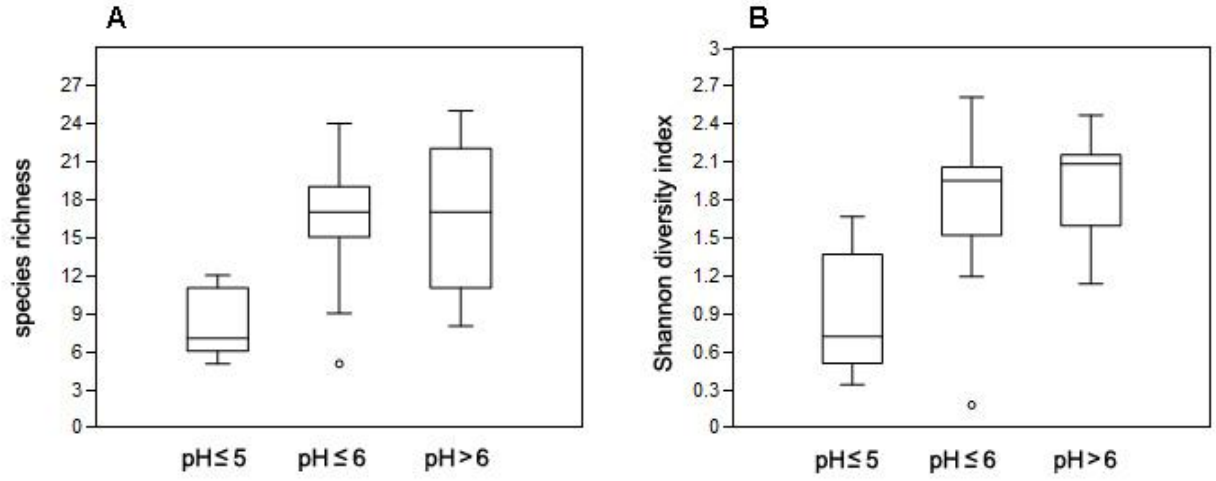

Fig. 14 Illustrated comparison of (A) species richness and (B) Shannon diversity indices of desmids among the categories of pH. In both cases, the overall significance (Kruskal-Wallis tests, $P < 0.001$) was determined by the differences between the first category (pH less or equal to 5) and the other categories (the post-hoc Mann-Whitney comparison, $P < 0.001$).

Nevertheless, dividing into separate categories based on pH is generally unnatural because pH itself is a continuous parameter. Moreover, it was not really possible to set the categories based on conductivity without a reflected spatial effect. It is clearly visible in Fig. 15 that certain values of conductivity were measured repeatedly (at the same site, at the sites lying in close proximity to each other, or at the sites of similar type) and other values are missing. Thus, it was appropriate to test the relationship between diversity indices and these environmental variables by using linear regression.

The results indicated that neither diatom species richness nor Shannon diversity indices were significantly dependent on pH. Further, there was no relationship between diatom species richness and conductivity ($r = 0.08$, $R^2 = 0.006$, $P = 0.59$; Fig. 15), unlike in PERMANOVA where conductivity explained 5 % of variation in the diatom species richness ($P < 0.05$). Finally, linear regression did not show any significant relationship between diatom Shannon diversity indices and conductivity. With regard to desmids, species richness ($r = 0.52$, $R^2 = 0.27$, $P < 0.001$) and Shannon diversity indices ($r = 0.64$, $R^2 = 0.42$, $P < 0.001$) significantly depended on pH (Fig. 16), whereas neither species richness nor Shannon diversity indices were dependent on conductivity.

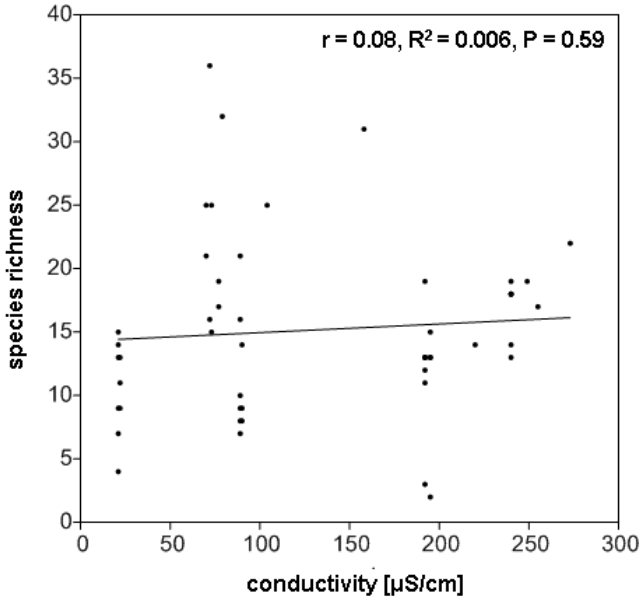

Fig. 15 Linear regression of diatom species richness depending on conductivity. The relationship turned out to be non-significant in contrast to the result of PERMANOVA.

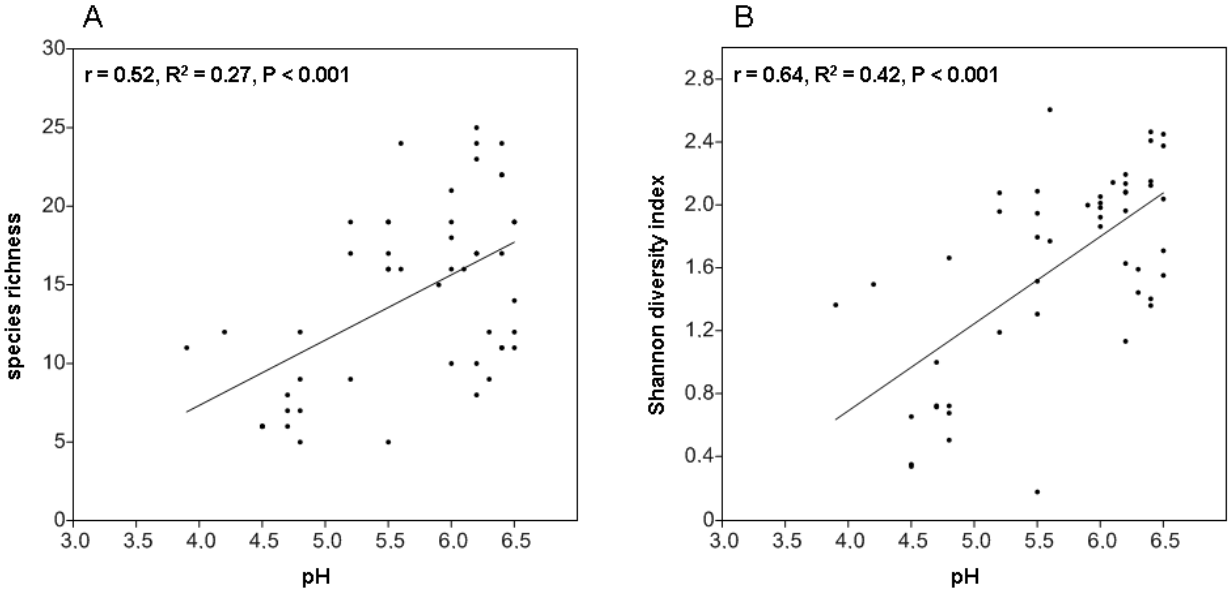

Fig. 16 Linear regression of (A) species richness and (B) Shannon diversity indices of desmid communities depending on pH. The relationships were significant in both cases ($P < 0.001$).

To sum the results of linear regression up, only desmid species richness and Shannon diversity indices increased with pH, other relationships were not appreciable. The noticeable influence of pH on desmid communities was also demonstrated by comparing the diversity indices among the categories of pH. The inconsistency in the results of PERMANOVA and these analyses might be caused by the fact that PERMANOVA shows the pure effects of individual factors in contrast to the performed analyses of diversity indices, which cannot adequately separate the effects of individual factors. Environmental parameters, such as pH and conductivity, are often correlated with other factors that possibly enhance or hide the pure effect of pH and conductivity.

3.6 Substrate specificity of epiphytic species

Even though host plant as a factor seems to be unimportant for determining associated epiphytic communities in comparison to the spatial effect (i.e. differences among sites), few algal species could show substrate specificity. To get reliable results, it was decided to work only with the 25 % most abundant species from the reduced datasets and with those species that were found at two sites at least. Thus, only the abundances of chosen 25 diatom species and 18 desmid species were correlated with the investigated genera of host plants (*Sphagnum*, *Utricularia*, *Potamogeton* and *Nymphaea*). The results are summarized in Table 8 for diatoms and Table 9 for desmids, showing only significant correlations between particular algal taxa and host plants.

Out of 25 diatom species, only *Frustulia saxonica* did not show any substrate preference, in contrast to as many as 9 out of 18 desmid species that were not substrate specific in any way. The rest of the species were either positively or negatively correlated with some of host plants. Considering just the most significant results (i.e. $P < 0.001$), diatoms species showed several substrate preferences, whereas from the desmid community *Staurastrum punctulatum* alone had a strong positive relationship to *Sphagnum* ($r = 0.51$, $P < 0.001$) and a marginally significant negative correlation with the host plants *Utricularia* and *Nymphaea* (both $r = -0.22$, $P < 0.05$).

By looking at the sums of all recorded significant correlations for each host plant, it is likely that there is no striking difference between the substrate types. Only in case of *Sphagnum* there are more negative correlations, and there are more positive correlations in case of *Nymphaea*, but the numbers of significant correlations were very low in general, so no

conclusion could be made based on such a result. To sum up, these findings again support the results of PERMANOVA that host plant does not play any crucial role in determining epiphytic community.

Table 8 Substrate specificity of diatom species. Only the significant results of correlation using Kendall´s tau are presented, giving correlation coefficients and significance. *** P < 0.001, ** P < 0.01, * P < 0.05

diatom species	**host plant**			
	Sphagnum	***Utricularia***	***Nymphaea***	***Potamogeton***
Achnanthidium minutissimum	-0.20 *			
Brachysira brebissonii	-0.23 *		0.23 *	
Brachysira neoexilis (morphotyp 1)	-0.28 **			0.20 *
Brachysira neoexilis (morphotyp 2)	-0.29 **		0.23 *	
Encyonopsis cf. *delicatissima*	-0.40 ***		0.30 **	
Eunotia bilunaris var. *bilunaris*	0.29 **			
Eunotia cf. *arcubus*	-0.27 **		0.33 ***	
Eunotia exigua	0.39 ***			-0.37 ***
Eunotia glacialis				-0.21 *
Eunotia implicata			0.36 ***	
Eunotia incisa		-0.21 *	0.24 *	
Eunotia paludosa	0.47 ***			-0.30 **
Fragilaria construens		0.22 *		
Fragilaria nanana	-0.25 *			
Frustulia saxonica				
Gomphonema acuminatum	-0.21 *	0.22 *		
Gomphonema gracile	-0.22 *		-0.25 *	0.35 ***
Gomphonema parvulum			-0.20 *	
Kobayasiella sp.	-0.24 *		0.21 *	
Navicula radiosa		0.31 **	-0.24 *	
Nitzschia sp. (morphotyp 1)				0.28 **
Nitzschia sp. (morphotyp 4)				0.25 **
Pinnularia pseudogibba	0.39 ***			-0.41 ***
Pinnularia subcapitata var. *elongata*	0.41 ***		-0.28 **	
Tabellaria flocculosa		-0.23 *	0.25 **	
no. of species - positive correlation	5	3	8	4
no. of species - negative correlation	10	2	4	4

Table 9 Substrate specificity of desmid species. Only the significant results of correlation using Kendall´s tau are presented, giving correlation coefficients and significance. *** P < 0.001, ** P < 0.01, * P < 0.05

desmid species	**host plant**			
	Sphagnum	***Utricularia***	***Nymphaea***	***Potamogeton***
Closterium calosporum var. *maius*				
Closterium incurvum				
Closterium parvulum		0.28 **		
Closterium striolatum				-0.27 **
Cosmarium amoenum	-0.24 *	-0.21 *	0.25 *	0.21 *
Cosmarium discrepans	-0.27 **	0.22 *		
Cosmarium goniodes				
Cosmarium impressulum				
Cosmarium regnellii				
Cosmarium sp. (morphotyp 1)				
Cosmarium sp. (morphotyp 2)				
Cosmarium sphagnicolum	-0.23 *			
Cosmarium subcostatum var. *minus*				0.21 *
Pleurotaenium trabecula				
Staurastrum punctulatum	0.51 ***	-0.22 *	-0.22 *	
Staurastrum tetracerum	-0.27 **			
Tetmemorus granulatus				
Tetmemorus laevis				-0.29 **
no. of species - positive correlation	1	2	1	2
no. of species - negative correlation	4	2	1	2

3.7 Comparison of algal group strategies

Logically, all previously described results revealed mostly the same pattern in both diatom and desmid communities. The general trends are obvious, even though all analyses were performed separately for both algal groups and the resulting numbers may have differed slightly. Nevertheless, to support such a strong statement, the direct comparison of diatom and desmid epiphyton was done by the Proscrustes statistic. The analysis was first carried out using the reduced datasets (49 samples where both 200 diatom cells and 200 desmid cells were found). The Procrustes statistic essentially confirmed the previous indirect comparisons by demonstration of non-random congruence of both NMDS ordinations (r = 0.76, P = 0.001). Secondly, edited complete datasets (140 samples where both 200 diatom cells and 200 desmid cells were found) was used as well and verified the generalisation of similar algal community strategies (r = 0.68, P = 0.001).

The non-random congruence of diatom and desmid datasets is demonstrated in the resulting diagrams (Fig. 17). In majority, the changes of sample positions are organized. This can be easily seen in the diagram of the reduced dataset (Fig. 17, plot A). For instance, the samples within distinct groups stick more or less together, are kept in the same place, or move the same direction. The outlying samples with the clearly different community structure remain separated within both cases, i.e. diatoms (circles in the plots) and desmids (arrow ends in the plots). In conclusion, the trends within both algal groups are clearly similar. Thus, because diatoms and desmids are monophyletic and unrelated algal groups which usually represent the dominant in freshwater benthic microhabitats, there is an evidence for that these patterns are general for the entire microphytobenthos.

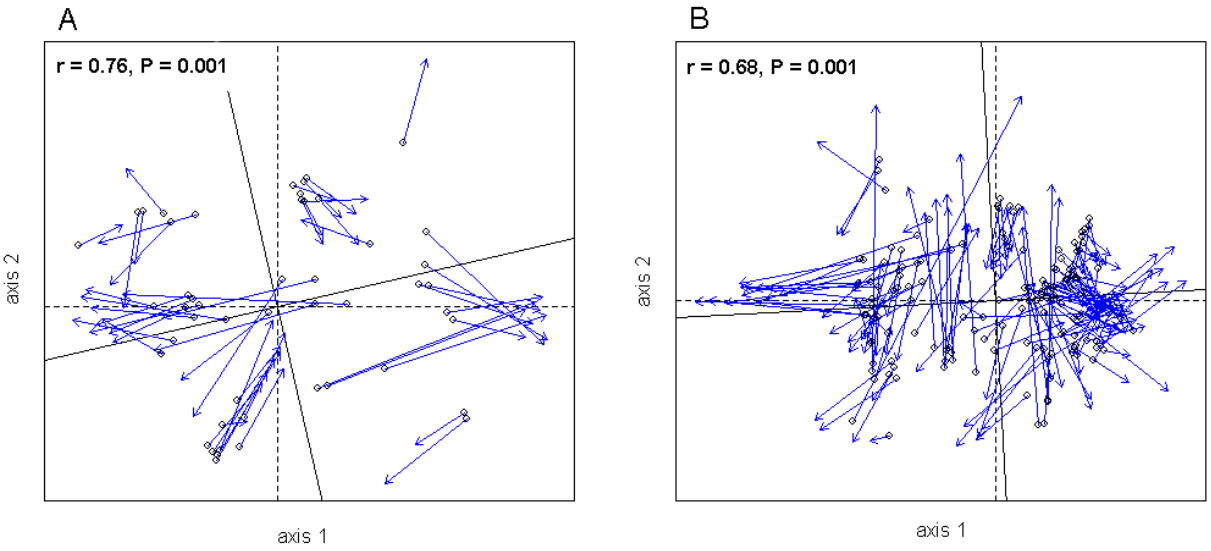

Fig. 17 Graphical visualisation of the Procrustes analysis (using 999 permutations). The NMDS ordination plots reflect the superimposition of diatom (circles) and desmid (arrow ends) samples, and in this case the similarity of the group strategies. Only the samples where both 200 diatom cells and 200 desmid cells were found were used for the analyses, meaning (A) 49 samples from the reduced dataset that was used for PERMANOVA, (B) 140 samples from the complete dataset.

4. Discussion

4.1 Factors affecting epiphyton

Despite the fact that there are many published studies assuming that host plant affects associated epiphytic community, probably through biological or chemical influences, there has always been a question whether there is any significant influence of host plant in comparison to other factors that substantially affect freshwater algal benthos. This Master´s thesis has documented the overwhelming effect of site and mild, but still noticeable, effect of environmental parameters on community structure of algal epiphyton. The effect of host plant appears to be almost imperceptible. This result concurs with already published studies that similarly investigated the effects of several factors on epiphyton at once, thus providing more objective and general view on ecology of epiphyton.

At this point, it should be, however, made clear that the term *site* does not mean only the spatial pattern itself. It also incorporates the history of the locality, as well as any undetected abiotic and biotic factors with a spatial distribution (Borcard et al., 1992; Anderson & Gribble, 1998). Generally, freshwater benthic communities are spatially structured (Soininen et al., 2004; Pals et al., 2006; Machová-Černá & Neustupa, 2009; Krivograd Klemenčič et al., 2010; Neustupa et al., 2012, 2013; Svoboda et al., 2014), but unfortunately it is impossible to separate the pure spatial effect from the other unexamined factors in the analysis. Still, it could be claimed that it is most probably space that largely influenced the epiphytic communities, and thus the term *site*, respectively *space*, is referred in the thesis as the main driving force. Yet it might include any other undetected factor that is spatially structured.

On the whole, the host plant itself had a negligible influence on associated epiphytic communities contrary to the remarkable influence of site and mild, but still noticeable, influence of environmental parameters. This finding matches to the studies concerning diatom or desmid epiphytic community structure (Eminson & Moss, 1980; Millie & Lowe, 1983; Pals et al., 2006; Cejudo-Figueiras et al., 2010). It is important to mention that the graphs in Pals et al. (2006) could be misleading. Although they present the differences among epiphyton associated with individual types of substrate, the illustrations were always made for each site separately, due to the occasional presence of different substrates at particular sites. Still there were reported much greater dissimilarities between epiphyton at different sites. On the other hand, Gough & Woelkerling (1976) and Woelkerling (1976) emphasized the host

plant effect even more, despite the fact that they found remarkable spatial effects as well. In this thesis, host plants were compared across the sites and the plots were made likewise to get more general trends. Nevertheless, it is apparent that if a more limited dataset (for example only the samples from one site) is used in the analysis, the effect of host plant on associated epiphyton could be occasionally enhanced and found as significant. The pattern might be well explained by stochasticity or various environmental conditions in the immediate vicinity of individual macrophyte, which are, however, hard to detect. This was partly corroborated within this thesis by performing preliminary analyses, which at each time included the subset of data from a single site. But, because the results lacked consistency among the sites, they were not reported here. Generally speaking, such methodology would possibly lead to the overestimation of host plant influence on associated epiphyton and subsequently to the speculation about the direct biological or chemical interactions between macrophytes and epiphytes, respectively about the indirect effect of plant architecture on epiphyton (as suggested in Blindow, 1987; Cattaneo et al., 1998; Laugaste & Reunanen, 2005). However, Siver (1977) and Cattaneo & Kalff (1979) found no influence of macrophytes, even though they sampled epiphyton at a single site, suggesting that the pattern is truly inconsistent among sites.

With regard to strongly spatially structured epiphyton, it is obvious that host plants represent a lower level of spatial factor, below the level of the whole water bodies. Host plants are basically types of microhabitats, which could be found within a site. It was suggested in Taniguchi & Tokeshi (2004), and it appears to be logical, that diversity and abundance of microorganisms increase with complexity of habitat at a smaller scale. This is probably not valid for epiphyton investigated within this thesis. At first, it was also planned to incorporate the plant architecture (i.e. substrate complexity) as a factor to PERMANOVA, but later it was not necessary to do so. Theoretically, only if the host plant explained significant variation in the epiphytic community, it could be worth starting to search for a macrophyte characteristic that possibly produces the dissimilarity. Also, other analyses did not reveal any compelling influence of plant architecture on associated epiphytic community, which considerably supported the decision not to include plant architecture as a factor to PERMANOVA. Such a result is not in line with the studies reporting the effect of plant architecture (Bland & Brook, 1974; Kuczyńska-Kippen et al., 2005; Messyasz & Kuczyńska-Kippen, 2006), even though all of them, including this thesis, concerned epiphytic community structure at several water bodies. However, Kuczyńska-Kippen et al. (2005) and Messyasz &

Kuczyńska-Kippen (2006) added that there was likely some combination of physico-chemical or biological factors closely related to the macrophytes which eventually affected epiphytic community.

Even though there was no significant effect of host plant and its architecture on epiphytic community, this outcome does not refute the influence of microhabitat on benthic communities as such. It has already been shown that there are cases of substantial differences between microhabitats (Poulíčková et al., 2004; Soininen & Eloranta, 2004; Townsend & Gell, 2005; Veselá, 2009), and that heterogeneity of microhabitats substantially contributes to high local diversity of microorganisms (Ács et al., 2003; Zheng & Stevenson, 2006; Veselá & Johansen, 2009). The dissimilarities might be well enhanced if two very distinct types of microhabitats (i.e. biologically active macrophytes versus biologically inert substrates e.g. rock, sand, mud or wood) are studied. Other example of such a pattern was presented in the study of Pals et al. (2006), where it was found out that frequently desmid community on sand was significantly different from those associated with macrophytes. Further, there are studies recording the dissimilarity between epiphytic community associated with natural plant substrates and with the plastic models or slides (Siver, 1977; van Dijk, 1993; Albay & Akcaalan, 2003). The results of Soininen & Eloranta (2004) and Townsend & Gell (2005) indicated that host plants, as living, thus biologically active and instantly growing, organisms, are far more dynamic microhabitats and often undergo the changes which are not common for inert, hard substrates. Thus, the turnover of epiphytic community must have been profound, as well as repeated colonisation and primary succession. If all of this is valid, the effect of microhabitat might not be revealed within this thesis, because host plants (biologically active substrates) were compared just with each other. In that case, macrophytes would have the same effect on epiphyton, which would be obviously distinct from the effect of inert substrates. For instance, every macrophyte would release some nutrients for epiphyton.

The studies concerning algal diversity in freshwater ecosystems usually emphasize on stronger effects of pH and conductivity on benthic algal communities, thus in general both of these environmental variables are surely important. In this study, the mild effect of pH and conductivity was reported, however it was not that unexpected since the sampling sites were chosen to be as similar as possible, i.e. to fall into the category of oligotrophic and mesotrophic sites. This criterion allowed findings that required overlap of host plants that were chosen for the study. It is supposed that if the sites with higher nutritional status or with other remarkably different limnological characteristic were involved in the study, the

environmental parameters would be more important, as described in Eminson & Moss (1980), Lalonde & Downing (1991) and Cejudo-Figueiras et al. (2010). However, Fránková et al. (2009) emphasized the importance of environmental gradients even within peatlands, i.e. sites still relatively alike, compared to the difference between peatlands and ponds which were explored within this thesis. Neustupa et al. (2013) found out that although the peatland sampling sites were very similar in terms of environmental parameters, the effect of pH and conductivity on diatom and desmid benthic communities was significant, and even greater than the spatial effect.

With regard to the algal diversity indices, Mataloni (1999), Štěpánková et al. (2008) and Neustupa et al. (2009) presented the positive correlation between algal diversity and both pH and conductivity, in contrast to this thesis and the study of Neustupa et al. (2013), where algal species richness being rather positively correlated with pH, but not related to conductivity. Coesel (1982) claimed that the highest algal diversity should be detected in the middle part of trophic range thanks to the presence of both oligotrophic and eutrophic species. In this thesis, where trophy is partly reflected by the conductivity values, the pure effect of conductivity explained barely 5 % of variation in the diatom species richness (PERMANOVA, $P < 0.05$), otherwise the analysis did not reveal any remarkable effects. Similarly, no relationship of any algal diversity indices to conductivity was revealed by linear regression. Still, the entire trophy range of fresh waters was not investigated, so it is not possible to decide whether Coesel´s assumption (1982) would be relevant in case of algal epiphyton.

4.2 Substrate specificity of epiphytic species

The differences in epiphyton between host plants (both between types of host plants and plant individuals) may occasionally appear due to the distinct environmental conditions in the vicinity of every plant. Such conditions could be caused either by the environment itself (Pals et al., 2006), which is more probable regarding the results of this thesis, or by some plant influence (Gough & Woelkerling, 1976; Woelkerling, 1976). Both aspects might eventually explain the differences among the macrophytes within one water body, as reported in the studies where only single site was sampled (Blindow, 1987; Cattaneo et al., 1998; Laugaste & Reunanen, 2005). The distinct environmental conditions related to every plant must have subsequently led to the presence of infrequent "substrate specialists".

As mentioned in the introduction, *Sphagnum* should be a substrate type that hosts relatively higher numbers of substrate specific taxa. It is probably through acidification of immediate *Sphagnum* proximity (Clymo, 1964; reviewed in Andrus, 1986). Therefore, individual algal species could prefer such conditions, or try to avoid them. According to the results of this thesis, the sums of recorded significant correlations for each host plant did not confirm the difference between *Sphagnum* and other macrophytes. Secondly, there are not many remarkable correlations between *Utricularia* and epiphytic algae. In the study of Prowse (1959), it was found out that *Utricularia* favour the presence of *Gomphonema* in contrast to the obvious absence of *Eunotia* species. In this thesis, there were many species of both genera recorded on *Utricularia*, thus Prowse´s results are most probably site specific. Ulanowicz (1995) suggested that *Utricularia* should enhance the growth of epiphyton on its surface, so that *Utricularia* can catch zooplankton that is attracted to swim closer to the plant traps. Ulanowicz´s model does not assume that particular algal species would preferably live on *Utricularia*, but it says that the growth of epiphytic community should be supported in general. If Ulanowicz´s idea is reliable, it might rather be the size and shape structure of the algal community that is more influenced by *Utricularia* to offer the best cell forms for predators to consume. Further, it is likely that algae associated with *Nymphaea*, respectively *Potamogeton*, need to be able to remain on the plant surface and to withstand more perturbations in the water column, in comparison to complex substrate that provide more closed or semiclosed refuges. The ability to form mucilage stalks is an example of the adaptation for cell attachment to the surface that could be found e.g. within the diatom genera *Gomphonema* or *Eunotia*. The performed correlation analyses, nevertheless, did not reveal the presence of such specialized algal species. Anyhow, there are no exceptionally different sums of significant correlations of algal taxa to particular macrophyte. The numbers are rather similar, but generally too low to come up with any obvious conclusion.

Although the results of this thesis indicate that there were some significant correlations between algal species and particular host plant, the substrate specificity seems rather unlikely to be true. Concerning diatoms, for example *Achnanthidium minutissimum*, belongs to the frequent species occurring in many types of microhabitats where it is usually recorded in considerably high abundances (e.g. Eminson & Moss, 1980; Blindow, 1987; Poulíčková et al., 2004; Townsend & Gell, 2005; Cejudo-Figueiras et al., 2010). Therefore, no substrate specificity of *Achnanthidium minutissimum* could be assumed. The same applies for the majority of other diatom and desmid species since there are published studies reporting that

the species was positively correlated with other type of microhabitat or occurred there in higher abundances (all summarized in Table 10). Concerning desmid species, the thesis assumes e.g. no substrate specificity of *Cosmarium regnelii* with regard to macrophytes, because there were no relevant correlations with any host plants. It is in good agreement with Messyasz & Kuczyńska-Kippen (2006) that reported significantly higher biomass of *Cosmarium regnelii* on *Chara*, and moreover the authors noted that *Cosmarium regnelii* is often present in the free water column. During the investigation within the thesis, there were planktonic species found from time to time, but they are believed to appear randomly in the samples, or they are present in the epiphyton which could serve as a temporal refuge (reviewed in Schindler & Scheuerell, 2002).

The exceptions, i.e. taxa with significant preference to particulate macrophyte, based on the congruence of the thesis results and already published data, are *Eunotia bilunaris*, *Eunotia exigua*, *Eunotia paludosa* and *Staurastrum punctulatum*. They are likely specific to *Sphagnum*, but only in case when only the category of a host plant is considered. However, these species are also commonly presented in the sediment microhabitats (Pals et al., 2006; Machová-Černá & Neustupa, 2009; Veselá, 2009). The highly significant substrate preferences of *Encyonopsis* cf. *delicatissima*, *Eunotia implicata* and *Pinnularia pseudogibba* (see Table 8) are quite uncertain since there were found no references in the literature.

Many authors (Eminson & Moss, 1980; Blindow, 1987; Messyasz & Kuczyńska-Kippen, 2006; Cejudo-Figueiras et al., 2010) came with the unsurprising statement that some epiphytic species show substrate specificity, others do not. On the other hand, Siver (1977) found no substrate specificity of microalgae at all. It is hard to say if the findings of other mentioned studies are reliable because it is not always clear whether they worked with more abundant species presented at several sites, meaning whether species were not unique to a sample or site. Moreover, the reported so called substrate specificity could be rather connected to stochasticity associated with algal colonisation or competitive exclusion of species by better adapted taxa (Townsend & Gell, 2005). The conflicting results might have been also caused by the investigation of water bodies with distinct limnological characteristics as stressed by Eminson & Moss (1980). The authors suggested that the substrate specificity of epiphytic organisms should stronger in oligotrophic waters where limited nutrients force epiphytic organisms to adapt more effective nutrient uptake. This might explain the conflict within the evidence in already published data, but it is hard to assess since many papers do not include detailed limnological information.

Table 10 The list of diatom and desmid species that were significantly correlated with some host plant within this thesis and at the same time there were some substrate preferences reported in already published studies.

THIS MASTER´S THESIS			REFERENCES
diatom/desmid species	**positive correlation**	**negative correlation**	**positive correlation or higher abundances**
Achnanthidium minutissimum	-	*Sphagnum* -0.2 *	frequent dominant species (for references see the text)
Brachysira brebissonii	*Nymphaea* 0.23 *	*Sphagnum* -0.23 *	bryophytes (Veselá, 2009)
Eunotia bilunaris var. *bilunaris*	*Sphagnum* 0.29 **	-	*Sphagnum* and sediment (Machová-Černá & Neustupa, 2009)
Eunotia exigua	*Sphagnum* 0.39 ***	*Potamogeton* -0.37 ***	*Sphagnum* and sediment (Machová-Černá & Neustupa, 2009)
Eunotia incisa	*Nymphaea* 0.24 *	*Uticularia* -0.21 *	glass slides (Siver, 1977)
Eunotia paludosa	*Sphagnum* 0.47 ***	*Potamogeton* -0.30 **	*Sphagnum* (Machová-Černá & Neustupa, 2009) bryophytes (Veselá, 2009)
Gomphonema gracile	*Potamogeton* 0.35 ***	*Sphagnum* -0.22 * *Nymphaea* -0.25 *	*Typha latifolia* (Cejudo-Figueiras et al., 2010)
Gomphonema parvulum	-	*Nymphaea* -0.20 *	stone (Soininen & Eloranta, 2004), *Myriophyllum alterniflorum* (Cejudo-Figueiras et al., 2010)
Navicula radiosa	*Utricularia* 0.31 **	*Nymphaea* -0.24 *	*Phragmintes autralis* (Albay & Akcaalan, 2003)
Pinnularia subcapitata var. *elongata*	*Sphagnum* 0.41 ***	*Nymphaea* -0.28 **	sediment (Veselá, 2009)
Closterium parvulum	*Utricularia* 0.28 **	-	sand (Pals et al., 2006)
Closterium striolatum		*Potamogeton* -0.27 **	sand (Pals et al., 2006)
Cosmarium regnellii	-	-	*Chara* (Messyasz & Kuczyńska-Kippen, 2006)
Staurastrum punctulatum	*Sphagnum* 0.51 ***	*Utricularia* -0.22 * *Nymphaea* -0.22 *	*Sphagnum* and sand (Pals et al., 2006), *Sphagnum* and sediment (Machová-Černá & Neustupa, 2009)

In case of the thesis, there was no exceptional number of substrate specialists recorded even though many sampling sites were oligotrophic. On the whole, there were more than 100 diatom species and 100 desmid species determined within the reduced datasets, still fairly low number of relevant substrate specificity was found. Therefore, the outcome of substrate specificity analysis reinforces the finding that the effect of host plant on associated epiphyton is almost negligible.

4.3 Generalization of algal group strategies

Since diatoms and desmids are monophyletic and unrelated algal groups, and they represent the dominants of microbenthos in given ecosystems (Chapter 1.6), the revealed group strategies are fairly suitable for further generalization. All indirect comparisons and the results of Procrustes statistics, which directly compared the diatom and desmid communities, indicated that the algal group strategies were generally identical. Such a congruence of benthic diatoms and desmids was previously reported in Neustupa et al. (2013). Therefore, both these studies conclude that the factors determining the community structure of microphytobenthos are mainly space (generally speaking of remarkable influence of site) and partly environmental conditions (pH and conductivity). The exceptions that should be considered are flagellates that are able to move easily over relatively bigger distances to the place with more favourable conditions (Happey-Wood, 1988; Hall & Pearl, 2011). However, diatoms and desmids are able to move as well and it still remains questionable whether flagellates could migrate to another microhabitat. Thus, it is believed that even benthic flagellates should follow the similar trends like diatoms and desmids.

The species richness of diatoms and desmids seemed virtually the same when comparing datasets with similar numbers of samples. Yet more desmid samples had to be excluded from analysis because of a low algal abundance. Out from 171 samples that were collected for the purpose of this thesis, only a single diatom sample (2-TK-UT2) did not contain sufficient number of cells. In case of desmids, there were as many as 29 inapplicable samples. With regard to more often absence of desmids in the epiphytic community, one question appeared. How to treat the samples where particular algal group do not occur in adequate density? To my knowledge, it is feasible to take account of such samples if the absolute cell numbers or biomass is measured. If the relative abundances of species in the community are recorded, none of widely used statistical methods would take account for such

samples in a dataset appropriately. Therefore, these samples are not usually included in analysis, but the absence of cells itself seems to be relevant information about community pattern anyway. Unfortunately, no solution to this problem exists so far. In terms of this thesis, the absence of epiphytic desmids could possibly reflect unfavourable environmental condition at site (i.e. Ďáblík 2 and Rybníček u Studeného where all desmid samples were found to be inapplicable) or in the vicinity of individual macrophyte.

The difference in number of diatom and desmid samples suitable for the investigation within the thesis can be explained by their quite different responses to extreme environmental stress conditions. Desmid communities usually shift to a stage with lower diversity when there are more stress-tolerant species present. Eventually desmids may completely disappear under such circumstances (Coesel, 1982; Mataloni, 1999). The results of the thesis support the pattern by showing decreased desmid diversity in more acidic conditions. This contradicts diatoms that can successfully cope with the extreme conditions. Diatom species composition is typically changed but they still remain in dense populations (Admiraal & Peletier, 1980; Peterson & Stevenson, 1992). Such a pattern makes diatoms perfect for biomonitoring of habitats even with very distinct environmental conditions (Dixit et al., 1992; Charles et al., 2006; Blanco et al., 2014), whereas desmids, even though they have a great potential for biomonitoring, are found mainly in moderately acidic and oligo-mesotrophic sites (Coesel, 1982, 2001, 2003). Nevertheless, the thesis showed that the trends of both groups and the factors driving algal community structure are very similar, regarding the common conditions in which both diatoms and desmids occurred in high densities. The low desmid densities in some samples just support the finding that they are affected by environmental parameters in addition to spatial factor.

Despite this work indicated that the factor site showed the most significant effect on freshwater algal epiphyton, the studies reporting some noticeable effect of host plant might also have a point. The reason is, and it has to be stressed again, that the thesis focused on the community structure of epiphyton. The results could be different if other characteristics of epiphyton, e.g. biomass, chlorophyll *a* content, or absolute densities of algae, would be investigated. In the research of Laugaste & Reunanen (2005) that was done within a single site, chlorophyll *a* content and biomass were reported to be lower on emergent macrophytes and on those with floating leaves, in contrast to submergent macrophytes. Considering more appropriate studies that were done at several sites, Lalonde & Downing (1991) found out that macrophytes with different architecture supported significantly different biomass of

epiphyton, but the effect was not as powerful as the effect of environmental parameters. The results of Kuczyńska-Kippen et al. (2005) presented that total algal densities and biomass were remarkably higher on *Chara* than on *Typha*. These two substrates also differed in dominant algal species, but not in terms of species richness. Still the substrate complexity of *Chara* and *Typha* seem to be relatively similar, thus in Kuczyńska-Kippen et al. (2005) it should have been emphasized even more that there were other factors closely related to the host plants and these factors eventually affected epiphyton.

In general, this thesis, focusing on epiphytic community structure, supports the neutral substrate hypothesis (Chapter 1.4). Likely, it is not the plant architecture that determines the community structure of algal epiphyton, but more indirect influences associated with host plant. That is, for instance, the influence of environmental parameters closely related to the host plant rather than the influence of substrate itself. Firstly, it might be important where the host plant grows, for example where in a water column in terms of depth and distance from a shore. Secondly, if there is any occasional movement of the plant in the water column or any movement of water masses around the plant surface. Water must then have brought new nutrients to the vicinity of host plant and those nutrients are eventually available for epiphyton. Thirdly, density of vegetation influences light conditions. The diversity of the possible indirect effects of the host plant might have caused the stochastic differences between samples. Unfortunately it is extremely hard to detect it within any research. However on the whole, it is the spatial effect that drives the epiphytic community. This outcome is highly relevant for the use in biomonitoring. Epiphyton can be used for the analysis regardless of substrate type, as suggested in Siver (1977) and Cejudo-Figueiras et al. (2010). It does not matter from which macrophyte within one site the epiphyton is sampled, but where (i.e. from which of the sites) the sample is taken. All the dissimilarities between epiphyton from different macrophytes within one site might well be random.

4.4 Future work suggestions

The presented thesis focused mainly on the community structure of algal epiphyton, including species richness and composition. However, it is unclear how the factors (space, host plant and environmental parameters) would affect other characteristics of epiphyton (e.g. biomass, chlorophyll *a* content, then phylogenetic, size and shape structure of algal epiphyton). Thus, future work should perform similar research, but with an extended reach. It

is advised to replicate the methodology used within this study. That means to sample epiphyton on several natural plant substrates at several water bodies, and investigate more characteristics of epiphytic community at once. The other aspect is to include temporal variability of epiphyton, which is not probably so enhanced in terms of changing of benthic community structure (Machová-Černá & Neustupa, 2009; Neustupa et al., 2012; Svoboda et al., 2014), but could be if biomass measurement is involved (Gons, 1982; Lazarek, 1982; Karosienė & Kasperovičienė, 2008; Toporowska et al., 2008).

Also, it would be worthwhile to investigate the phylogenetic structure, as well as size and shape of algal cells in the epiphytic community. These aspects have not yet been studied in detail. Firstly, only the proposition has appeared that size structure of epiphyton could be used in biomonitoring, in the same way as taxonomic structure (Cattaneo et al., 1995; Wunsam et al., 2002). The aspects of algal cell shape and possible associated adaptations are not known at all. The study of size and shape of epiphytic cells (as in Neustupa et al., 2009, 2013), or epiphytic biomass, should also make clear if Ulanowicz´s model (1995) of *Utricularia*, changing associated epiphyton to be more attractive to zooplankton, could be found in the nature. Secondly, the contribution of phylogenetic data involved in ecological researches leads to the feasible determination of processes that form communities, for instance if it is rather environmental filtering or competition between organisms (reviewed in Webb et al., 2002; Emerson & Gillespie, 2008; Hardy, 2008). These processes cannot be detected just by traditional data including presences and abundances of species in the community (Martin, 2002). Thus in addition to epiphyton biomass and chlorophyll *a* content, future work should focus more on these aspects of any microorganismal community, since on the whole they have not been sufficiently explored yet.

Additionally, it could be also interesting to use the artificial models of chosen macrophytes (likewise in Siver, 1977; Cattaneo & Kalff, 1979; Burkholder & Wetzel, 1990) in order to check again whether there is any influence of plant architecture on associated epiphyton, respectively any biological or chemical influence of host plants, as biologically active substrates. Further, the succession of benthic community on morphologically different microhabitats can be explored. The best way would be to place the artificial plant models at several sites, so that it would be decided if the pattern could be generalized.

By studying epiphyton in running waters (partly in e.g. Winter & Duthie, 2000; Soininen & Eloranta, 2004; Veselá & Johansen, 2009), it should be revealed whether the factor of current velocity (Peterson & Stevenson, 1992; Ghosh & Gaur, 1998; Battin et al.,

2003) is also crucial for the epiphytic community. Higher current velocity is expected to lead to the presence of more adapted species for living in an environment with such perturbation. For example there may be more species that are firmly attached to the surface by mucilage stalks.

Other research opportunities are promising too. The information about heterotrophic protists in freshwater epiphyton (so far partly in Carrias et al., 1998; Mitchell et al., 2003; Mieczan, 2007; Mieczan & Adamczuk, 2015), as well as the information about the whole epiphytic microbial biofilm (Sekar et al., 2002; Ács et al., 2003; Barranguet et al., 2004; Domozych & Domozych, 2008) are still very fragmentary. In particular, it would be interesting to investigate the succession of epiphyton in the relationship to bacteria which are known to be the first organisms that colonise the substrate, thus providing the base for the creation of epiphytic layer (Bruckner et al., 2008), or in the relationship to the production of diatom mucilage as a type of secondary substrate (Hoagland et al., 1982; Tuji, 2000).

Such huge projects are surely challenging, unless many researchers were involved at the same time. However, the main recommendation coming out of this thesis is more straightforward. Any even less ambitious projects concerning epiphyton should take account of spatial factor (sites) together with the factor of host plant, and should not perform the study within a single water body. If the spatial factor is not incorporated in a study, the results could be site specific and subsequently cannot be generalized.

5. Conclusion

This Master´s thesis explored freshwater algal epiphyton on several types on natural plant substrates at several sites. Therefore, variation in epiphyton among and within water bodies, as well among and within host plant types could have been investigated in details. By applying such an approach, the thesis provides considerable and accurate insight into ecology of freshwater microphytobenthos.

On the whole, the neutral substrate hypothesis is highly favoured by the outcomes of this thesis, leading to the conclusion that epiphyton can be used in biomonitoring regardless of substrate type (host plant). The results have demonstrated the overwhelming effect of site factor and mild, but still noticeable, effect of environmental parameters (pH and conductivity) on community structure of freshwater algal epiphyton. The influence of the host plant and its architecture appears to be negligible. The occasional differences between epiphyton associated with different macrophytes within one site are believed to be random, or caused by the influence of environmental conditions that are in the immediate vicinity of individual host plant. But it is more probable that these distinct environmental conditions are determined by the environment itself or by host plant indirectly. In terms of substrate preferences, there was no substantial evidence of substrate specificity of any particular algal taxon. Thus, every individual macrophyte is considered to be truly independent. The majority of algal species that showed positive correlations with some of the host plants are often reported to be present in higher abundances in other types of microhabitats. Only a few algal species (*Encyonopsis* cf. *delicatissima, Eunotia implicata* and *Pinnularia pseudogibba*), out of more than 200 identified species, are perhaps substrate specialists, however it should be validated.

These results are in good agreement with already published studies that similarly investigated the effects of several factors on epiphyton at once. The value of the research relied on the comparison between epiphytic diatom and desmid communities. The results have indicated that the patterns of both algal groups were virtually the same and are potentially generalized for the entire microphytobentic community.

Finally, it is strongly recommended to include spatial distance as a factor (i.e. to investigate more than one site) in future works concerning epiphytic community. Only by this methodology, it is possible to determine whether discovered patterns can be generalized. Otherwise the obtained data could be site specific and may lead to overestimation of macrophyte influence on the associated epiphyton.

6. References

Ács, É., A. K. Borsodi, J. Makk, P. Molnár, A. Mózes, A. Rusznyák, M. N. Reskóné, & K. T. Kiss, 2003. Algological and bacteriological investigations on reed periphyton in Lake Velencei, Hungary. Hydrobiologia 506-509: 549–557.

Adl, S. M., A. G. Simpson, C. E. Lane, J. Lukeš, D. Bass, S. S. Bowser, M. Brown, F. Burki, M. Dunthorn, V. Hampl, A. Heiss, M. Hoppenrath, E. Lara, L. LeGall, D. H. Lynn, H. McManus, E. A. D. Mitchell, S. E. Mozley-Stanridge, L. W. Parfrey, J. Pawlowski, S. Rueckert, L. Shadwick, C. Schoch, A. Smirnov, & F. W. Spiegel, 2012. The revised classification of eukaryotes. The Journal of Eukaryotic Microbiology 59: 429–493.

Admiraal, W., & H. Peletier, 1980. Distribution of diatom species on an estuarine mud flat and experimental analysis of the selective effect of stress. Journal of Experimental Marine Biology and Ecology 46: 157–175.

Albay, M., & R. Akcaalan, 2003. Comparative study of periphyton colonisation on common reed (*Phragmites australis*) and artificial substrate in a shallow lake, Manyas, Turkey. Hydrobiologia 506-509: 531–540.

Allen, H. L., 1971. Primary productivity, chemo-organotrophy, and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral of a lake. Ecological Monographs 41: 97–127.

Anderson, M., & N. Gribble, 1998. Partitioning the variation among spatial, temporal and environmental components in a multivariate data set. Australian Journal of Ecology 23: 158–167.

Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson, M. J., 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand.

Andrus, R. E., 1986. Some aspects of *Sphagnum* ecology. Canadian Journal of Botany 64: 416–426.

Asaeda, T., M. Sultana, J. Manatunge, & T. Fujino, 2004. The effect of epiphytic algae on the growth and production of *Potamogeton perfoliatus* L. in two light conditions. Environmental and Experimental Botany 52: 225–238.

Barranguet, C., S. van Beusekom, B. Veuger, T. Neu, E. Manders, J. Sinke, & W. Admiraal, 2004. Studying undisturbed autotrophic biofilms: still a technical challenge. Aquatic Microbial Ecology 34: 1–9.

Battarbee, R. W., 1986. Diatom analysis. - In: Berglund, B. E. (eds.), Handbook of Holocene Palaeoecology and Palaeohydrology. John Wiley & Sons, Chichester: 527–570.

Battin, T. J., L. A. Kaplan, J. D. Newbold, X. Cheng, & C. Hansen, 2003. Effects of current velocity on the nascent architecture of stream microbial biofilms. Applied and Environental Microbiology 69: 5443–5452.

Blanco, S., C. Cejudo-Figueiras, I. Álvarez-Blanco, E. van Donk, E. M. Gross, L.-A. Hansson, K. Irvine, E. Jeppesen, T. Kairesalo, B. Moss, T. Nõges, & E. Bécares, 2014. Epiphytic diatoms along environmental gradients in western Europeans Shallow lakes. CLEAN - Soil, Air, Water 42: 229–235.

Bland, R., & A. Brook, 1974. The spatial distribution of desmids in lakes in northern Minnesota, USA. Freshwater Biology 4: 543–556.

Blindow, I., 1987. The composition and density of epiphyton on several species of submerged macrophytes - the neutral substrate hypothesis tested. Aquatic Botany 29: 157–168.

Borcard, D., P. Legendre, & P. Drapeau, 1992. Partialling out the spatial component of ecological variation. Ecology 73: 1045–1055.

Borg, I., & P. Groenen, 2005. Modern multidimensional scaling: theory and applications. Springer, New York: 614.

Bray, J. R., & J. Curtis, 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27: 325–249.

Brock, T. D., 1970. Photosynthesis by algal epiphytes of *Utricularia* in Everglades National Park. Bulletin of Marine Science 20: 952–956.

Brönmark, C., 1985. Interactions between macrophytes, epiphytes and herbivores: an experimental approach. Oikos 45: 26–30.

Bruckner, C. G., R. Bahulikar, M. Rahalkar, B. Schink, & P. G. Kroth, 2008. Bacteria associated with benthic diatoms from Lake Constance: phylogeny and influences on diatom growth and secretion of extracellular polymeric substances. Applied and Environmental Microbiology 74: 7740–7749.

Bukhtiyarova, L. N., 2007. Revision of the genus *Achnanthes* Bory s. lato (Bacillariophyta). 1. Genera *Achnanthes* Bory s. str. and *Achnanthidium* Kützing s. str. International Journal on Algae 9: 328–341.

Bukhtiyarova, L. N., 2008. Revision of the genus *Achnanthes* Bory s. lato (Bacillariophyta). 2. New monosutural genera and the key for their identification. International Journal on Algae 10: 50–72.

Burkholder, J. M., & R. G. Wetzel, 1990. Epiphytic alkaline phosphatase on natural and artificial plants in an oligotrophic lake: re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. Limnology and Oceanography 35: 736–747.

Burkholder, J. M., R. G. Wetzel, & K. L. Klomparens, 1990. Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intact biofilm matrix. Applied and Environental Microbiology 56: 2882–2890.

Cabin, R. J., & R. J. Mitchell, 2000. To Bonferroni or not to Bonferroni: when and how are the questions. Bulletin of the Ecological Society of America 81: 246–248.

Carrias, J.-F., C. Amblard, C. Quiblier-Lloberas, & G. Bourdier, 1998. Seasonal dynamics of free and attached heterotrophic nanoflagellates in an oligomesotrophic lake. Freshwater Biology 39: 91–101.

Cattaneo, A., 1983. Grazing on epiphytes. Limnology and Oceanography 28: 124–132.

Cattaneo, A., & M. C. Amireault, 1992. How artificial are artificial substrata for periphyton? Jounal of the North American Benthological Society 11: 244–256.

Cattaneo, A., G. Galanti, S. Gentinetta, & S. Romo, 1998. Epiphytic algae and macroinvertebrates on submerged and floating-leaved macrophytes in an Italian lake. Freshwater Biology 39: 725–740.

Cattaneo, A., & J. Kalff, 1979. Primary production of algae growing on natural and artificial aquatic plants: a study of interactions between epiphytes and their substrate. Limnology and Oceanography 24: 1031–1037.

Cattaneo, A., & J. Kalff, 1980. The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. Limnology and Oceanography 25: 280–289.

Cattaneo, A., G. Méthot, B. Pinel-Alloul, T. Niyonsenga, & L. Lapierre, 1995. Epiphyte size and taxonomy as biological indicators of ecological and toxicological factors in lake Saint-François (Québec). Environmental Pollution 87: 357–372.

Cejudo-Figueiras, C., I. Álvarez-Blanco, E. Bécares, & S. Blanco, 2010. Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited. Marine and Freshwater Research 61: 1457–1467.

Clarke, K. R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143.

Clymo, R., 1964. The origin of acidity in *Sphagnum* bogs. The Bryologist 67: 427–431.

Coesel, P. F., 1982. Structural characteristics and adaptations of desmid communities. Journal of Ecology 70: 163–177.

Coesel, P. F., 2001. A method for quantifying conservation value in lentic freshwater habitats using desmids as indicator organisms. Biodiversity and Conservation 10: 177–187.

Coesel, P. F., 2003. Desmid flora data as a tool in conservation management of Dutch freshwater wetlands. Biologia 58: 717–722.

Coesel, P.F., & K. J. Meesters, 2007. Desmids of the lowlands, Mesotaeniaceae and Desmidiaceae of the European lowlands. KNNV Publishing, Zeist, the Netherlands.

Dixit, S., J. Smol, J. Kingston, & D. Charles, 1992. Diatoms: powerful indicators of environmental change. Environmental Science & Technology 26: 23–33.

Domozych, D. S., & C. R. Domozych, 2008. Desmids and biofilms of freshwater wetlands: development and microarchitecture. Microbial Ecology 55: 81–93.

dos Santos, T. R., C. Ferragut, & C. E. de Mattos Bicudo, 2013. Does macrophyte architecture influence periphyton? Relationships among *Utricularia foliosa*, periphyton assemblage structure and its nutrient (C, N, P) status. Hydrobiologia 714: 71–83.

Dudley, T. L., 1992. Beneficial effects of herbivores on stream macroalgae via epiphyte removal. Oikos 65: 121–127.

Emerson, B. C., & R. G. Gillespie, 2008. Phylogenetic analysis of community assembly and structure over space and time. Trends in Ecology & Evolution 23: 619–630.

Eminson, D., & B. Moss, 1980. The composition and ecology of periphyton communities in freshwaters. British Phycological Journal 15: 429–446.

Finlay, B. J., & K. J. Clarke, 1999. Apparent global ubiquity of species in the protist genus *Paraphysomonas*. Protist Elsevier 150: 419–430.

Fitzgerald, G. P., 1969. Some factors in the competition or antagonism among bacteria, algae, and aquatic weeds. Journal of Paleolimnology 5: 351–359.

Flemming, W. D., 1954. Naphrax: a synthetic mounting medium of high refractive index. New and improved methods of preparation. Royal Microscopical Society 74: 42–44.

Fontaneto, D., G. F. Ficetola, R. Ambrosini, & C. Ricci, 2006. Patterns of diversity in microscopic animals: are they comparable to those in protists or in larger animals? Global Ecology and Biogeography 15: 153–162.

Fránková, M., J. Bojková, A. Poulíčková, & M. Hájek, 2009. The structure and species richness of the diatom assemblages of the Western Carpathian spring fens along the gradient of mineral richness. Fottea 9: 355–368.

Gaiser, E. E., & J. Johansen, 2000. Freshwater diatoms from Carolina Bays and other isolated wetlands on the Atlantic Coastal Plain of South Carolina, U.S.A., with descriptions of seven taxa new to science. Diatom Research 15: 75–130.

Ghosh, M., & J. P. Gaur, 1998. Current velocity and the establishment of stream algal periphyton communities. Aquatic Botany 60: 1–10.

Gons, H. J., 1982. Structural and functional characteristics of epiphyton and epipelon in relation to their distribution in Lake Vechten. Hydrobiologia 95: 79–114.

Gontcharov, A., & M. Melkonian, 2008. In search of monophyletic taxa in the family Desmidiaceae (Zygnematophyceae, Viridiplantae): The genus *Cosmarium*. American Journal of Botany 95: 1079–1095.

Gontcharov, A., & M. Melkonian, 2011. A study of conflict between molecular phylogeny and taxonomy in the Desmidiaceae (Streptophyta, Viridiplantae): Analyses of 291 rbcL sequences. Protist 162: 253–267.

Gough, S. B., & L. P. Gough, 1981. Comment on "Primary production of algae growing on natural artificial aquatic plants: a study of interactions between epiphytes and their substrate" (Cattaneo and Kalff). Limnology and Oceanography 25: 987–988.

Gough, S. B., & W. J. Woelkerling, 1976. Wisconsin desmids. II. Aufwuchs and plankton communities of selected soft water lakes, hard water lakes and calcareous spring ponds. Hydrobiologia 49: 3–25.

Gower, J. C., 1971. Statistical methods of comparing different multivariate analyses of the same data. - In: Hodson, F.R., D.G. Kendall & P. Tautu (eds.): Mathematics in the archaeological and historical sciences. Edinburgh University Press, Edinburgh: 138–149.

Gross, E. M., 2003. Allelopathy of aquatic autotrophs. Critical Reviews in Plant Science 22: 313–339.

Guiry, M. D., & G. M. Guiry, 2015. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 11 March 2015

Hall, N., & H. Pearl, 2011. Vertical migration patterns of phytoflagellates in relation to light and nutrient availability in a shallow microtidal estuary. Marine Ecology Progress Series 425: 1–19.

Hammer, Ø., D. A. T. Harper, P. D. Ryan, 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Happey-Wood, C. M., 1988. Vertical-migration patterns of flagellates in a community of freshwater benthic algae. Hydrobiologia 161: 99–123.

Hardy, O. J., 2008. Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. Journal of Ecology 96: 914–926.

Hart, E. A., & J. R. Lovvorn, 2003. Algal vs. macrophyte inputs to food webs of inland saline wetlands. Ecology 84: 3317–3326.

Havens, K. E., J. Hauxwell, A. C. Tyler, S. Thomas, K. J. McGlathery, J. Cebrian, I. Valiela, A. D. Steinman, & S.-J. Hwang, 2001. Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress. Environmental Pollution 113: 95–107.

Heino, J., & J. Soininen, 2010. Are common species sufficient in describing turnover in aquatic metacommunities along environmental and spatial gradients? Limnology and Oceanography 55: 2397–2402.

Hillebrand, H., 2005. Light regime and consumer control of autotrophic biomass. Journal of Ecology 93: 758–769.

Hilt, S., 2006. Allelopathic inhibition of epiphytes by submerged macrophytes. Aquatic Botany 85: 252–256.

Hoagland, K. D., S. C. Roemer, & J. R. Rosowski, 1982. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany 69: 188–213.

Hutchinson, G., 1975. A treatise on limnology, vol. 3: Limnological botany. John Wiley & Sons, New York: 547–548.

Chambers, P. A., P. Lacoul, K. J. Murphy, & S. M. Thomaz, 2008. Global diversity of aquatic macrophytes in freshwater. Hydrobiologia 595: 9–26.

Charles, D. F., F. W. Acker, D. D. Hart, C. W. Reimer, & P. B. Cotter, 2006. Large-scale regional variation in diatom-water chemistry relationships: rivers of the eastern United States. Hydrobiologia 561: 27–57.

James, M. R., I. Hawes, M. Weatherhead, C. Stanger, & M. Gibbs, 2000. Carbon flow in the littoral food web of an oligotrophic lake. Hydrobiologia 441: 93–106.

Jones, J. I., B. Moss, J. W. Eaton, & J. O. Young, 2000. Do submerged aquatic plants influence periphyton community composition for the benefit of invertebrate mutualists? Freshwater Biology 43: 591–604.

Joniak, T., N. Kuczyńska-Kippen, & B. Nagengast, 2007. The role pf aquatic macrophytes on microhabitual transformation of physical-chemical features of small ware bodies. Hydrobiologia 584: 101–109.

Kahlert, M., & K. Pettersson, 2002. The impact of substrate and lake trophy on the biomass and nutrient status of benthic algae. Hydrobiologia 489: 161–169.

Karosienė, J., & J. Kasperovičienė, 2008. Seasonal succession of epiphyton algal communities on *Phragmites australis* (Cav.) Trin. ex Stend. in a mesoeutrophic lake. Ekologija 54: 32–39.

Kendall, M. G., 1938. A new measure of rank corelation. Biometrika 30: 81–93.

Kitting, C. L., B. Fry, & M. D. Morgan, 1984. Detection of inconspicuous epiphytic algae supporting food webs in seagrass meadows. Oecologia 62: 145–149.

Köhler, J., J. Hachoł, & S. Hilt, 2010. Regulation of submersed macrophyte biomass in a temperate lowland river: interactions between shading by bank vegetation, epiphyton and water turbidity. Aquatic Botany 92: 129–136.

Krammer, K., 2000. Diatoms of Europe, vol. 1: The genus *Pinnularia*. - In: Lange-Bertalot, H. (eds): Diatoms of the European inland water and comparable habitats. A.R.G. Gantner Verlag K.G.

Krammer, K., 2002. Diatoms of Europe, vol. 3: *Cymbella*. - In: Lange-Bertalot, H. (eds): Diatoms of the European inland water and comparable habitats. A.R.G. Gantner Verlag K.G.

Krammer, K., 2003. Diatoms of Europe, vol. 4: *Cymbopleura*, *Delicata*, *Navicymbula*, *Gomphocymbellopsis*, *Afrocymbella*. - In: Lange-Bertalot, H. (eds): Diatoms of the European inland water and comparable habitats. A.R.G. Gantner Verlag K.G.

Krammer, K., & H. Lange-Bertalot, 1986. Bacillariophyceae, 1. Teil. - In: Ettl, H., J. Gerloff, H. Heynig & D. Mollenhauer (eds.): Süsswasserflora von Mitteleuropa 2/1. G. Fischer Verlag, Jena.

Krammer, K., & H. Lange-Bertalot, 1988. Bacillariophyceae, 2. Teil. - In: Ettl, H., J. Gerloff, H. Heynig & D. Mollenhauer (eds.): Süsswasserflora von Mitteleuropa 2/1. G. Fischer Verlag, Jena

Krammer, K., & H. Lange-Bertalot, 1991a. Bacillariophyceae, 3. Teil. - In: Ettl, H., J. Gerloff, H. Heynig & D. Mollenhauer (eds.): Süsswasserflora von Mitteleuropa 2/1. G. Fischer Verlag, Jena.

Krammer, K., & H. Lange-Bertalot, 1991b. Bacillariophyceae, 4. Teil. - In: Ettl, H., J. Gerloff, H. Heynig & D. Mollenhauer (eds.): Süsswasserflora von Mitteleuropa 2/1. G. Fischer Verlag, Jena.

Krivograd Klemenčič, A., N. Smolar-Žvanut, D. Istenič, & T. Griessler-Bulc, 2010. Algal community patterns in Slovenian bogs along environmental gradients. Biologia 65: 422–437.

Kruskal, J. B., 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29: 1–27.

Kuczyńska-Kippen, N., B. Messyasz, B. Nagengast, S. Celewicz, & M. Klimko, 2005. A comparative study of periphyton communities on reed complex and *Chara tementosa* in three shallow lakes of Wielkopolska area, Poland. Biologia 60: 349–355.

Lalonde, S., & J. A. Downing, 1991. Epiphyton biomass is related to lake trophic status, depth, and macrophyte architecture. Canadian Journal of Fisheries and Aquatic Sciences 48: 2285–2291.

Lange-Bertalot, H., 2001. Diatoms of Europe, vol. 2: *Navicula* sensu stricto, 10 Genera Separated from *Navicula* sensu lato, *Frustulia*. - In: Lange-Bertalot, H. (eds.): Diatoms of the European inland water and comparable habitats. A.R.G. Gantner Verlag K.G.

Lange-Bertalot, H., M. Bąk, A. Witkowski & N. Tagliaventi, 2011. Diatoms of Europe, vol. 6: *Eunotia* and some related taxa. - In: Lange-Bertalot, H. (eds.): Diatoms of the European inland water and comparable habitats. A.R.G. Gantner Verlag K.G.

Lange-Bertalot, H., & D. Metzeltin, 1996. Iconographia Diatomologica, vol. 2: Indicators of oligotrophy. 800 taxa representative of three ecologically distinct lake types: carbonate buffered, oligodystrophic, weakly buffered soft water. - In: Lange-Bertalot, H. (eds.). Koeltz Scientific Books.

Laugaste, R., & M. Reunanen, 2005. The composition and density of epiphyton on some macrophyte species in the partly meromictic Lake Verevi. Hydrobiologia 547: 137–150.

Lazarek, S., 1982. Structure and productivity of epiphytic aldal communities on *Lobelia dortmanna* L. in acidified and limed lakes. Water, Air and Soil Pollution 18: 333–342.

Legendre, P., & M. J. Anderson, 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecological Monographs 69: 1–24.

Legendre, P., & L. Legendre, 1998. Numerical ecology, 2nd English edition. Elsevier Science, Amsterdam, The Netherlands.

Lenzenweger, R., 1996. Desmidiaceenflora von Österreich. 1.Teil. J. Cramer, Berlin.

Lenzenweger, R., 1997. Desmidiaceenflora von Österreich. 2.Teil. J. Cramer, Berlin.

Lenzenweger, R., 1999. Desmidiaceenflora von Österreich. 3.Teil. J. Cramer, Berlin.

Lenzenweger, R., 2003. Desmidiaceenflora von Österreich. 4.Teil. J. Cramer, Berlin.

Liboriussen, L., & E. Jeppesen, 2003. Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshwater Biology 48: 418–431.

Machová-Černá, K., & J. Neustupa, 2009. Spatial distribution of algal assemblages in a temperate lowland peat bog. International Review of Hydrobiology 94: 40–56.

Mann, H. B., & D. R. Whitney, 1947. On a test of whether one of two random variables is stochastically larger than the other. The Annals of Mathematical Statistics 8: 50–60.

Martin, A. P., 2002. Phylogenetic approaches for describing and comparing the diversity of microbial communities. Applied and Environmental Microbiology 68: 3673–3682.

Mataloni, G., 1999. Ecological studies on algal communities from Tierra del Fuego peat bogs. Hydrobiologia 391: 157–171.

McArdle, B. H., & M. J. Anderson, 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82: 290–297.

Messyasz, B., & N. Kuczyńska-Kippen, 2006. Periphytic algal communities: a comparison of *Typha angustifolia* L. and *Chara tomentosa* L. beds in three shallow lakes (west Poland). Polish Journal of Ecology 54: 15–27.

Mieczan, T., 2007. Epiphytic protozoa (testate amoebae and ciliates) associated with *Sphagnum* in peatbogs: relationship to chemical parameters. Polish Journal of Ecology 55: 79–90.

Mieczan, T., & M. Adamczuk, 2015. Ecology of testate amoebae (Protists) in mosses: distribution and relation of species assemblages with environmental parameters (King George Island, Antarctica). Polar Biology 38: 221–230.

Millie, D. F., & R. L. Lowe, 1983. Studies on Lake Erie's littoral algae: host specificity and temporal periodicity of epiphytic diatoms. Hydrobiologia 99: 7–18.

Mitchell, E. A. D., D. Gilbert, A. Buttler, C. Amblard, P. Grosvernier, & J.-M. Gobat, 2003. Structure of microbial communities in *Sphagnum* peatlands and effect of atmospheric carbon dioxide enrichment. Microbial Ecology 46: 187–199.

Molisch, H., 1937. Der Einfluss einer Pflanze auf die Anderd- Allelopathie. Fischer, Jena, Germany.

Morin, J. O´N., & K. D. Kimb, 1983. Relationship of macrophyte-mediated changes in the water column to periphyton composition and abundance. Freshwater Biology 13: 403–414.

Mormul, R. P., S. M. Thomaz, M. J. da Silveira, & L. Rodrigues, 2010. Epiphyton or macrophyte: which primary producer attracts the snail *Hebetancylus moricandi*? American Malacological Bulletin 28: 127–133.

Müller, U., 1995. Vertical zonation and production rates of epiphytic algae on *Phragmites australis*. Freshwater Biology 34: 69–80.

Müller, U., 1999. The vertical zonation of adpressed diatoms and other epiphytic algae on *Phragmites australis*. European Journal of Phycology 34: 487–496.

Nemjová, K., J. Neustupa, J. Šťastný, P. Škaloud, & J. Veselá, 2011. Species concept and morphological differentiation of strains traditionally assigned to *Micrasterias truncata*. Phycological Research 59: 208–220.

Neustupa, J., K. Černá, & J. Šťastný, 2009. Diversity and morphological disparity of desmid assemblages in Central European peatlands. Hydrobiologia 630: 243–256.

Neustupa, J., K. Černá, & J. Šťastný, 2011. The effects of aperiodic desiccation on the diversity of benthic desmid assemblages in a lowland peat bog. Biodiversity and Conservation 20: 1695–1711.

Neustupa, J., K. Černá, & J. Šťastný, 2012. Spatio-temporal community structure of peat bog benthic desmids on a microscale. Aquatic Ecology 46: 229–239.

Neustupa, J., J. Veselá, & J. Šťastný, 2013. Differential cell size structure of desmids and diatoms in the phytobenthos of peatlands. Hydrobiologia 709: 159–171.

Newson, R., 2002. Parameters behind "nonparametric" statistics: Kendall´s tau, Somers´D and median differences. The Stata Journal 2: 45–64.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, & H. Wagner, 2013. vegan: community ecology package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan

Pals, A., D. Elst, K. Muylaert, & J. Van Assche, 2006. Substrate specificity of periphytic desmids in shallow softwater lakes in Belgium. Hydrobiologia 568: 159–168.

Peres-Neto, P. R., & D. A. Jackson, 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. Oecologia 129: 169–178.

Peres-Neto, P. R., P. Legendre, S. Dray, & D. Borcard, 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 87: 2614–2625.

Peterson, C. G., & R. J. Stevenson, 1992. Resistance and resilience of lotic algal communities: importance of disturbance timing and current. Ecology 73: 1445–1461.

Phillips, G. L., D. Eminson, & B. Moss, 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquatic Botany 4: 103–126.

Poulíčková, A., M. Duchoslav, & M. Dokulil, 2004. Littoral diatom assemblages as bioindicators of lake trophic status: a case study from perialpine lakes in Austria. European Journal of Phycology 39: 143–152.

Poulíčková, A., P. Hašler, M. Lysáková, & B. Spears, 2008. The ecology of freshwater epipelic algae: an update. Phycologia 47: 437–450.

Prowse, G., 1959. Relationship between epiphytic algal species and their macrophytic hosts. Nature 183: 1204–1205.

R Core Team, 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Razali, N. M., & Y. B. Wah, 2011. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. Journal of Statistical Modeling and Analytics 2: 21–33.

Riber, H. H., J. P. Sørensen, & A. Kowalczewski, 1983. Exchange of phosphorus between water, macrophytes and epiphytic periphyton in the littoral of Mikolajskie Lake, Poland. - In:

Wetzel, R. G. (eds.), Periphyton of Freshwater Ecosystems. Dr. W. Junk Publishers, Hague: 326–243.

Rice, W. R., 1989. Alalyzing tables of statistical tests. Evolution 43: 223–225.

Roberts, E., J. Kroker, S. Körner, & A. Nicklisch, 2003. The role of periphyton during the re-colonization of a shallow lake with submerged macrophytes. Hydrobiologia: 525–530.

Růžička, J., 1977. Die Desmidiaceen Mitteleuropas, Bd. 1/1. - Schweizerbart, Stuttgart.

Růžička, J., 1981. Die Desmidiaceen Mitteleuropas, Bd. 1/2. - Schweizerbart, Stuttgart.

Sand-Jensen, K., 1990. Epiphyte shading: its role in resulting depth distribution of submerged aquatic macrophytes. Folia Geobotanice & Phytotaxonomica 25: 315–320.

Sand-Jensen, K., & J. Borum, 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquatic Botany 41: 137–175.

Sekar, R., K. V. K. Nair, V. N. R. Rao, & V. P. Venugopalan, 2002. Nutrient dynamics and successional changes in a lentic freshwater biofilm. Freshwater Biology 47: 1893–1907.

Shannon, C. E., & W. Weaver, 1949: The mathematical theory of communication. Urbana, IL, University of Illinois Press.

Shapiro, S. S., & M. B. Wilk, 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591–611.

Shelford, V., 1918. Conditions of existence. - In: Ward, H. B., and G. C. Whipple (eds.), Freshwater Biology. Wiley: 21–60.

Schindler, D. E., & M. D. Scheuerell, 2002. Habitat coupling in lake ecosystems. Oikos 98: 177–189.

Siver, P. A., 1977. Comparison of attached diatom communities on natural and artificial substrates. Jounal of Phycology 13: 402–406.

Snoeijs, P., S. Busse, & M. Potapova, 2002. The importance of diatom cell size in community analysis. Journal of Phycology 38: 265–272.

Soininen, J., & P. Eloranta, 2004. Seasonal persistence and stability of diatom communities in rivers: are there habitat specific differences? European Journal of Phycology 39: 153–160.

Soininen, J., R. Paavola, & T. Muotka, 2004. Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. Ecography 27: 330–342.

Soudzilovskaia, N., J. Ornelissen, & H. J. D. Uring, 2010. Similar cation exchange capacities among bryophyte species refute a presumed mechanism of peatland acidification. Ecology 91: 2716–2726.

Sultana, M., T. Asaeda, M. E. Azim, & T. Fujino, 2010. Morphological responses of a submerged macrophyte to epiphyton. Aquatic Ecology 44: 73–81.

Svoboda, P., J. Kulichová, & J. Šťastný, 2014. Spatial and temporal community structure of desmids on a small spatial scale. Hydrobiologia 722: 291–303.

Štěpánková, J., J. Vavrušková, P. Hašler, P. Mazalová, & A. Poulíčková, 2008. Diversity and ecology of desmids of peat bogs in the Jizerské hory Mts. Biologia 63: 895–900.

Taniguchi, H., & M. Tokeshi, 2004. Effects of habitat complexity on benthic assemblages in a variable environment. Freshwater Biology 49: 1164–1178.

Thomas, J., D. Nwanko, & P. Sterry, 1985. The feeding strategies of juvenile and adult *Biomphalaria glabrata* (Say) under simulated natural conditions and their relevance to ecological theory and snail control. Proceedings of the Royal Society of London B. 226: 117–209.

Toporowska, M., B. Pawlik-Skowrońska, & A. Z. Wojtal, 2008. Epiphytic algae on *Stratiotes aloides* L., *Potamogeton lucens* L., *Ceratophyllum demersum* L. and *Chara* spp. in a macrophyte-dominated lake. Oceanological and Hydrobiological Studies 37: 51–63.

Townsend, S. A., & P. A. Gell, 2005. The role of substrate type on benthic diatom assemblages in the Daly and Roper Rivers of the Australian wet/dry tropics. Hydrobiologia 548: 101–115.

Tuji, A., 2000. Observation of developmental processes in loosely attached diatom (Bacillariophyceae) communities. Phycological Research 48: 75–84.

Ulanowicz, R. E., 1995. Utricularia's secret: the advantage of positive feedback in oligotrophic environments. Ecological Modelling 79: 49–57.

van Dijk, G. M., 1993. Dynamics and attenuation characteristics of periphyton upon artificial substratum under various light conditions and some additional observations on periphyton upon *Potamogeton pectinatus* L. Hydrobiologia 252: 143–161.

van Donk, E., & W. J. van de Bund, 2002. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. Aquatic Botany 72: 261–274.

Veselá, J., 2009. Spatial heterogeneity and ecology of algal communities in an ephemeral sandstone stream in the Bohemian Switzerland National Park, Czech Republic. Nova Hedwigia 88: 531–547.

Veselá, J., & J. R. Johansen, 2009. The diatom flora of ephemeral headwater streams in the Elbsandsteingebirge region of the Czech Republic. Diatom Research 24: 443–477.

Webb, C. O., D. D. Ackerly, M. a. McPeek, & M. J. Donoghue, 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33: 475–505.

Wetzel, R. G., 1993. Microcommunities and microgradients: linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. Netherlands Journal of Aquatic Ecology 27: 3–9.

Wilcock, R. J., P. D. Champion, J. W. Nagels, & G. F. Croker, 1999. The influence of aquatic macrophytes on the hydraulic and physico-chemical properties of a New Zealand lowland stream. Hydrobiologia 416: 203–214.

Williams, D. M., & F. E. Round, 1987. Revision of the genus *Fragialria*. Diatom Research 2: 267–288.

Williamson, D. F., R. A. Parker, & J. S. Kendrick, 1989. The box plot: a simple visual method to interpret data. Annals of Internal Medicine 110: 916–921.

Winter, J. G., & H. C. Duthie, 2000. Stream epilithic, epipelic and epiphytic diatoms: habitat fidelity and use in biomonitoring. Aquatic Ecology 34: 345–353.

Woelkerling, W. J., 1976. Wisconsin desmids. I. Aufwuchs and plankton communities of selected acid bogs, alkaline begs, and closed bogs. Hydrobiologia 48: 209–232.

Wunsam, S., A. Cattaneo, & N. Bourassa, 2002. Comparing diatom species, genera and size in biomonitoring: a case study from streams in the Laurentians (Québec, Canada). Freshwater Biology 47: 325–340.

Young, I. T., 1977. Proof without for the prejudice: use of the analysis of histograms and other Kolmogorov-Smirnov from flow systems and other sources. The Journal of Histochemistry and Cytochemistry 25: 953–941.

Zheng, L., & R. J. Stevenson, 2006. Algal assemblages in multiple habitats of restored and extant wetlands. Hydrobiologia 561: 221–238.

7. Appendix

List of appendixes

Appendix 1 Overview of sampling sites with additional information.

Appendix 2 Overview of all samples with sampling dates and related factors.

Appendix 3 Overview of datasets.

Appendix 4 Diatom species list from complete dataset.

Appendix 5 Desmid species list from complete dataset.

Appendix 1 Overview of sampling sites with additional information. ([a]) The sites that were not included to the analysis of desmid communities due to low number of desmids cells found in the samples.

site	abbr.	GPS N (°)	GPS E (°)	trophic state
Swamp 1	S1	50.57584	14.670978	oligotrophic
Swamp 2	S2	50.575783	14.670375	oligotrophic
Swamp 3	S3	50.5789	14.667397	oligotrophic
tůň u Klůčku	TK	50.577356	14.661661	oligo-mesotrophic
Kozohlůdky	KO	49.216361	14.648664	oligotrophic
Borkovická blata	BB	49.235583	14.623514	oligotrophic
pískovny Cep 1	C1	48.917978	14.883856	oligotrophic
pískovny Cep 2	C2	48.917083	14.882594	oligotrophic
pískovny Cep 3	C3	48.923533	14.839153	oligotrophic
Rybníčky u Podbořánek 1	P1	50.043147	13.440767	mesotrophic
Rybníčky u Podbořánek 2	P2	50.044681	13.436136	mesotrophic
Horní Kracle	HK	50.140422	13.843089	mesotrophic
Ďáblík 1	D1	48.828142	14.597161	mesotrophic
Ďáblík 2 [a]	D2	48.828183	14.596436	mesotrophic
Rybníček u Studeného [a]	RS	49.601056	15.127856	mesotrophic

site	PRE-STUDY		MAIN STUDY	
	sampling date	no. of samples	sampling date	no. of samples
Swamp 1	15.10.2011	6	28.8.2012	6
Swamp 2	-	-	28.8.2012	6
Swamp 3	15.10.2011	3	28.8.2012	9
tůň u Klůčku	15.10.2011	3	28.8.2012	12
Kozohlůdky	30.9.2011	6	10.9.2012	9
Borkovická blata	-	-	10.9.2012	6
pískovny Cep 1	-	-	8.9.2012	9
pískovny Cep 2	-	-	8.9.2012	5
pískovny Cep 3	-	-	8.9.2012	9
Rybníčky u Podbořánek 1	1.10.2011	12	27.8.2012	18
Rybníčky u Podbořánek 2	-	-	27.8.2012	12
Horní Kracle	-	-	5.9.2012	9
Ďáblík 1	-	-	8.9.2012	18
Ďáblík 2 [a]	-	-	8.9.2012	7
Rybníček u Studeného [a]	17.10.2011	6	-	-

Appendix 2 Overview of all samples with sampling dates and related factors. The last two columns show in which samples 200 cells of particular algal group were found. Those samples could be included in the analyses and thus created the complete datasets. The numbers in brackets reflect recorded species richness.

no.	sample code	date	site	host	pH	conductivity [µS/cm]	200 diatoms (spp.)	200 desmids (spp.)
1	1-S1-UT1	15.10.2011	S1	UT	4.4	81	yes (17)	yes (12)
2	1-S1-UT2	15.10.2011	S1	UT	5.1	91	yes (8)	yes (9)
3	1-S1-UT3	15.10.2011	S1	UT	5.0	79	yes (14)	yes (14)
4	1-S1-SP1	15.10.2011	S1	SP	4.4	81	yes (16)	yes (8)
5	1-S1-SP2	15.10.2011	S1	SP	5.1	91	yes (8)	yes (7)
6	1-S1-SP3	15.10.2011	S1	SP	5.0	79	yes (15)	yes (16)
7	1-S3-SP1	15.10.2011	S3	SP	4.1	104	yes (7)	yes (4)
8	1-S3-SP2	15.10.2011	S3	SP	4.1	102	yes (7)	yes (4)
9	1-S3-SP3	15.10.2011	S3	SP	4.2	97	yes (6)	yes (4)
10	1-TK-SP1	15.10.2011	TK	SP	4.7	168	yes (7)	yes (4)
11	1-TK-SP2	15.10.2011	TK	SP	5.4	254	yes (10)	yes (18)
12	1-TK-SP3	15.10.2011	TK	SP	4.6	157	yes (9)	yes (15)
13	1-KO-EQ1	30.9.2011	KO	EQ	4.5	33	yes (9)	not found
14	1-KO-EQ2	30.9.2011	KO	EQ	4.5	33	yes (12)	not found
15	1-KO-EQ3	30.9.2011	KO	EQ	4.5	33	yes (5)	not found
16	1-KO-UT1	30.9.2011	KO	UT	4.5	33	yes (8)	yes (11)
17	1-KO-UT2	30.9.2011	KO	UT	4.5	33	yes (6)	yes (11)
18	1-KO-UT3	30.9.2011	KO	UT	4.5	33	yes (8)	not found
19	1-P1-SP1	1.10.2011	P1	SP	3.6	142	yes (19)	yes (8)
20	1-P1-SP2	1.10.2011	P1	SP	3.6	161	yes (21)	yes (10)
21	1-P1-SP3	1.10.2011	P1	SP	3.6	200	yes (24)	not found
22	1-P1-UT1	1.10.2011	P1	UT	7.1	250	yes (23)	yes (14)
23	1-P1-UT2	1.10.2011	P1	UT	6.7	237	yes (30)	yes (14)
24	1-P1-UT3	1.10.2011	P1	UT	6.7	237	yes (33)	yes (17)
25	1-P1-NY1	1.10.2011	P1	NY	9.5	249	yes (19)	yes (8)
26	1-P1-NY2	1.10.2011	P1	NY	9.0	248	yes (12)	yes (7)
27	1-P1-NY3	1.10.2011	P1	NY	7.0	244	yes (12)	not found
28	1-P1-CA1	1.10.2011	P1	CA	7.1	250	yes (21)	not found
29	1-P1-CA2	1.10.2011	P1	CA	7.1	250	yes (30)	yes (16)
30	1-P1-CA3	1.10.2011	P1	CA	6.7	237	yes (18)	yes (17)
31	1-RS-CA1	17.10.2011	RS	CA	6.5	173	yes (19)	not found
32	1-RS-CA2	17.10.2011	RS	CA	6.5	171	yes (23)	not found
33	1-RS-CA3	17.10.2011	RS	CA	6.6	166	yes (20)	not found
34	1-RS-TY1	17.10.2011	RS	TY	6.5	173	yes (18)	not found
35	1-RS-TY2	17.10.2011	RS	TY	6.5	171	yes (13)	not found
36	1-RS-TY3	17.10.2011	RS	TY	6.6	166	yes (16)	not found
37	2-P1-SP1	27.8.2012	P1	SP	3.9	220	yes (14)	yes (11)
38	2-P1-SP2	27.8.2012	P1	SP	4.2	104	yes (25)	yes (12)
39	2-P1-SP3	27.8.2012	P1	SP	4.8	158	yes (31)	yes (12)

no.	sample code	date	site	host	pH	conductivity [µS/cm]	200 diatoms (spp.)	200 desmids (spp.)
40	2-P1-UT1	27.8.2012	P1	UT	6.5	249	yes (19)	yes (11)
41	2-P1-UT2	27.8.2012	P1	UT	6.5	255	yes (17)	yes (12)
42	2-P1-UT3	27.8.2012	P1	UT	6.5	273	yes (21)	yes (14)
43	2-P1-CH1	27.8.2012	P1	CH	6.5	249	yes (9)	yes (14)
44	2-P1-CH2	27.8.2012	P1	CH	6.5	255	yes (15)	yes (15)
45	2-P1-CH3	27.8.2012	P1	CH	6.5	273	yes (20)	yes (14)
46	2-P1-NY1	27.8.2012	P1	NY	6.4	240	yes (19)	yes (11)
47	2-P1-NY2	27.8.2012	P1	NY	6.3	240	yes (18)	yes (12)
48	2-P1-NY3	27.8.2012	P1	NY	6.2	240	yes (13)	yes (17)
49	2-P1-PO1	27.8.2012	P1	PO	6.4	240	yes (14)	yes (11)
50	2-P1-PO2	27.8.2012	P1	PO	6.3	240	yes (18)	yes (9)
51	2-P1-PO3	27.8.2012	P1	PO	6.2	240	yes (18)	yes (10)
52	2-P1-CA1	27.8.2012	P1	CA	6.5	249	yes (26)	yes (12)
53	2-P1-CA2	27.8.2012	P1	CA	6.5	255	yes (29)	not found
54	2-P1-CA3	27.8.2012	P1	CA	6.5	273	yes (26)	yes (18)
55	2-P2-UT1	27.8.2012	P2	UT	6.7	260	yes (14)	yes (18)
56	2-P2-UT2	27.8.2012	P2	UT	6.7	260	yes (18)	yes (21)
57	2-P2-UT3	27.8.2012	P2	UT	6.7	260	yes (17)	yes (15)
58	2-P2-CA1	27.8.2012	P2	CA	6.7	260	yes (19)	yes (17)
59	2-P2-CA2	27.8.2012	P2	CA	6.7	260	yes (19)	yes (20)
60	2-P2-CA3	27.8.2012	P2	CA	6.7	260	yes (16)	yes (19)
61	2-P2-PO1	27.8.2012	P2	PO	6.5	270	yes (16)	yes (13)
62	2-P2-PO2	27.8.2012	P2	PO	6.5	270	yes (12)	yes (12)
63	2-P2-PO3	27.8.2012	P2	PO	6.5	270	yes (17)	yes (8)
64	2-P2-CH1	27.8.2012	P2	CH	6.7	260	yes (22)	yes (18)
65	2-P2-CH2	27.8.2012	P2	CH	6.7	260	yes (16)	yes (16)
66	2-P2-CH3	27.8.2012	P2	CH	6.7	260	yes (11)	yes (15)
67	2-S1-SP1	28.8.2012	S1	SP	6.1	70	yes (11)	yes (17)
68	2-S1-SP2	28.8.2012	S1	SP	5.5	79	yes (14)	yes (8)
69	2-S1-SP3	28.8.2012	S1	SP	4.9	98	yes (16)	yes (22)
70	2-S1-UT1	28.8.2012	S1	UT	6.1	70	yes (17)	yes (15)
71	2-S1-UT2	28.8.2012	S1	UT	5.5	79	yes (17)	yes (16)
72	2-S1-UT3	28.8.2012	S1	UT	4.9	98	yes (14)	yes (19)
73	2-S2-SP1	28.8.2012	S2	SP	5.0	46	yes (10)	yes (18)
74	2-S2-SP2	28.8.2012	S2	SP	5.4	54	yes (12)	yes (21)
75	2-S2-SP3	28.8.2012	S2	SP	5.7	55	yes (9)	yes (16)
76	2-S2-UT1	28.8.2012	S2	UT	5.0	46	yes (10)	yes (20)
77	2-S2-UT2	28.8.2012	S2	UT	5.4	54	yes (15)	yes (25)
78	2-S2-UT3	28.8.2012	S2	UT	5.7	55	yes (14)	yes (18)
79	2-S3-SP1	28.8.2012	S3	SP	4.7	89	yes (7)	yes (8)
80	2-S3-SP2	28.8.2012	S3	SP	4.8	89	yes (8)	yes (9)
81	2-S3-SP3	28.8.2012	S3	SP	4.5	90	yes (9)	yes (6)
82	2-S3-UT1	28.8.2012	S3	UT	4.7	89	yes (10)	yes (7)

no.	sample code	date	site	host	pH	conductivity [µS/cm]	200 diatoms (spp.)	200 desmids (spp.)
83	2-S3-UT2	28.8.2012	S3	UT	4.8	89	yes (9)	yes (5)
85	2-S3-NY1	28.8.2012	S3	NY	4.7	89	yes (16)	yes (6)
86	2-S3-NY2	28.8.2012	S3	NY	4.8	89	yes (21)	yes (7)
87	2-S3-NY3	28.8.2012	S3	NY	4.5	90	yes (14)	yes (6)
88	2-TK-SP1	28.8.2012	TK	SP	6.2	192	yes (3)	yes (8)
89	2-TK-SP2	28.8.2012	TK	SP	6.4	192	yes (13)	yes (17)
90	2-TK-SP3	28.8.2012	TK	SP	6.0	195	yes (2)	yes (10)
91	2-TK-UT1	28.8.2012	TK	UT	6.2	192	yes (13)	yes (24)
92	2-TK-UT2	28.8.2012	TK	UT	6.4	192	not found	yes (22)
93	2-TK-UT3	28.8.2012	TK	UT	6.0	195	yes (13)	yes (18)
94	2-TK-PO1	28.8.2012	TK	PO	6.2	192	yes (11)	yes (23)
95	2-TK-PO2	28.8.2012	TK	PO	6.4	192	yes (12)	yes (24)
96	2-TK-PO3	28.8.2012	TK	PO	6.0	195	yes (13)	yes (19)
97	2-TK-NY1	28.8.2012	TK	NY	6.2	192	yes (13)	yes (25)
98	2-TK-NY2	28.8.2012	TK	NY	6.4	192	yes (19)	yes (22)
99	2-TK-NY3	28.8.2012	TK	NY	6.0	195	yes (15)	yes (21)
100	2-D1-SP1	8.9.2012	D1	SP	5.6	70	yes (21)	yes (24)
101	2-D1-SP2	8.9.2012	D1	SP	5.6	70	yes (25)	yes (16)
102	2-D1-SP3	8.9.2012	D1	SP	6.4	72	yes (36)	not found
103	2-D1-UT1	8.9.2012	D1	UT	6.5	79	yes (32)	yes (19)
104	2-D1-UT2	8.9.2012	D1	UT	6.2	77	yes (17)	yes (17)
105	2-D1-UT3	8.9.2012	D1	UT	6.5	73	yes (25)	yes (19)
106	2-D1-CH1	8.9.2012	D1	CH	6.5	79	yes (32)	yes (18)
107	2-D1-CH2	8.9.2012	D1	CH	6.2	77	yes (17)	yes (15)
108	2-D1-CH3	8.9.2012	D1	CH	6.5	73	yes (29)	yes (21)
109	2-D1-PO1	8.9.2012	D1	PO	6.1	77	yes (19)	yes (16)
110	2-D1-PO2	8.9.2012	D1	PO	6.0	72	yes (16)	yes (16)
111	2-D1-PO3	8.9.2012	D1	PO	5.9	73	yes (15)	yes (15)
112	2-D1-TY1	8.9.2012	D1	TY	6.1	77	yes (27)	yes (17)
113	2-D1-TY2	8.9.2012	D1	TY	6.0	72	yes (14)	yes (21)
114	2-D1-TY3	8.9.2012	D1	TY	5.9	73	yes (21)	yes (18)
115	2-D1-CA1	8.9.2012	D1	CA	6.5	79	yes (33)	not found
116	2-D1-CA2	8.9.2012	D1	CA	6.2	77	yes (17)	yes (13)
117	2-D1-CA3	8.9.2012	D1	CA	6.5	73	yes (30)	not found
118	2-D2-CA1	8.9.2012	D2	CA	6.2	106	yes (26)	not found
119	2-D2-CA2	8.9.2012	D2	CA	6.2	81	yes (30)	not found
120	2-D2-CA3	8.9.2012	D2	CA	6.2	86	yes (35)	not found
121	2-D2-UT1	8.9.2012	D2	UT	6.2	106	yes (20)	not found
122	2-D2-UT2	8.9.2012	D2	UT	6.2	81	yes (34)	not found
123	2-D2-UT3	8.9.2012	D2	UT	6.2	86	yes (27)	not found
124	2-D2-CH1	8.9.2012	D2	CH	6.2	81	yes (23)	not found
125	2-HK-UT1	5.9.2012	HK	UT	7.0	230	yes (19)	yes (14)
126	2-HK-UT2	5.9.2012	HK	UT	7.0	228	yes (13)	yes (15)

no.	sample code	date	site	host	pH	conductivity [µS/cm]	200 diatoms (spp.)	200 desmids (spp.)
127	2-HK-UT3	5.9.2012	HK	UT	7.0	228	yes (18)	yes (13)
129	2-HK-EQ2	5.9.2012	HK	EQ	7.0	228	yes (16)	yes (10)
130	2-HK-EQ3	5.9.2012	HK	EQ	7.0	228	yes (20)	yes (12)
131	2-HK-NY1	5.9.2012	HK	NY	7.0	230	yes (21)	not found
132	2-HK-NY2	5.9.2012	HK	NY	7.0	230	yes (19)	not found
133	2-HK-NY3	5.9.2012	HK	NY	7.0	228	yes (22)	yes (13)
134	2-KO-UT1	10.9.2012	KO	UT	4.3	42	yes (11)	yes (13)
135	2-KO-UT2	10.9.2012	KO	UT	4.3	41	yes (10)	yes (10)
136	2-KO-UT3	10.9.2012	KO	UT	4.4	38	yes (3)	not found
137	2-KO-EQ1	10.9.2012	KO	EQ	4.3	42	yes (13)	yes (6)
138	2-KO-EQ2	10.9.2012	KO	EQ	4.3	41	yes (12)	yes (7)
139	2-KO-EQ3	10.9.2012	KO	EQ	4.4	38	yes (10)	not found
140	2-KO-SP1	10.9.2012	KO	SP	3.8	51	yes (22)	yes (15)
141	2-KO-SP2	10.9.2012	KO	SP	4.3	41	yes (7)	yes (8)
142	2-KO-SP3	10.9.2012	KO	SP	4.4	38	yes (5)	yes (6)
143	2-BB-SP1	10.9.2012	BB	SP	6.3	96	yes (21)	yes (16)
144	2-BB-SP2	10.9.2012	BB	SP	6.3	90	yes (17)	not found
145	2-BB-SP3	10.9.2012	BB	SP	6.4	90	yes (17)	yes (10)
146	2-BB-PO1	10.9.2012	BB	PO	6.3	96	yes (17)	yes (11)
147	2-BB-PO2	10.9.2012	BB	PO	6.4	88	yes (19)	yes (9)
148	2-BB-PO3	10.9.2012	BB	PO	6.2	88	yes (15)	yes (10)
149	2-C1-UT1	8.9.2012	C1	UT	6.8	250	yes (7)	yes (16)
150	2-C1-UT2	8.9.2012	C1	UT	6.8	250	yes (12)	yes (18)
151	2-C1-UT3	8.9.2012	C1	UT	6.8	250	yes (16)	yes (15)
152	2-C1-PO1	8.9.2012	C1	PO	6.8	250	yes (7)	yes (17)
153	2-C1-PO2	8.9.2012	C1	PO	6.8	250	yes (13)	yes (18)
154	2-C1-PO3	8.9.2012	C1	PO	6.8	250	yes (13)	yes (23)
155	2-C1-TY1	8.9.2012	C1	TY	6.8	250	yes (15)	yes (15)
156	2-C1-TY2	8.9.2012	C1	TY	6.8	250	yes (21)	yes (16)
157	2-C1-TY3	8.9.2012	C1	TY	6.8	250	yes (13)	yes (18)
158	2-C2-TY1	8.9.2012	C2	TY	6.5	26	yes (17)	yes (17)
159	2-C2-TY2	8.9.2012	C2	TY	6.6	26	yes (12)	yes (18)
160	2-C2-TY3	8.9.2012	C2	TY	6.5	26	yes (14)	yes (20)
161	2-C2-NY1	8.9.2012	C2	NY	6.5	26	yes (16)	yes (28)
162	2-C2-NY2	8.9.2012	C2	NY	6.6	26	yes (17)	yes (27)
163	2-C3-SP1	8.9.2012	C3	SP	5.5	21	yes (4)	yes (5)
164	2-C3-SP2	8.9.2012	C3	SP	5.2	22	yes (9)	yes (19)
165	2-C3-SP3	8.9.2012	C3	SP	5.5	21	yes (15)	yes (16)
166	2-C3-NY1	8.9.2012	C3	NY	5.5	21	yes (13)	yes (16)
167	2-C3-NY2	8.9.2012	C3	NY	5.5	21	yes (7)	yes (19)
168	2-C3-NY3	8.9.2012	C3	NY	5.5	21	yes (14)	yes (19)
169	2-C3-PO1	8.9.2012	C3	PO	5.5	21	yes (9)	yes (17)
170	2-C3-PO2	8.9.2012	C3	PO	5.2	22	yes (13)	yes (9)
171	2-C3-PO3	8.9.2012	C3	PO	5.2	22	yes (11)	yes (17)

Appendix 3 Overview of datasets. The numbers in brackets refer to the number of sample in each dataset. The complete datasets contain the applicable samples where 200 cells of particular algal group were found (see Appendix 2) and were pruned to the reduced datasets which provide the maximum possible overlap of host plants. The datasets for the Procrustes statistic contain only the samples in which both 200 diatom cells and 200 desmid cells were found.

no.	sample code	COMPLETE DATASETS			REDUCED DATASETS		
		diatoms (170)	desmids (141)	Procrustes (140)	diatoms (50)	desmids (50)	Procrustes (49)
1	1-S1-UT1	yes	yes	yes	excluded	excluded	excluded
2	1-S1-UT2	yes	yes	yes	excluded	excluded	excluded
3	1-S1-UT3	yes	yes	yes	excluded	excluded	excluded
4	1-S1-SP1	yes	yes	yes	excluded	excluded	excluded
5	1-S1-SP2	yes	yes	yes	excluded	excluded	excluded
6	1-S1-SP3	yes	yes	yes	excluded	excluded	excluded
7	1-S3-SP1	yes	yes	yes	excluded	excluded	excluded
8	1-S3-SP2	yes	yes	yes	excluded	excluded	excluded
9	1-S3-SP3	yes	yes	yes	excluded	excluded	excluded
10	1-TK-SP1	yes	yes	yes	excluded	excluded	excluded
11	1-TK-SP2	yes	yes	yes	excluded	excluded	excluded
12	1-TK-SP3	yes	yes	yes	excluded	excluded	excluded
13	1-KO-EQ1	yes	excluded	excluded	excluded	excluded	excluded
14	1-KO-EQ2	yes	excluded	excluded	excluded	excluded	excluded
15	1-KO-EQ3	yes	excluded	excluded	excluded	excluded	excluded
16	1-KO-UT1	yes	yes	yes	excluded	excluded	excluded
17	1-KO-UT2	yes	yes	yes	excluded	excluded	excluded
18	1-KO-UT3	yes	excluded	excluded	excluded	excluded	excluded
19	1-P1-SP1	yes	yes	yes	excluded	excluded	excluded
20	1-P1-SP2	yes	yes	yes	excluded	excluded	excluded
21	1-P1-SP3	yes	excluded	excluded	excluded	excluded	excluded
22	1-P1-UT1	yes	yes	yes	excluded	excluded	excluded
23	1-P1-UT2	yes	yes	yes	excluded	excluded	excluded
24	1-P1-UT3	yes	yes	yes	excluded	excluded	excluded
25	1-P1-NY1	yes	yes	yes	excluded	excluded	excluded
26	1-P1-NY2	yes	yes	yes	excluded	excluded	excluded
27	1-P1-NY3	yes	excluded	excluded	excluded	excluded	excluded
28	1-P1-CA1	yes	excluded	excluded	excluded	excluded	excluded
29	1-P1-CA2	yes	yes	yes	excluded	excluded	excluded
30	1-P1-CA3	yes	yes	yes	excluded	excluded	excluded
31	1-RS-CA1	yes	excluded	excluded	excluded	excluded	excluded
32	1-RS-CA2	yes	excluded	excluded	excluded	excluded	excluded
33	1-RS-CA3	yes	excluded	excluded	excluded	excluded	excluded
34	1-RS-TY1	yes	excluded	excluded	excluded	excluded	excluded
35	1-RS-TY2	yes	excluded	excluded	excluded	excluded	excluded
36	1-RS-TY3	yes	excluded	excluded	excluded	excluded	excluded
37	2-P1-SP1	yes	yes	yes	yes	yes	yes

no.	sample code	COMPLETE DATASETS			REDUCED DATASETS		
		diatoms (170)	desmids (141)	Procrustes (140)	diatoms (50)	desmids (50)	Procrustes (49)
38	2-P1-SP2	yes	yes	yes	yes	yes	yes
39	2-P1-SP3	yes	yes	yes	yes	yes	yes
40	2-P1-UT1	yes	yes	yes	yes	yes	yes
41	2-P1-UT2	yes	yes	yes	yes	yes	yes
42	2-P1-UT3	yes	yes	yes	yes	yes	yes
43	2-P1-CH1	yes	yes	yes	excluded	excluded	excluded
44	2-P1-CH2	yes	yes	yes	excluded	excluded	excluded
45	2-P1-CH3	yes	yes	yes	excluded	excluded	excluded
46	2-P1-NY1	yes	yes	yes	yes	yes	yes
47	2-P1-NY2	yes	yes	yes	yes	yes	yes
48	2-P1-NY3	yes	yes	yes	yes	yes	yes
49	2-P1-PO1	yes	yes	yes	yes	yes	yes
50	2-P1-PO2	yes	yes	yes	yes	yes	yes
51	2-P1-PO3	yes	yes	yes	yes	yes	yes
52	2-P1-CA1	yes	yes	yes	excluded	excluded	excluded
53	2-P1-CA2	yes	excluded	excluded	excluded	excluded	excluded
54	2-P1-CA3	yes	yes	yes	excluded	excluded	excluded
55	2-P2-UT1	yes	yes	yes	excluded	excluded	excluded
56	2-P2-UT2	yes	yes	yes	excluded	excluded	excluded
57	2-P2-UT3	yes	yes	yes	excluded	excluded	excluded
58	2-P2-CA1	yes	yes	yes	excluded	excluded	excluded
59	2-P2-CA2	yes	yes	yes	excluded	excluded	excluded
60	2-P2-CA3	yes	yes	yes	excluded	excluded	excluded
61	2-P2-PO1	yes	yes	yes	excluded	excluded	excluded
62	2-P2-PO2	yes	yes	yes	excluded	excluded	excluded
63	2-P2-PO3	yes	yes	yes	excluded	excluded	excluded
64	2-P2-CH1	yes	yes	yes	excluded	excluded	excluded
65	2-P2-CH2	yes	yes	yes	excluded	excluded	excluded
66	2-P2-CH3	yes	yes	yes	excluded	excluded	excluded
67	2-S1-SP1	yes	yes	yes	excluded	excluded	excluded
68	2-S1-SP2	yes	yes	yes	excluded	excluded	excluded
69	2-S1-SP3	yes	yes	yes	excluded	excluded	excluded
70	2-S1-UT1	yes	yes	yes	excluded	excluded	excluded
71	2-S1-UT2	yes	yes	yes	excluded	excluded	excluded
72	2-S1-UT3	yes	yes	yes	excluded	excluded	excluded
73	2-S2-SP1	yes	yes	yes	excluded	excluded	excluded
74	2-S2-SP2	yes	yes	yes	excluded	excluded	excluded
75	2-S2-SP3	yes	yes	yes	excluded	excluded	excluded
76	2-S2-UT1	yes	yes	yes	excluded	excluded	excluded
77	2-S2-UT2	yes	yes	yes	excluded	excluded	excluded
78	2-S2-UT3	yes	yes	yes	excluded	excluded	excluded
79	2-S3-SP1	yes	yes	yes	yes	yes	yes
80	2-S3-SP2	yes	yes	yes	yes	yes	yes

no.	sample code	COMPLETE DATASETS			REDUCED DATASETS		
		diatoms (170)	desmids (141)	Procrustes (140)	diatoms (50)	desmids (50)	Procrustes (49)
81	2-S3-SP3	yes	yes	yes	yes	yes	yes
82	2-S3-UT1	yes	yes	yes	yes	yes	yes
83	2-S3-UT2	yes	yes	yes	yes	yes	yes
84	2-S3-UT3	yes	yes	yes	yes	yes	yes
85	2-S3-NY1	yes	yes	yes	yes	yes	yes
86	2-S3-NY2	yes	yes	yes	yes	yes	yes
87	2-S3-NY3	yes	yes	yes	yes	yes	yes
88	2-TK-SP1	yes	yes	yes	yes	yes	yes
89	2-TK-SP2	yes	yes	yes	yes	yes	yes
90	2-TK-SP3	yes	yes	yes	yes	yes	yes
91	2-TK-UT1	yes	yes	yes	yes	yes	yes
92	2-TK-UT2	excluded	yes	excluded	excluded	yes	excluded
93	2-TK-UT3	yes	yes	yes	yes	yes	yes
94	2-TK-PO1	yes	yes	yes	yes	yes	yes
95	2-TK-PO2	yes	yes	yes	yes	yes	yes
96	2-TK-PO3	yes	yes	yes	yes	yes	yes
97	2-TK-NY1	yes	yes	yes	yes	yes	yes
98	2-TK-NY2	yes	yes	yes	yes	yes	yes
99	2-TK-NY3	yes	yes	yes	yes	yes	yes
100	2-D1-SP1	yes	yes	yes	yes	yes	yes
101	2-D1-SP2	yes	yes	yes	yes	yes	yes
102	2-D1-SP3	yes	excluded	excluded	yes	excluded	excluded
103	2-D1-UT1	yes	yes	yes	yes	yes	yes
104	2-D1-UT2	yes	yes	yes	yes	yes	yes
105	2-D1-UT3	yes	yes	yes	yes	yes	yes
106	2-D1-CH1	yes	yes	yes	excluded	excluded	excluded
107	2-D1-CH2	yes	yes	yes	excluded	excluded	excluded
108	2-D1-CH3	yes	yes	yes	excluded	excluded	excluded
109	2-D1-PO1	yes	yes	yes	yes	yes	yes
110	2-D1-PO2	yes	yes	yes	yes	yes	yes
111	2-D1-PO3	yes	yes	yes	yes	yes	yes
112	2-D1-TY1	yes	yes	yes	excluded	excluded	excluded
113	2-D1-TY2	yes	yes	yes	excluded	excluded	excluded
114	2-D1-TY3	yes	yes	yes	excluded	excluded	excluded
115	2-D1-CA1	yes	excluded	excluded	excluded	excluded	excluded
116	2-D1-CA2	yes	yes	yes	excluded	excluded	excluded
117	2-D1-CA3	yes	excluded	excluded	excluded	excluded	excluded
118	2-D2-CA1	yes	excluded	excluded	excluded	excluded	excluded
119	2-D2-CA2	yes	excluded	excluded	excluded	excluded	excluded
120	2-D2-CA3	yes	excluded	excluded	excluded	excluded	excluded
121	2-D2-UT1	yes	excluded	excluded	excluded	excluded	excluded
122	2-D2-UT2	yes	excluded	excluded	excluded	excluded	excluded

no.	sample code	COMPLETE DATASETS			REDUCED DATASETS		
		diatoms (170)	desmids (141)	Procrustes (140)	diatoms (50)	desmids (50)	Procrustes (49)
123	2-D2-UT3	yes	excluded	excluded	excluded	excluded	excluded
124	2-D2-CH1	yes	excluded	excluded	excluded	excluded	excluded
125	2-HK-UT1	yes	yes	yes	excluded	excluded	excluded
126	2-HK-UT2	yes	yes	yes	excluded	excluded	excluded
127	2-HK-UT3	yes	yes	yes	excluded	excluded	excluded
128	2-HK-EQ1	yes	excluded	excluded	excluded	excluded	excluded
129	2-HK-EQ2	yes	yes	yes	excluded	excluded	excluded
130	2-HK-EQ3	yes	yes	yes	excluded	excluded	excluded
131	2-HK-NY1	yes	excluded	excluded	excluded	excluded	excluded
132	2-HK-NY2	yes	excluded	excluded	excluded	excluded	excluded
133	2-HK-NY3	yes	yes	yes	excluded	excluded	excluded
134	2-KO-UT1	yes	yes	yes	excluded	excluded	excluded
135	2-KO-UT2	yes	yes	yes	excluded	excluded	excluded
136	2-KO-UT3	yes	excluded	excluded	excluded	excluded	excluded
137	2-KO-EQ1	yes	yes	yes	excluded	excluded	excluded
138	2-KO-EQ2	yes	yes	yes	excluded	excluded	excluded
139	2-KO-EQ3	yes	excluded	excluded	excluded	excluded	excluded
140	2-KO-SP1	yes	yes	yes	excluded	excluded	excluded
141	2-KO-SP2	yes	yes	yes	excluded	excluded	excluded
142	2-KO-SP3	yes	yes	yes	excluded	excluded	excluded
143	2-BB-SP1	yes	yes	yes	excluded	excluded	excluded
144	2-BB-SP2	yes	excluded	excluded	excluded	excluded	excluded
145	2-BB-SP3	yes	yes	yes	excluded	excluded	excluded
146	2-BB-PO1	yes	yes	yes	excluded	excluded	excluded
147	2-BB-PO2	yes	yes	yes	excluded	excluded	excluded
148	2-BB-PO3	yes	yes	yes	excluded	excluded	excluded
149	2-C1-UT1	yes	yes	yes	excluded	excluded	excluded
150	2-C1-UT2	yes	yes	yes	excluded	excluded	excluded
151	2-C1-UT3	yes	yes	yes	excluded	excluded	excluded
152	2-C1-PO1	yes	yes	yes	excluded	excluded	excluded
153	2-C1-PO2	yes	yes	yes	excluded	excluded	excluded
154	2-C1-PO3	yes	yes	yes	excluded	excluded	excluded
155	2-C1-TY1	yes	yes	yes	excluded	excluded	excluded
156	2-C1-TY2	yes	yes	yes	excluded	excluded	excluded
157	2-C1-TY3	yes	yes	yes	excluded	excluded	excluded
158	2-C2-TY1	yes	yes	yes	excluded	excluded	excluded
159	2-C2-TY2	yes	yes	yes	excluded	excluded	excluded
160	2-C2-TY3	yes	yes	yes	excluded	excluded	excluded
161	2-C2-NY1	yes	yes	yes	excluded	excluded	excluded
162	2-C2-NY2	yes	yes	yes	excluded	excluded	excluded
163	2-C3-SP1	yes	yes	yes	yes	yes	yes
164	2-C3-SP2	yes	yes	yes	yes	yes	yes
165	2-C3-SP3	yes	yes	yes	yes	yes	yes

no.	sample code	COMPLETE DATASETS			REDUCED DATASETS		
		diatoms (170)	desmids (141)	Procrustes (140)	diatoms (50)	desmids (50)	Procrustes (49)
166	2-C3-NY1	yes	yes	yes	yes	yes	yes
167	2-C3-NY2	yes	yes	yes	yes	yes	yes
168	2-C3-NY3	yes	yes	yes	yes	yes	yes
169	2-C3-PO1	yes	yes	yes	yes	yes	yes
170	2-C3-PO2	yes	yes	yes	yes	yes	yes
171	2-C3-PO3	yes	yes	yes	yes	yes	yes

Appendix 4 Diatom species list from the complete dataset, i.e. 170 applicable samples. In total, there were 171 species identified.

Achnanthes cf. *stolida*
Achnanthes cf. *tuma*
Achnanthes sp. (morphotyp 1)
Achnanthes sp. (morphotyp 2)
Achnanthidium pyrenaicum
Achnanthidium minutissimum
Achnanthidium subatomoides
Amphora ovalis
Brachysira brebissonii
Brachysira neoexilis (morphotyp 1)
Brachysira neoexilis (morphotyp 2)
Brachysira procera
Brachysira serians
Caloneis tenuis
Cocconeis placentula var. *lineata*
Cocconeis placentula var. *placentula*
Cymbella aspera
Cymbella cf. *lange-bertalotii*
Cymbella cymbiformis
Cymbopleura naviculiformis
Denticula kuetzingii
Encyonema elginense
Encyonema gracile
Encyonema minutum
Encyonema silesiacum
Encyonopsis cf. *delicatissima*
Encyonopsis falaisensis
Epithemia adnata
Eunotia ambivalens
Eunotia arculus
Eunotia bilunaris var. *bilunaris*
Eunotia boreotenuis
Eunotia cf. *arcubus*
Eunotia cf. *pomeranica*
Eunotia circumborealis
Eunotia dianae-stitinensis
Eunotia elegans
Eunotia exigua
Eunotia formicina
Eunotia glacialis
Eunotia implicata
Eunotia incisa
Eunotia intermedia
Eunotia meisteri
Eunotia minor
Eunotia mucophila
Eunotia muscicola
Eunotia naegelii
Eunotia nymanniana
Eunotia paludosa
Eunotia paludosa s.l.
Eunotia pectinalis var. *ventricosa*
Eunotia rhomboidea
Eunotia tenella
Eunotia trinacria
Fallacia vitrea
Fragilaria acus
Fragilaria brevistriata
Fragilaria capucina
Fragilaria construens
Fragilaria crotonensis
Fragilaria nanana
Fragilaria nitzschioides
Fragilariforma bicapitata
Fragilariforma virescens
Frustulia crassinervia
Frustulia saxonica
Gomphonema acuminatum
Gomphonema acuminatum var. *brebissonii*
Gomphonema anjae
Gomphonema augur
Gomphonema cf. *clavatum*
Gomphonema gracile
Gomphonema jadwigiae
Gomphonema minuta
Gomphonema parvulum
Gomphonema truncatum
Gomphonema intricatum var. *vibrio*
Hippodonta capitata
Chamaepinnularia mediocris
Kobayasiella sp.
Kobayasiella subtilissima
Lemnicola hungarica
Luticola sp.
Navicula cf. *tenelloides*
Navicula cryptocephala

Navicula lanceolata
Navicula leptostriata
Navicula molestiformis
Navicula radiosa
Navicula rhynchocephala
Navicula trivialis
Neidium ampliatum
Neidium bisulcatum
Neidium hercynicum
Nitzschia dissipata var. *media*
Nitzschia insignis
Nitzschia sp. (morphotyp 1)
Nitzschia sp. (morphotyp 2)
Nitzschia sp. (morphotyp 3)
Nitzschia sp. (morphotyp 4)
Nitzschia sp. (morphotyp 5)
Nitzschia sp. (morphotyp 6)
Nitzschia sp. (morphotyp 7)
Nitzschia sp. (morphotyp 8)
Nitzschia sp. (morphotyp 9)
Pinnularia acrosphaeria
Pinnularia acuminata
Pinnularia anglica
Pinnularia angusta var. *rostrata*
Pinnularia biceps
Pinnularia borealis var. *borealis*
Pinnularia brauniana
Pinnularia cf. *anglica*
Pinnularia cf. *frequentis*
Pinnularia cf. *obscura*
Pinnularia cf. *subcommutata*
Pinnularia cf. *tirolensis* (mophotyp 1)
Pinnularia cf. *tirolensis* (mophotyp 2)
Pinnularia cf. *tirolensis* var. *julma*
Pinnularia complexa
Pinnularia cruxarea
Pinnularia frequentis
Pinnularia gibba
Pinnularia gibbiformis
Pinnularia isselana
Pinnularia macilenta
Pinnularia neomajor
Pinnularia nodosa
Pinnularia nodosa var. *percapitata*
Pinnularia pisciculus
Pinnularia polyonca var. *similis*
Pinnularia pseudogibba
Pinnularia rhombarea
Pinnularia sinistra
Pinnularia sp.
Pinnularia stomatophora
Pinnularia subcapitata var. *elongata*
Pinnularia subcapitata var. *subrostrata*
Pinnularia subfalaiseana
Pinnularia subgibba
Pinnularia subgibba var. *undulata*
Pinnularia transversa
Pinnularia undula
Pinnularia viridiformis
Pinnularia viridis
Placoneis elginensis
Planothidium frequentissimum
Pseudostaurosira parasitica var. *subconstricta*
Rhopalodia gibba
Rossithidium nodosum
Rossithidium petersenii
Rossithidium pusillum
Sellaphora americana
Sellaphora pupula
Stauroneis anceps
Stauroneis cf. *kriegeri*
Stauroneis cf. *agrestis*
Stauroneis cf. *thermicola*
Stauroneis gracilis
Stauroneis thermicola
Staurosira construens var. *exigua*
Staurosirella pinnata
Stenopterobia curvula
Stenopterobia delicatissima
Surirella sp.
Surirella angusta
Tabellaria fenestrata
Tabellaria flocculosa
Ulnaria biceps
Ulnaria ulna

Appendix 5 Desmid species list from the complete dataset, i.e. 141 applicable samples. In total, there were 161 species identified.

Actinotaenium cf. *gelidum*
Actinotaenium cf. *phymatosporum*
Actinotaenium crassiusculum
Actinotaenium cruciferum
Actinotaenium cucurbita
Actinotaenium inconspicuum
Actinotaenium turgidum
Bambusina borreri
Closterium abruptum
Closterium acutum
Closterium baillyanum
Closterium calosporum var. *brasiliense*
Closterium calosporum var. *maius*
Closterium cf. *archerianum* var. *minus*
Closterium cf. *parvulum* var. *angustum*
Closterium cf. *turgidum*
Closterium cf. v*enus*
Closterium cornu
Closterium dianae
Closterium directum
Closterium ehrenbergii
Closterium exiguum
Closterium gracile
Closterium incurvum
Closterium intermedium
Closterium juncidum
Closterium limneticum
Closterium lineatum
Closterium moniliferum
Closterium parvulum
Closterium pritchardianum
Closterium rostratum
Closterium setaceum
Closterium striolatum
Closterium venus
Cosmarium abbreviatum var. *germanicum*
Cosmarium amoenum
Cosmarium cf. *botrytis*
Cosmarium cf. *depressum*
Cosmarium cf. *difficile*
Cosmarium cf. *margaritiferum*
Cosmarium cf. *pseudoornatum*
Cosmarium cf. *punctulatum*
Cosmarium cf. *subtumidum*
Cosmarium cf. *trilobulatum var. depressum*
Cosmarium cf. *turpinii* var. *eximium*
Cosmarium connatum
Cosmarium contractum
Cosmarium contractum var. ellipsoideum
Cosmarium difficile
Cosmarium discrepans
Cosmarium formosulum
Cosmarium goniodes
Cosmarium impressulum
Cosmarium laeve
Cosmarium laeve var. *octangulare*
Cosmarium margaritiferum
Cosmarium obsoletum
Cosmarium obtusatum
Cosmarium pachydermum
Cosmarium paragranatoides
Cosmarium polygonatum
Cosmarium portianum
Cosmarium pseudopyramidatum
Cosmarium pyramidatum
Cosmarium quadratulum
Cosmarium quadratum
Cosmarium ralfsii var. *montanum*
Cosmarium regnellii
Cosmarium regnesii
Cosmarium reniforme
Cosmarium sp. (morphotyp 1)
Cosmarium sp. (morphotyp 2)
Cosmarium sp. (morphotyp 3)
Cosmarium sp. (morphotyp 4)
Cosmarium sp. (morphotyp 5)
Cosmarium sphagnicola
Cosmarium subcostatum var. *minus*
Cosmarium subcrenatum
Cosmarium subquadrans var. *minor*
Cosmarium subtumidum
Cosmarium tetraophthalmum
Cosmarium thwaitesii var. *penioides*
Cosmarium tinctum
Cosmarium wittrockii
Cylindrocystis brebissonii

Cylindrocystis gracilis
Euastrum ansatum
Euastrum ansatum var. *rhomboidale*
Euastrum ansatum var. *robustum*
Euastrum bidentatum
Euastrum binale
Euastrum binale var. *papilliferum*
Euastrum denticulatum
Euastrum gayanum
Euastrum intermedium
Euastrum luetkemuelleri
Euastrum obesum
Euastrum pectinatum
Euastrum sp.
Euastrum verrucosum
Haplotaenium rectum
Hyalotheca dissiliens
Micrasterias americana
Micrasterias crux-melitensis
Micrasterias jenneri
Micrasterias rotata
Micrasterias thomasiana
Micrasterias truncata
Micrasterias truncata var. *semiradiata*
Netrium digitus
Penium cylindrus
Pleurotaenium ehrenbergii (morphotyp 1)
Pleurotaenium ehrenbergii (morphotyp 2)
Pleurotaenium nodosum
Pleurotaenium trabecula
Spondylosium pulchellum
Staurastrum alternans
Staurastrum avicula var. *avicula*
Staurastrum avicula var. *lunatum*
Staurastrum bieneanum
Staurastrum bohlinianum
Staurastrum crenatum
Staurastrum dispar
Staurastrum gladiosum
Staurastrum gracile
Staurastrum gradiosum
Staurastrum hirsutum
Staurastrum chaetoceras
Staurastrum lapponicum
Staurastrum margaritaceum
Staurastrum micron
Staurastrum orbiculare var. *depressum*
Staurastrum planctonicum
Staurastrum polymorphum
Staurastrum polytrichum
Staurastrum punctulatum
Staurastrum simonyi var. *semicirculare*
Staurastrum simonyi
Staurastrum sp.
Staurastrum striolatum
Staurastrum teliferum
Staurastrum tetracerum
Staurodesmus convergens
Staurodesmus cuspidatus
Staurodesmus dejectus var. *apiculatus*
Staurodesmus dickiei
Staurodesmus extensus var. *vulgaris*
Staurodesmus glaber
Staurodesmus octocornis
Staurodesmus omearae
Staurodesmus patens
Staurodesmus sp.
Teilingia granulata
Tetmemorus brebissonii var. *minor*
Tetmemorus granulatus
Tetmemorus laevis
Tetmemorus laevis var. *minutus*
Xanthidium antilopaeum
Xanthidium armatum
Xanthidium bifidum