University of South Bohemia, Faculty of Biological Sciences

Bachelor Thesis

Selective formation of algal and cyanobacterial assemblages on different substrates in a small oligotrophic pond in the Czech-Moravian Uplands

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2006

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KAPITULČINOVÁ, D., (2006): Selective formation of algal and cyanobacterial assemblages on different substrates in a small oligotrophic pond in the Czech-Moravian Uplands. BSc Thesis, University of South Bohemia, Faculty of Biological Sciences, České Budějovice, Czech Republic

Annotation: Periphyton assemblages on natural and artificial substrates were studied in a small oligotrophic pond in the Czech-Moravian Uplands. Artificial assemblages developed on suspended glass and PVC panels which were fixed to half-shaded floats. The panels were exposed in the pond for about one month in four different periods (autumn 2004, spring 2005, late summer 2005 and winter 2006). Differences in the relative species composition between experimentally darkened and naturally illuminated conditions, glass and PVC surface as well as natural (*Equisetum fluviatile*, floating twigs etc.) and artificial substrates, were monitored.

Acknowledgements: I would like to thank Hanys, supervisor of this thesis, for useful discussion and advice. Pavel Hrouzek and his parents provided me the opportunity to do the research on their pond. My thanks belong to them as well as to many other people who helped me in the field. I also wish to thank to Prof. Jiří Komárek and T. B. for their help with the identification of some problematic species. I am grateful to my friends, especially MDP ☺, and my family, who have kept supporting me in my work.

Hereby I declare that I worked up this thesis by myself only with a help of literature listed in References and people mentioned in Acknowledgements.

04/05/2006, České Budějovice & Bristol

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1. Introduction

1.1. Periphytic communities

Algae growing attached to substrates are generally known as *periphyton*. However, this word has obscure etymology and its usage was discussed several times (SLÁDEČKOVÁ, 1962; WETZEL, 1983 in ALOI, 1990). The term may have been introduced in the 1920s by Russian limnologists (in SLÁDEČKOVÁ, 1962) to refer to the microalgal community living upon the surfaces of submersed objects in water (WETZEL, 1983 in ALOI, 1990). The definition does not include fungal, bacterial, protozoan and other animal components, which are included in the German *Aufwuchs* (meaning 'to grow upon'). Yet this distinction is not made by many authors (e.g. WEBER, 1973 in ALOI, 1990) and the term *Aufwuchs* has rather been replaced by *biofilm* (KALFF, 2002).

Periphytic communities can be divided into a few groups according to the nature of the substrate which they colonise. Those growing on rock substrates are known as *epilithon*, whereas *epiphyton* refers to those growing on aquatic macrophytes. Specific communities can be found on necrotising parts of plants (e.g. leaves of aquatic plants or near-shore trees). A group of periphyton growing on mud or silt substrates is referred to as *epipelon* and the one growing on sand substrates is called *epipsammon* (JAVORNICKÝ et al., 1978). *Metaphyton* is a term used for communities not directly attached to substrates but derived from, and associated with substrates in areas protected from waves and currents. They are typically composed of clumps of primarily filamentous green algae entangled among macrophytes or trapped between the sediment and water surface near shore (KALFF, 2002).

Periphyton in inland waters at all latitudes is typically dominated by a variety of diatoms, green algae and cyanobacteria. The absolute and relative abundance of each group changes over the course of the season and is linked to seasonally altering nutrient supply, light conditions, scouring in streams during floods, external nutrient supply rates in wetlands and predation. Surprisingly, much less is known about the ecology and physiology of periphyton communities than about their phytoplankton counterparts, even though periphyton production is higher than phytoplankton production in many water bodies, especially shallow lakes (e.g. LIBORIUSSEN & JEPPESEN, 2003). Apparently, periphyton has received less attention from limnologists than the phytoplankton because of sampling difficulties including a heterogeneous periphyton distribution in nature and the difficulty of separating the periphyton from the substrates on which they grow (KALFF, 2002).

1.2. Artificial substrates

1.2.1. Types and use of artificial substrates

Because of the problems with sampling of periphytic algae, artificial substrates have been introduced. Advantages include a reduced variability, known surface area, standardised conditions (e.g. uniform colonisation time, material, texture, size) (CATTANEO & AMIREAULT, 1992) and no nutritional or chlorophyll artefacts from host plants of epiphyton (WEHR & SHEATH, 2003). Artificial substrates can be easily manipulated and they simplify both the detachment of the assemblage and the determination of the assemblage and the sample area (CATTANEO & AMIREAULT, 1992). In addition, standardised surface enables comparisons from site to site and estimations of the statistical variability. These characteristics are especially important when quantifying impacts of point sources are of concern (BARBIERO, 2000).

An artificial substrate has been defined by CAIRNS (1982 in ALOI, 1990) as a 'device placed in an aquatic ecosystem to study colonisation by indigenous organisms' (ALOI, 1990). Since HENTSCHEL (1916 in CATTANEO & AMIREAULT, 1992) first suspended artificial substrates in a lake (glass slides), many different materials and anchoring devices have been used. Among them are glass cover slips and culture tubes, rocks, tiles, bricks, Styrofoam, SEM tabs, metal plates, plastic (Plexiglas, PVC, artificial plants) and nutrient diffusing substrates (ALOI, 1990). They have been used for studying colonisation, succession, productivity dynamics and pollution assessment, as well as for strict taxonomic surveys of algae in aquatic habitats (TUCHMAN & STEVENSON, 1980). Periphytic algae can also be successfully used for removal of excessive nutrients (e.g. phosphorus), metals and toxic substances, hence improving water quality (VYMAZAL, 1988; JÖBGEN et al., 2004). Nowadays, periphyton communities are exploited mainly in water quality monitoring.

The ideal artificial substrate has several characteristics. It should be replicatable, not discriminate for or against any particular organism, or group of organisms, not influencing the growth dynamics of attached communities, and finally, it should be easily submersible in the water mass and subsequently readily retrievable without significant loss of the sampled organisms (AUSTIN et al., 1981). In case the experimental design considers artificial substrate a substitute for the natural substrate, the periphytic community must accurately represent the community composition and abundance on the naturally occurring substrate (rock, plant etc.) (ALOI, 1990). Yet, the question of whether artificial substrates satisfactorily mimic natural ones remains open, because results of various comparative studies are contradictory (BROWN, 1976; CATTANEO & KALFF, 1979; SCHAGERL & DONABAUM 1998; LANE et al., 2003).

1.2.2. Factors affecting periphyton development

There are several factors that affect the development of the periphytic community in addition to the type of artificial substrate used. Artificial substrates must be in place for a sufficient time to allow representative communities to develop on the substrate surface (ALOI, 1990). The exposure time depends on geographical location and altitude of the investigated site, quality and temperature of water, type of the water body, time of year and the aim of the study (observation of a primary biofilm, mature periphyton community etc.). In order to obtain a well developed periphyton community on an artificial substrate in Central Europe during the warmer periods of the year, one, two and four weeks are commonly sufficient exposure times for eutrophic, mesotrophic and oligotrophic water bodies, respectively. In winter the times should be somewhat longer (JAVORNICKÝ et al., 1978).

Siting of the artificial substrates (depth, orientation, shading, disturbance etc.) is also very important. The water depth at which an artificial substrate is placed will affect the light conditions and, where water level fluctuates, whether the substrates remain submerged for the duration of the incubation period. Most studies report placing an artificial substrate between 0.16 m and 4 m below the water level (BROWN, 1976; GONS, 1982; HOAGLAND et al. 1982; SEKAR et al. 1998; BARBIERO, 2000; DANILOV & EKELUND, 2001).

The orientation of artificial substrates, with regards both to vertical or horizontal positioning and the angle to the current, varies between studies (review by ALOI, 1990). Slides and tiles are often orientated vertically to reduce detrital accumulation and restrict algal growth to truly periphytic species (BROWN, 1976; TUCHMAN & STEVENSON, 1980), however, this appears to slow down the colonisation rates (ALOI, 1990).

Shading is another factor which markedly affects the development of periphyton communities on artificial substrates (STEINMAN & MCINTIRE, 1986; HOAGLAND & PETERSON, 1990; MARKS & LOWE, 1993; SEKAR et al., 2002; ROBERTS et al., 2004).

Water current also has a direct influence on the distribution of periphytic species. STEINMAN & MCINTIRE (1986) found out that a fast current inhibits initial colonisation, but once established, the growth of a periphyton assemblage may be enhanced by rapid exchanges of nutrients and dissolved gasses between algal cells and the flowing water.

SEKAR et al. (1998) reported that periphyton settlement (on Perspex panels in a freshwater reservoir) also differed with the size of the artificial substrate used. In their study algal densities were slightly higher on smaller panels (3 x 3 cm) than on the larger ones (10 x 7 cm). Moreover, they observed that the thickness of algal biofilms and the total algal density at the periphery of the panels were significantly different from those at the centre. However, the species richness was markedly higher at the centre of the panels and the species composition differed with the position on the panels as well. This phenomenon is known as the 'edge effect' and it can be defined as preferential settlement of sessile organisms towards the edges of panels as compared to their centres. This effect seems to be caused by factors such as enhanced eddy diffusion (DICKMAN & GOCHNAUER, 1978 IN SEKAR, 1998), current velocity (CATTANEO, 1990; BLENKINSOPP & LOCK, 1994) and grazing activity (CATTANEO, 1983).

In another experiment, SEKAR et al. (2004) proved that the surface wettability and roughness as well as the presence of organic and bacterial films markedly influenced the adhesion of all examined species (Chlorophyceae, Bacillariophyceae and Cyanobacteria). Culture age, density and cell viability were also substantially important factors.

1.2.3. Glass slides and plastic

Glass slides had been the standard in periphyton research, especially of diatoms, particularly after the development and popularisation of the Catherwood diatometer by PATRICK et al. (1954 in ALOI, 1990). Obviously, glass slides became so common because they are inexpensive, inert, of uniform surface, and periphyton may be easily removed by scraping (ALOI, 1990) or directly examined under a light microscope, which is enabled by the transparency of the substrate. A considerable advantage of the direct examination is the possibility to observe and count early-maturing development stages, loosely attached algae and cyanobacteria etc. which are usually damaged by the scraping from the slides (JAVORNICKÝ et al., 1978).

Although glass slides have been among the most commonly used artificial substrates, a lot of comparative studies has shown that the periphyton growing on glass substrates is significantly different from the natural assemblages in terms of biomass (BROWN, 1976), chlorophyll (ROSEMARIN & GELIN, 1978 IN ALOI, 1990), species composition (BROWN, 1976; BARBIERO, 2000) and also primary productivity (LOEB, 1981). On the other hand, there is still some evidence that the composition of communities on glass slides is representative of the community composition on the natural substrates (LANE et al., 2003). However, CATTANEO & AMIREAULT (1992) highlighted a trend over the past twenty years away from 'smooth' surfaces such as glass towards more 'natural' surfaces such as unglazed tiles. Yet smooth surfaces (e.g. plastic strips) may have some application (CORING, 1993 in KELLY et al., 1998) and many investigators used artificial plants made of plastic for periphyton research as well (CATTANEO & KALFF, 1978; CATTANEO & KALFF, 1979; BURKHOLDER & WETZEL, 1989; JONES et al., 2000). CATTANEO & KALFF (1979) studied primary production of algae growing on both natural and artificial aquatic plants and interactions between epiphytes and their substrate. They discovered that epiphytes growing on *Potamogeton richardsonii* were similar to those on morphologically similar plastic plants in both biomass (Chl *a*) and primary production per unit area, and that living plants are apparently a neutral substrate for algal growth (according to alkaline phosphatase activity measurements). The first point is in contrast with the findings of other similar studies which revealed that algal communities on natural leaves differed substantially from those on artificial ones (CATTANEO & KALFF, 1978; BURKHOLDER & WETZEL, 1989). Moreover, there are several studies which do not agree with the second conclusion that living plants are a neutral substrate for algae. KÖRNER & NICKLISCH (2002) brought evidence that *Myriophyllum spicatum* and *Ceratophyllum demersum* proved to inhibit the activity of photosystem II and so the growth of the investigated phytoplankton species (Cyanobacteria, Bacillariophyceae, Chlorophyceae). Substances which induce the inhibition are algicidal and cyanobactericidal polyphenols (LEU et al., 2002). JONES et al. (2000) used artificial aquatic plants to test the hypothesis that submerged aquatic plants can affect the periphyton which grows on their surfaces, making it nutritionally beneficial to snails. In return preferential feeding by snails clears the plants from a potential competitor, with both plants and grazers gaining from this mutualistic relationship. Nevertheless, they concluded that although submerged aquatic plants exert a control over the composition of their periphytic community, it is unlikely that the plants manipulate the periphyton to make it more attractive to grazers such as snails.

1.2.4. Water quality monitoring

Much of the published literature on periphyton only deals with diatom communities. This is in part due to the fact that diatoms are ubiquitous, diverse, and have defined ecological characteristics. Diatom frustules are taxonomically diagnostic and exhibit little phenotypic variation compared to other heteromorphic algae (STEVENSON & LOWE, 1986 in ALOI, 1990). In addition, separation of diatoms from large amounts of organic detritus or epiphyte samples is easily achieved by oxidising the samples. Consequently, diatoms have proven useful in water quality monitoring (LOWE, 1972 in ALOI, 1990). Diatoms growing on rocks and other hard surfaces are favoured for the monitoring throughout Europe. Since hard surfaces are not always naturally available, introduced artificial substrates have to be applied at some times (KELLY et al. 1998).

Many countries are now either using diatoms as a part of routine monitoring programmes, or are in the process of developing techniques. In recent years, diatom-based monitoring has also been used for the monitoring associated with directives of the European Union (EU). The great geographical diversity of European states raised obvious questions about the comparability of data from different regions and has led to a development of standards which should be used in water quality monitoring (KELLY et al. 1998). Two guidance standards dealing with diatoms in water quality assessment (routine sampling and pretreatment of benthic diatoms from rivers; identification, enumeration and interpretation of benthic diatom samples from running waters) have been established in the Czech Republic, based on the European Standard (EN 14407:2004). A guidance standard has been introduced for assessments of algal and cyanobacterial assemblages as well.

Although the standards are ordinarily used in water quality monitoring in the Czech Republic, they are not fully satisfactory. In addition, Czech specialists do not share the same opinions on existing methodics and there also are some problems with the interpretation of the data with regard to the ecological conditions of the investigated sites. Evidently, the present situation is caused by lack of practical projects which would compare current methodics and choose the most acceptable one (MARVAN et al., 2005).

1.3. Aims of the study

As mentioned above, some doubts still exist about the correct use of artificial substrates, the materials selected and the factors affecting the settlement of organisms upon them. This is of a special importance when the artificial substrates should represent the composition of organisms on naturally occurring substrates.

Three aims of the study were established: **1)** to compare periphyton assemblages on natural (e.g. wood, aquatic plants) and artificial (glass, plastic) substrates; **2)** to compare periphyton assemblages on glass slides and plastic panels (PVC) and **3)** to compare periphyton assemblages under naturally illuminated and experimentally darkened conditions in a small oligotrophic pond.

2. Materials and methods

2.1. Site description

The study was conducted in the oligotrophic pond Huntov (49°37'33.5" N, 15°07'14.3" E) near Kamenice nad Lipou in the western part of the Českomoravská vrchovina (Czech-Moravian Uplands) (Appendix 1). The bedrock is mainly composed of orthogneiss, biotite-gneiss, migmatite and granite. Annual mean air temperatures vary from 6 to 8°C depending on the site location. The annual total precipitation ranges from 500 to 700 mm. The pond is located at the altitude of 664 m a. s. l. (in HROUZEK, 1999)

Surrounding vegetation is mainly formed by *Picea excelsa* with *Frangula alnus* and *Fagus sylvatica*. Water in the pond springs from wells rising ca 300 meters above this pond. These wells form a system of smaller drains, which flows down into the pond through peat meadows. Endangered flower species, *Drosera rotundifolia* and *Dactylorhiza majalis,* grow in the meadows and a critically endangered crustacean, *Astacus astacus*, lives in the pond. Water horsetail, *Equisetum fluviatile*, is the species prevailing in the littoral zone (Appendix 2). The maximum depth of the pond is 3 metres and the thickness of the mud sediment layer on the bottom varies from 50 to 100 cm. The water is oligotrophic according to chemical analyses, with nitrates and nitrites present only in trace amounts (Table 1, from HROUZEK, 1999).

NH_4^+ (mg.l^{-1})	NO_2^- (mg.l^{-1})	NO_3^- (mg.l^{-1})	Fe (mg.l^{-1})	Ca^{2+} (mg.l^{-1})	pH
<0.05	<0.01	<2.0	1.74	7.5	6.4

Table 1. Water chemistry in the pond Huntov – western shore, 4/8/1998 (from HROUZEK, 1999)

2.2. Construction and exposition of floats

Hand-made floats were used for the comparative studies. Each float consisted of a bamboo stick (150 cm) and two types of artificial substrates; i.e. 6 microscope slides (7 x 3 x 0.3 cm) and 6 PVC panels of the same size. PVC and glass panels were fastened to the bamboo stick alternately 5 cm from each other by means of a 6 cm long fishing-line. In order to compare periphyton assemblages under naturally illuminated and experimentally darkened conditions, a half of the float was covered with a straw mat which sheltered the panels beneath it from light. One PET-bottle was fastened at each end of the stick to hold the construction on the water surface. The float was anchored with two bound stones deployed to the bottom of the pond (Figure 1).

In order to cover a potential temporal variability in the data, the floats were placed in the pond for four different periods. The exposure times were 28 days during the autumn 2004 (10/09 – 06/10/2004), spring 2005 (20/04 – 19/05/2005), late summer 2005 (29/07 – 26/08/2005) and 34 days during the winter 2006 (04/02 – 10/03/2006). Three floats were used during each period and suspended in different parts of the pond: near the dam, in the middle of the pond and near the littoral zone (Appendix 2). The winter experiment was conducted in order to reveal whether some viable algae and/or cyanobacteria are present in the pond and, if this is the case, whether they will colonise the glass/PVC panels under the ice cover. Therefore, the floats were slightly modified for the winter conditions. Only four glass slides and four PVC panels were fastened to the bamboo stick by a 35 cm long fishing-line so the substrates would not get frozen into the ice cover. Since light, one of the main limiting factors in winter, would apparently scarcely reach the artificial substrates through the layer of the ice and straw mats, the latter were not applied. Three holes (0.5 x 2 m) were cut into the ice cover by a chainsaw in approximately the same positions where the floats were placed during the previous experiments. The floats were sited into the prepared holes without binding them to the stones as this was not necessary in such conditions.

Originally, glass slides were fixed to the fishing-line by a transparent tape and a super glue during the first two experiments, whereas PVC panels were tied through a small hole at the top part of the panels. Since all glass slides got unglued and were lost during the second exposition time (spring 2005), another type of attachment was invented for the other investigations. Consequently, both glass and PVC panels were fastened by the fishing-line through two small V-cuts at the top part (Figure 2).

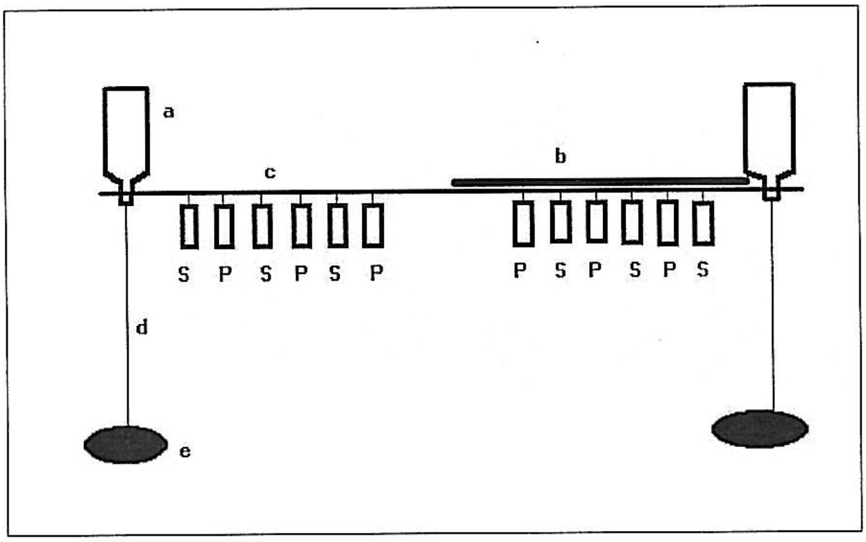

Figure 1. Used floats: a – PET-bottle; b – straw mat; c – bamboo stick; d – string; e – stone; S – glass slide; P – PVC panel

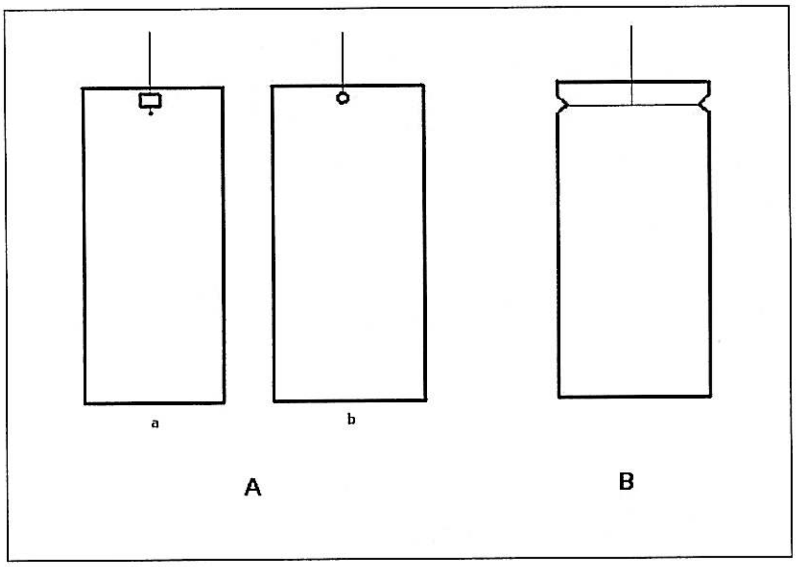

Figure 2. Panel attachments: A – original attachment, a – by tape and glue (glass slide); b – through a small hole (PVC panel); B – V-cut attachment (glass/PVC panels)

2.3. Periphyton sampling

All periphyton-covered panels (Appendix 2) were snipped by scissors directly from a boat after the first three exposure periods, whereas in winter 2006, the floats had to be cut out from the ice cover. All panels were stored separately in plastic bottles (50 ml) in order to preclude the loss of loosely attached periphytic communities and the mixing of assemblages from different panels. The bottles were filled with formaldehyde solution (final concentration 1.5%) to preserve the organic material. In the laboratory, the biofilms were scraped off the panels with a razor blade and kept in the respective bottles. In addition, periphyton growing on the non-panel parts of the floats, i.e. the illuminated parts of bamboo sticks and the top/bottom sides of the straw mats, was sampled in autumn 2004 and spring 2005.

Naturally occurring assemblages were sampled on the same day when the artificial substrates were removed. Algal and cyanobacterial communities growing on *Equisetum fluviatile* (Appendix 2) and plankton were sampled during all investigated periods (autumn 2004, spring 2005, late summer 2005 and winter 2006). During the first three periods, plankton was sampled near the dam, whereas in winter 2006 it was sampled from two holes made in the ice cover in the middle of the pond, and in the littoral zone. Assemblages occurring on floating wood (twigs) and between the submersed shore vegetation were collected in autumn 2004 and spring 2005. Periphyton growing on dead floating *Equisetum fluviatile* as well as on bryophytes was sampled in spring 2005, whereas epiphyton from *Carex rostrata* was collected in autumn 2004. Firmly attached algal and cyanobacterial assemblages were found on the bottom side of the cut ice cover after the removal of the floats in the littoral zone and they were examined as well. Different natural substrates were sampled in different periods because some of the substrates were not found during some periods. The periphyton assemblages were either preserved by formaldehyde solution (final concentration 1.5%) or examined immediately after the return to the laboratory.

2.4. Preparation of permanent slides

Permanent slides were prepared from the autumn samples after the all-species examination under a light microscope in order to identify diatoms to species. A simplified approach to the slide preparation was chosen. Concurrently, two cover slips were placed on a hotplate and a droplet of the sample from one bottle was dripped on each slip. Subsequently, three drops of hydrogen peroxide were applied one after another to remove organic material after the evaporation of the formaldehyde solution. Dried cover slips with the diatom frustules were transferred carefully onto a clean filter paper. A small droplet of Pleurax was placed on each cover slip and the slides were laid upon them. All created permanent slides were allowed to dry properly for a week's time.

2.5. Examination of samples

Three drops from each bottle were observed under the light microscopes Olympus CX – 40 or Olympus BX – 51. Photographs of the present species (Appendices 3, 4) were taken by the Camedia C5050 camera fastened to the microscope. The relative occurrence of the species was examined using a semiquantitative scale (Table 1).

The following studies were applied for the determination of the present taxa: Cyanobacteria – GEITLER, 1932; KOMÁREK & ANAGNOSTIDIS, 1999; KOMÁREK & ANAGNOSTIDIS, 2005; Glaucophyta – STARMACH, 1966; Bacillariophyceae – KRAMMER & LANGE-BERTALOT, 1997a, 1997b, 1991, 2000; HOUK, 2003; Chrysophyceae – STARMACH, 1985; Xanthophyceae – ETTL, 1978; Zygnemophyceae – LENZENWEGER, 1996; LENZENWEGER, 1997; LENZENWEGER, 1999; Chlorophyceae – HINDÁK et al., 1978; KOMÁREK & FOTT, 1983; Euglenophyta – WOLOWSKI, 1998; WOLOWSKI & HINDÁK, 2005; Dinoflagellata – POPOVSKÝ & PFIESTER, 1990).

Original value	Species	Values used in Canoco	Values used in pie charts (%)
6	Present on a mass scale, 90 – 100%	7	95
5	Very frequent, 50 – 90%	6	70
4	Frequent, 20 – 50%	5	35
3	Relatively frequent, 5 – 20%	4	12.5
2	Rare, 1 – 5%	3	3
1	Very rare, 0.1 – 1%	2	0.55
+	Sporadic, < 0.1%	1	0.05

Table 1. Semiquantitative scale for estimation of the relative species occurrence (modified from JAVORNICKÝ et al., 1978)

2.6. Data analyses

As the lengths of gradients in the classical ordination method (detrended correspondence analysis – DCA) varied around 1.2 in all cases, a linear constrained ordination, redundancy analysis (RDA), was used to create models explaining the variability in the periphyton community structure. Three hypotheses according to the aims of the study were tested: 1) the type of the substrate (natural – *Equisetum fluviatile*, wood (twigs), shore, and bryophytes; artificial – PVC/ glass panels) has no influence on the periphyton community structure; 2) periphyton community structure on glass slides does not differ from that on PVC panels; 3) periphyton community structure under naturally illuminated conditions does not differ from that under experimentally darkened conditions. The Monte-Carlo permutation tests (999 permutations) were applied to compute significance of hypothetical relations. Permutations in randomised blocks were used for testing of the second and third hypotheses. Algal and cyanobacterial community structure and semiquantitative data were used as predicted values in the model. A mean of three semiquantitative values (values 1 – 7 instead of + – 6, Table 1) from one sample was used. Species present only in one sample were eliminated from the calculations. Substrate types (natural/artificial, glass/PVC, illuminated/darkened) were used as predictors. The floats were applied as blocks (covariables) by testing the hypotheses 2) and 3). Light/dark conditions and glass/PVC panels were used as covariables by testing the hypotheses 2) and 3), respectively. The data from the non-panel parts of the floats were not included in the statistical analyses. All calculations were run using the multivariate data analysis software Canoco for Windows 4.5. The program Canodraw 4.0. (TER BRAAK & ŠMILAUER, 2002) was used for graphical presentation of ordination results. The results were summarised using biplot diagrams. In the biplot diagram, the relative length and position of arrows show the extent and direction of response of algal parameters to the environmental factors.

Pie charts showing the relative occurrence of the most abundant species on various substrates were created using the program Microsoft Office Excel 2003. The relative occurrences (in %) were calculated from the semiquantitative data using the percent mean values of the groups (Table 1).

3. Results

3.1. Species diversity

During the four investigated periods a total of 157 algal and cyanobacterial taxa were recorded at the locality on all sampled substrates. 15 belonged to Cyanobacteria, 1 to Glaucophyta, 55 to Chromophyta, 74 to Chlorophyta, 9 to Euglenophyta and 3 to Dinophyta.

In the autumn (2004), 107 and 63 species were found on artificial and natural substrates, respectively. During the spring (2005) observation, 80 species on artificial and 91 on natural substrates were recorded. In the late summer (2005), 99 species on artificial and 73 on natural substrates were identified. Finally, 11 and 44 species were observed on artificial and natural substrates, respectively, during the winter experiment (2006). A list of the present species for each sampling period and substrate is provided in Appendix 5. Photographs of some of the most frequent species, e.g. *Eunotia bilunaris*, *Fragilaria virescens*, *Gomphonema angustatum*, *Tabellaria flocculosa*, *Scenedesmus quadricauda*, *Zygnema* sp. steril., *Chroococcus aphanocapsoides* etc. are provided in Appendix 3. In Appendix 4 some 'interesting' species, e.g. *Cyanodictyon turfosum*, *Palmodictyon viride*, *Paulinella chromatophora* etc., are shown.

3.2. Periphytic communities on natural vs. artificial substrates

3.2.1. Autumn 2004

An RDA plot (Figure 3) shows significant differences ($p<0.001$) in relative cyanobacterial and algal abundances on natural and artificial substrates. The first axis explained 14.9% of the total variability in the species data. The green algae (Chlorophyta) *Bulbochaete* sp. steril. and *Pediastrum angulosum*, the diatom (Bacillariophyceae) *Eunotia implicata* and the euglenophyte *Trachelomonas* cf. *hispida* (Euglenophyta) inclined considerably to the natural substrates. The artificial substrates seemed to be preferred by the green algae *Monoraphidium contortum*, the diatom *Fragilaria ulna* and the blue-green algae (Cyanobacteria) *Chroococcus* cf. *aphanocapsoides*.

Significant differences ($p<0.001$) can be seen in an RDA plot (Figure 4) describing the relative species occurrences in relation to the substrate type. The whole model explained 25.4% of the total variability in the species data. The first axis explained 16.3% and the second one explained 4.1% of the total variability. The green algae *Oedogonium* sp. steril., *Pleurotaenium ehrenbergii* and *Netrium digitus*, the diatom *Gomphonema acuminatum*, and the blue-green (Cyanobacteria) *Merismopedia elegans* preferred *Equisetum fluviatile*. *Zygnema* sp. steril. (Zygnemophyceae – Chlorophyta), *Trachelomonas* cf. *hispida* and *Eunotia implicata*, inclined markedly to the shore vegetation, whereas the diatom *Anomoeoneis* cf. *vitrea* tended to plankton. The species composition on *Carex* sp. and floating twigs was similar. The green algae *Monoraphidium contortum*, *Scenedesmus* spp. and *Microthamnion* sp., the diatoms *Fragilaria virescens* and *Fragilaria ulna*, and the cyanobacteria *Cyanodictyon turfosum* and *Chroococcus* cf. *aphanocapsoides* seemed to prefer the artificial substrates.

The relative occurrences of the most abundant species on different substrates differed considerably as well, as can be seen in the pie charts (Appendix 6). Epiphyton on *Equisetum fluviatile* composed mainly of green algae (Chlorophyta – shades of green colour), blue-green algae (Cyanobacteria – shades of bluegreen colour) and diatoms (Bacillariophyceae – shades of yellow, orange and brown colours) (Appendix 6a).

The green alga *Bulbochaete* sp. steril. was the dominant species on both *Carex rostrata* and floating twigs. Generally, the species composition on twigs (Appendix 6b) and *Carex* (Appendix 6c) was similar only with slight differences in a few species and the relative occurrences. On twigs, diatoms made up just over 50%. In contrast, green algae slightly prevailed over diatoms on *Carex*.

The filamentous conjugating green alga (Zygnemophyceae – Chlorophyta) *Zygnema* sp. steril. was the dominant species occurring between the submersed shore vegetation (data not shown). As regards plankton, diatoms were the most abundant group. In plankton, diatoms, mainly *Fragilaria virescens* and *Tabellaria flocculosa*, prevailed substantially over green algae, such as *Scenedesmus quadricauda* and *Closterium dianae*, and cyanobacteria, e.g. *Cyanodictyon turfosum* and *Chroococcus* cf. *aphanocapsoides*. *Trachelomonas* cf. *hispida* was an important planktonic species as well (data not shown).

With respect to the artificial substrates (glass+PVC) (Appendix 6d), diatoms were the dominant species. Green algae, blue-greens and dinoflagellates were of a marked importance on artificial substrates as well.

Periphyton on the bamboo sticks composed mainly of diatoms, especially *Tabellaria flocculosa*, and green algae, mainly *Scenedesmus quadricauda* (data not shown). Results from the top/bottom sides of the straw mats are provided along with results from naturally illuminated/experimentally darkened conditions.

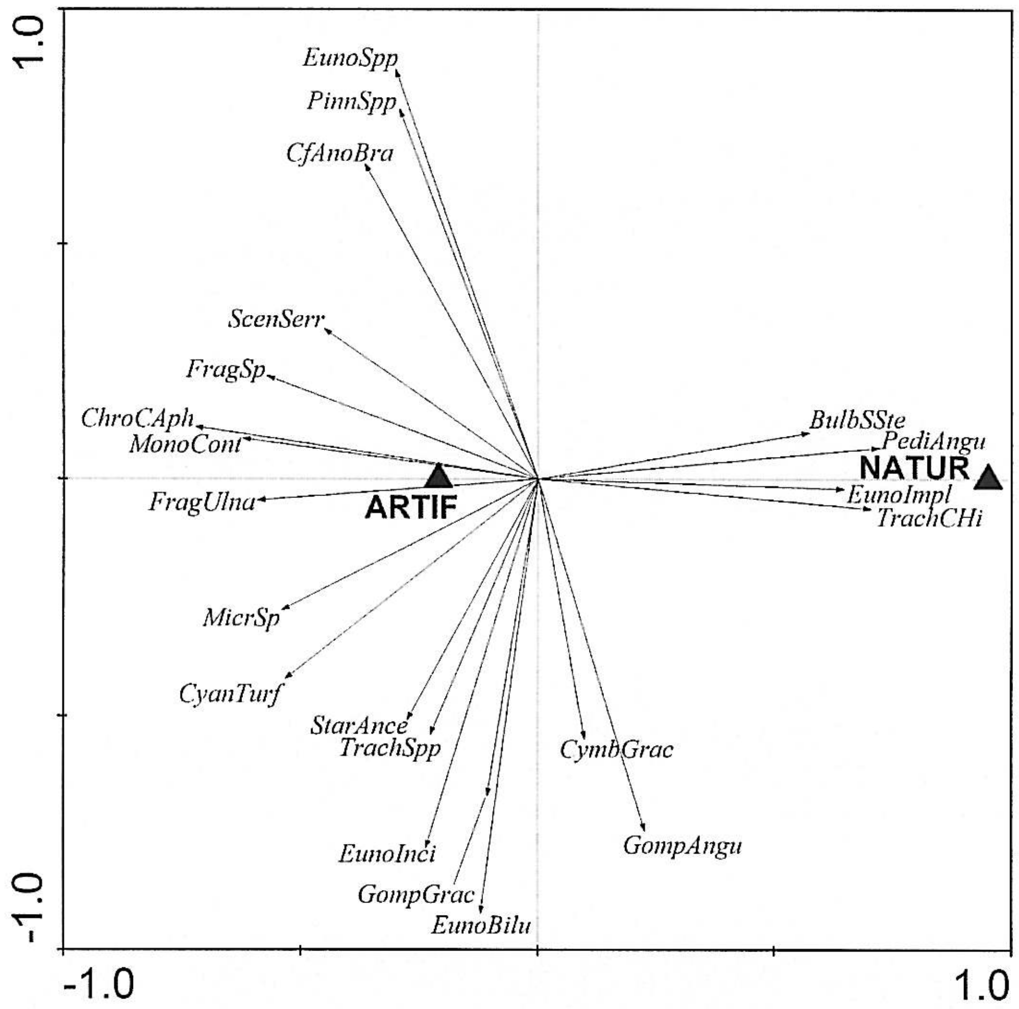

Figure 3. Results of a redundancy analysis (RDA) describing relative occurrences of species on natural and artificial substrates in autumn 2004.

ARTIF=artificial substrates, NATUR=natural substrates, CfAnoBra=cf. *Anomoeoneis brachysira*, BulbSSte=*Bulbochaete* sp. steril., ChroCAph=*Chroococcus* cf. *aphanocapsoides*, CyanTurf=*Cyanodictyon turfosum*, CymbGrac=*Cymbella gracilis*, EunoBilu=*Eunotia bilunaris*, EunoImpl=*Eunotia implicata*, EunoInci=*Eunotia incisa*, EunoSpp=*Eunotia* spp., FragSp=*Fragilaria* sp., FragUlna=*Fragilaria ulna*, GompAngu=*Gomphonema angustatum*, GompGrac=*Gomphonema gracile*, MicrSp=*Microthamnion* sp., MonoCont=*Monoraphidium contortum*, PediAngu=*Pediastrum angulosum*, PinnSpp=*Pinnularia* spp., ScenSerr=*Scenedesmus serratus*, StarAnce=*Stauroneis anceps*, TrachCHi=*Trachelomonas* cf. *hispida*, TrachSpp=*Trachelomonas* spp.

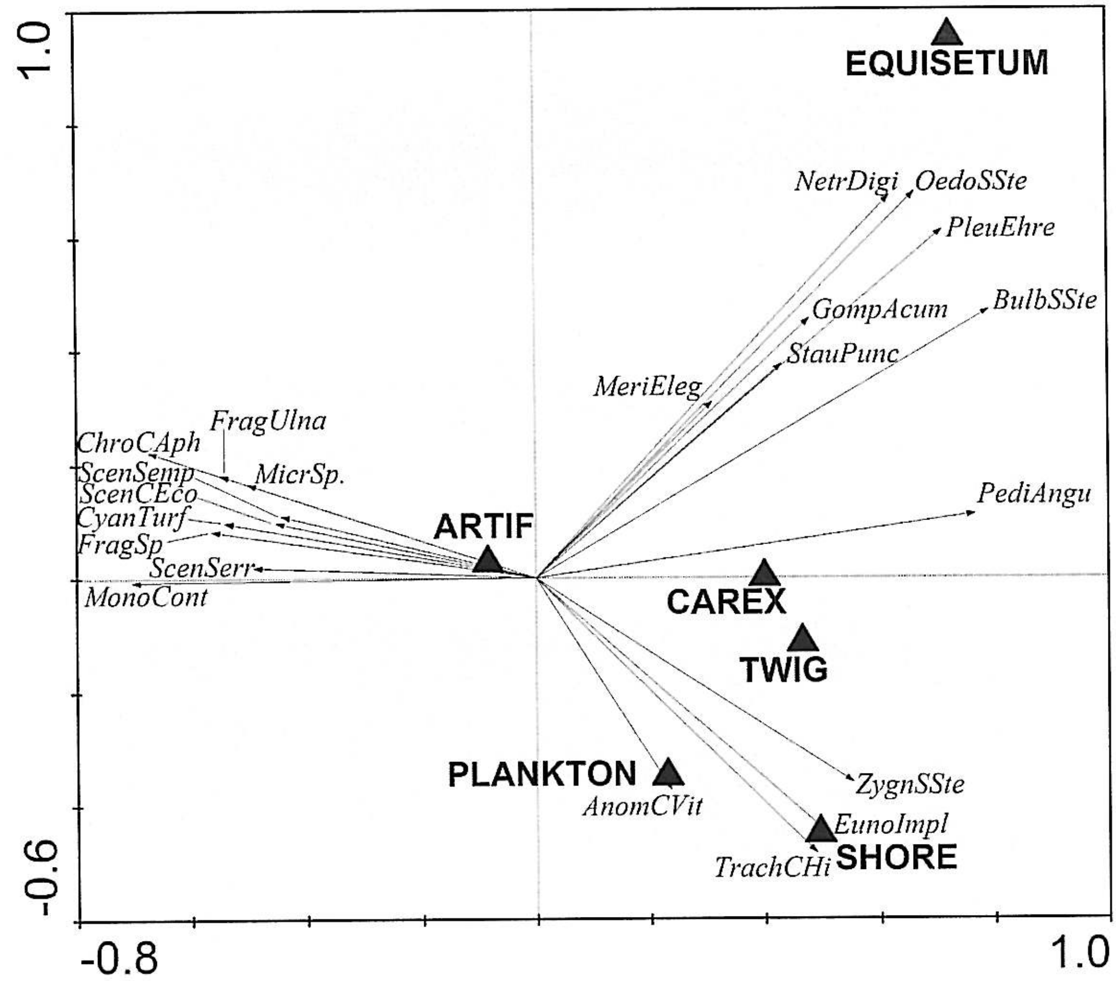

Figure 4. Results of a redundancy analysis (RDA) describing relative occurrences of species in relation to substrate types in autumn 2004.

ARTIF=artificial substrates, EQUISETUM=*Equisetum fluviatile*, SHORE=periphyton occurring between submersed shore vegetation; CAREX=*Carex rostrata*, AnomCVit=*Anomoeoneis* cf. *vitrea*, BulbSSte=*Bulbochaete* sp. steril., ChroCAph=*Chroococcus* cf. *aphanocapsoides*, CyanTurf=*Cyanodictyon turfosum*, EunoImpl=*Eunotia implicata*, FragSp=*Fragilaria* sp., FragUlna=*Fragilaria ulna*, GompAcum=*Gomphonema acuminatum*, MeriEleg=*Merismopedia elegans*, MicrSp=*Microthamnion* sp., MonoCont=*Monoraphidium contortum*, NetrDigi=*Netrium digitus*, OedoSSte=*Oedogonium* sp. steril., PediAngu=*Pediastrum angulosum*, PleuEhre=*Pleurotaenium ehrenbergii*, ScenCEco=*Scenedesmus* cf. *ecornis*, ScenSemp=*Scenedesmus sempervirens*, ScenSerr=*Scenedesmus serratus*, StauPunc=*Staurastrum punctulatum*, TrachCHi=*Trachelomonas* cf. *hispida*, ZygnSSte=*Zygnema* sp, steril.

3.2.2. Spring 2005

An RDA plot (Figure 5) describes significant differences ($p<0.001$) in species occurring on natural and artificial substrates (only PVC). The canonical axis (axis 1) explained 19.1% of the total variability in the species data. The diatom *Tabellaria fenestrata* and the green algae

Ankistrodesmus sp., *Pediastrum angulosum* etc. preferred the natural substrates, whereas the green alga *Microthamnion* sp. tended to the artificial substrates.

Significant preferences of species to various substrate types ($p<0.001$) are illustrated in an RDA plot (Figure 6). The whole model explained 47.7% of the total variability. The first axis explained 20.1% and the second axis explained 10.6% of the total variability. Small differences in the species composition occurred between viable and dead horsetails *Equisetum fluviatile*. *Bulbochaete* sp. steril., *Pleurotaenium ehrenbergii*, *Staurastrum punctulatum* etc. preferred the horsetails. *Pinnularia* cf. *interrupta* inclined to plankton, whereas *Arthrodesmus octocornis* tended to bryophytes. Artificial substrates (PVC) were preferred by *Microthamnion* sp.

According to the pie chart of the relative occurrences, diatom species were the dominants on viable *Equisetum fluviatile*, followed by green and blue-green algae (Appendix 7a). Although diatoms were predominant on dead floating *Equisetum fluviatile*, green algae were an important group as well (Appendix 7b). As for both bryophytes and assemblages between submersed shore vegetation, diatoms were the prevailing species (data not shown).

Diatoms, especially *Fragilaria virescens*, predominated in plankton (Appendix 7c). Green algae and dinoflagellates were of a great importance in plankton as well. Diatoms and green algae prevailed on artificial substrates (only PVC panels) (Appendix 7d). With respect to periphyton on bamboo sticks, diatoms were the most prevailing group (data not shown).

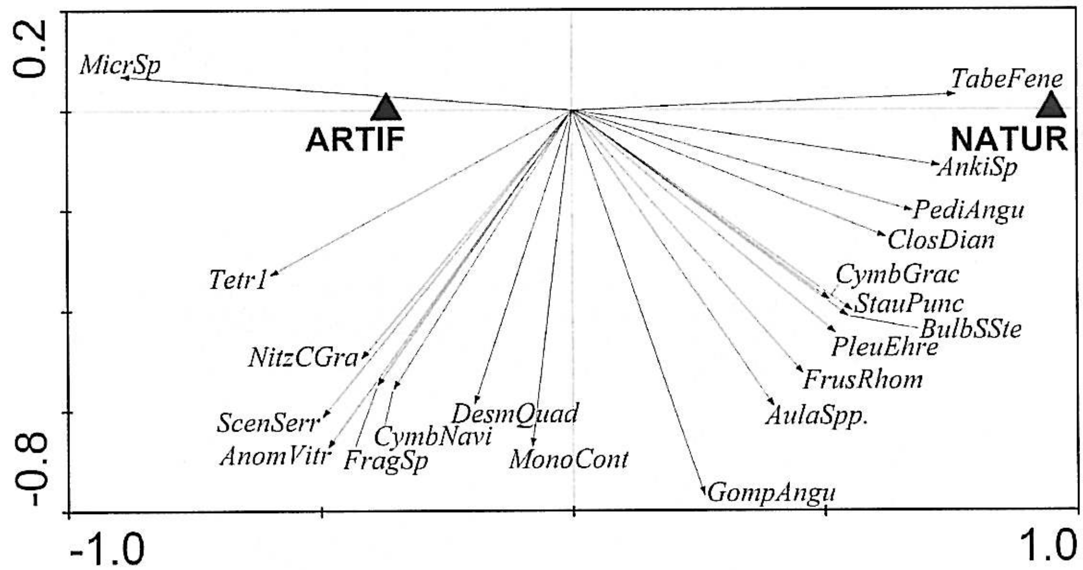

Figure 5. Results of a redundancy analysis (RDA) describing relative occurrences of species on natural and artificial substrates in spring 2005.

ARTIF=artificial substrates (PVC), NATUR=natural substrates, AnkiSp=*Ankistrodesmus* sp., AnomVitr=*Anomoeoneis vitrea*, AulaSpp=*Aulacoseira* spp., BulbSSte=*Bulbochaete* sp. steril., ClosDian=*Closterium dianae*, CymbGrac=*Cymbella gracilis*, CymbNavi=*Cymbella naviculiformis*, DesmQuad=*Scenedesmus quadricauda*, FragSp=*Fragilaria* sp., FrusRhom=*Frustulia rhomboides*, GompAngu=*Gomphonema angustatum*, MicrSp=*Microthamnion* sp., MonoCont=*Monoraphidium contortum*, NitzCGra=*Nitzschia* cf. *gracilis*, PediAngu=*Pediastrum angulosum*, PleuEhre=*Pleurotaenium ehrenbergii*, ScenSerr=*Scenedesmus serratus*, StauPunc=*Staurastrum punctulatum*, TabeFene=*Tabellaria fenestrata*, Tetr1=Tetrasporal 1

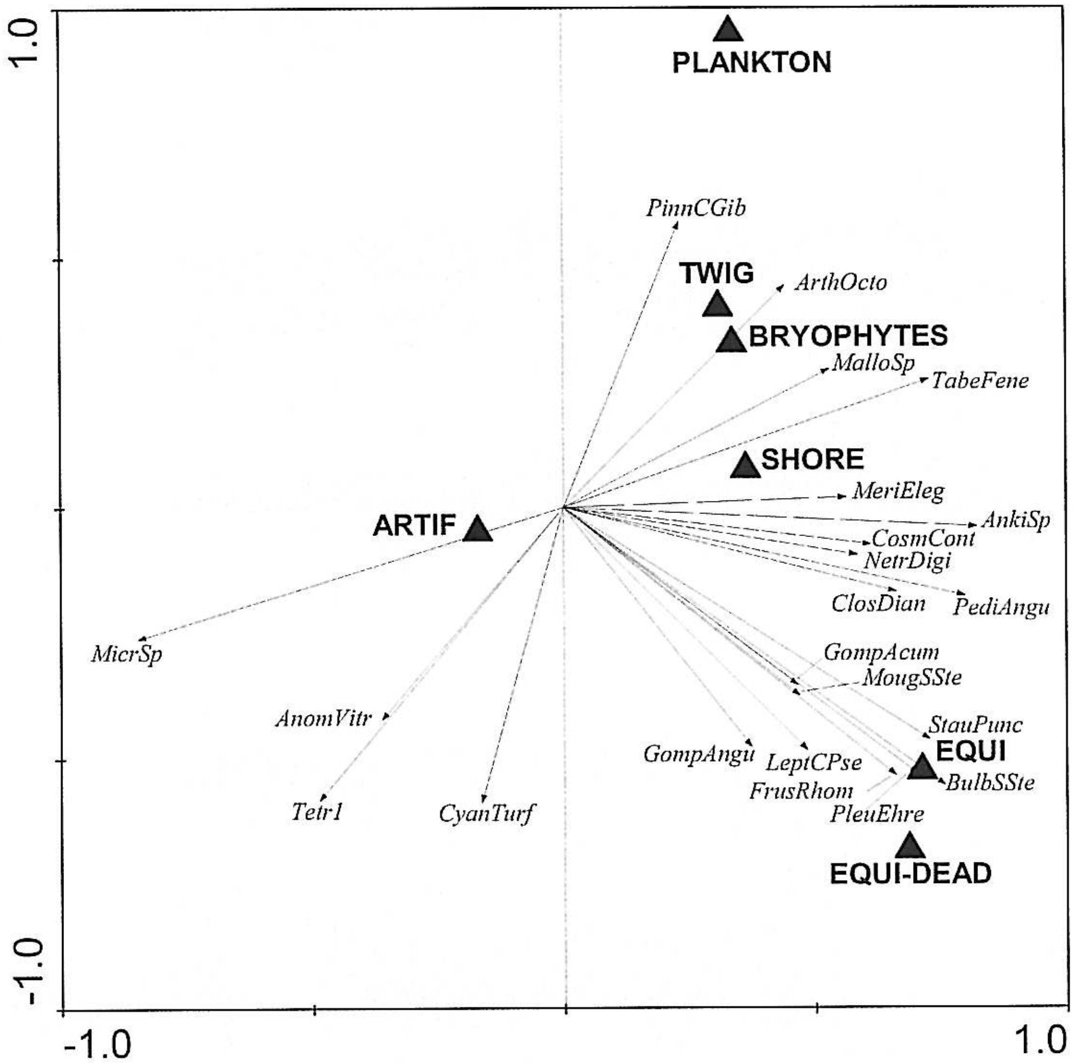

Figure 6. Results of a redundancy analysis (RDA) describing relative occurrences of species in relation to substrate types in spring 2005.

ARTIF=artificial substrates (PVC), EQIU =*Equisetum fluviatile*, EQUI-DEAD=dead floating *Equisetum fluviatile*, SHORE=periphyton occurring between submersed shore vegetation, AnkiSp=*Ankistrodesmus* sp., AnomVitr=*Anomoeoneis vitrea*, ArthOcto=*Arthrodesmus octocornis*, BulbSSte=*Bulbochaete* sp. steril., ClosDian=*Closterium dianae*, CosmCont=*Cosmarium contractum*, CyanTurf=*Cyanodictyon turfosum*, FrusRhom=*Frustulia rhomboides*, GompAcum=*Gomphonema acuminatum*, GompAngu=*Gomphonema angustatum*, LeptCPse=*Leptolyngbya* cf. *psedovaleriana*, MalloSp=*Mallomonas* sp., MeriEleg=*Merismopedia elegans*, MicrSp=*Microthamnion* sp., MougSSte=*Mougeotia* sp. steril., NetrDigi=*Netrium* *digitus*, PediAngu=*Pediastrum angulosum*, PinnCGib=*Pinnularia* cf. *gibba*, PleuEhre=*Pleurotaenium ehrenbergii*, StauPunc=*Staurastrum punctulatum*, TabeFene=*Tabellaria fenestrata*, Tetr1=Tetrasporal 1

3.2.3. Late summer 2005

Significant preferences of species to natural or artificial substrates ($p<0.001$) are illustrated in an RDA plot (Figure 7). The first axis explained 14.9% of the total variability in the species data. *Chlamydomonas* sp. (green algae) inclined to the natural substrates and *Oedogonium* sp. steril. (green algae) to the artificial ones.

An RDA plot (Figure 8) describes significant differences ($p<0.001$) in the species relative occurrences in relation to the types of substrates. The whole model explained 20.6% of the total variability. The first axis explained 12.8% and the second axis explained 4.8% of the total variability. *Bulbochaete* sp. steril. preferred *Equisetum fluviatile*, whereas *Crucigeniella pulchra*, *Crucigenia tetrapedia*, cf. *Diplochloris* etc., inclined to plankton, being their natural community. Evidently, communities growing on artificial substrates were dissimilar to the naturally occurring communities.

Relative occurrences of the most abundant species on various substrates differed considerably as well. Epiphyton on horsetails *Equisetum fluviatile* (Appendix 8a) was predominated by diatoms, mainly *Gomphonema angustatum*. However, the green alga *Bulbochaete* sp. steril. was the second most abundant species on horsetails in this period.

Cyanobacteria, and *green algae* were the prevailing groups in plankton (Appendix 8b). On artificial substrates (glass+PVC) (Appendix 8c) periphyton composed mainly of diatoms, green algae and cyanobacteria.

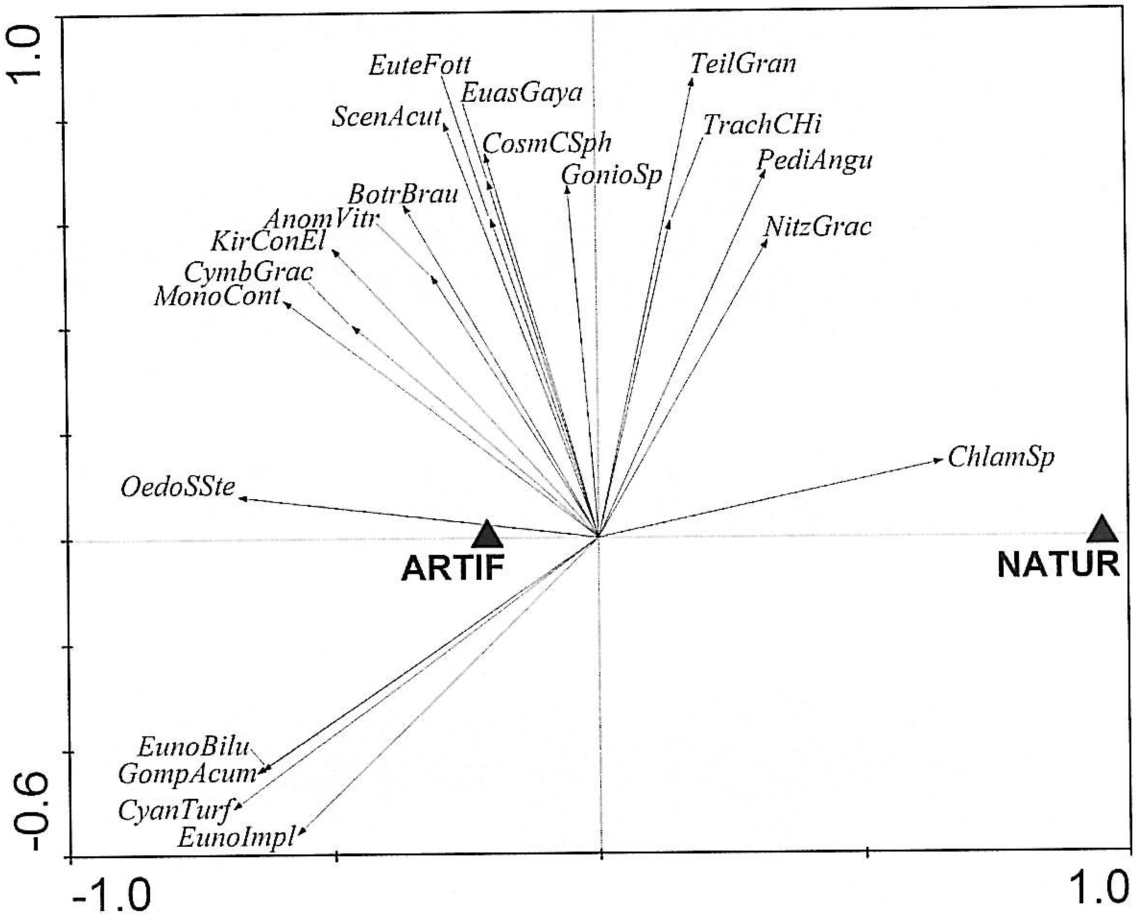

Figure 7. Results of a redundancy analysis (RDA) describing relative occurrences of species on natural and artificial substrates in late summer 2005.

ARTIF=artificial substrates, NATUR=natural substrates, AnomVitr=*Anomoeoneis vitrea*, BotrBrau=*Botryococcus braunii*, ChlamSp=*Chlamydomonas* sp., CosmCSph=*Cosmarium* cf. *sphagnicolum*, CyanTurf=*Cyanodictyon turfosum*, CymbGrac=*Cymbella gracilis*, EuasGaya=*Euastrum gayanum*, EunoBilu=*Eunotia bilunaris*, EunoImpl=*Eunotia implicata*, EuteFott=*Eutetramorus fottii*, GompAcum=*Gomphonema acuminatum*, GonioSp=*Goniochloris* sp., KirConEl=*Kirchneriella contorta* var. *elegans*, MonoCont=*Monoraphidium contortum*, NitzGrac=*Nitzschia gracilis*, OedoSSte=*Oedogonium* sp. steril., PediAngu=*Pediastrum angulosum*, ScenAcut=*Scenedesmus acutus*, TeilGran=*Teilingia granulata*, TrachCHi=*Trachelomonas* cf. *hispida*

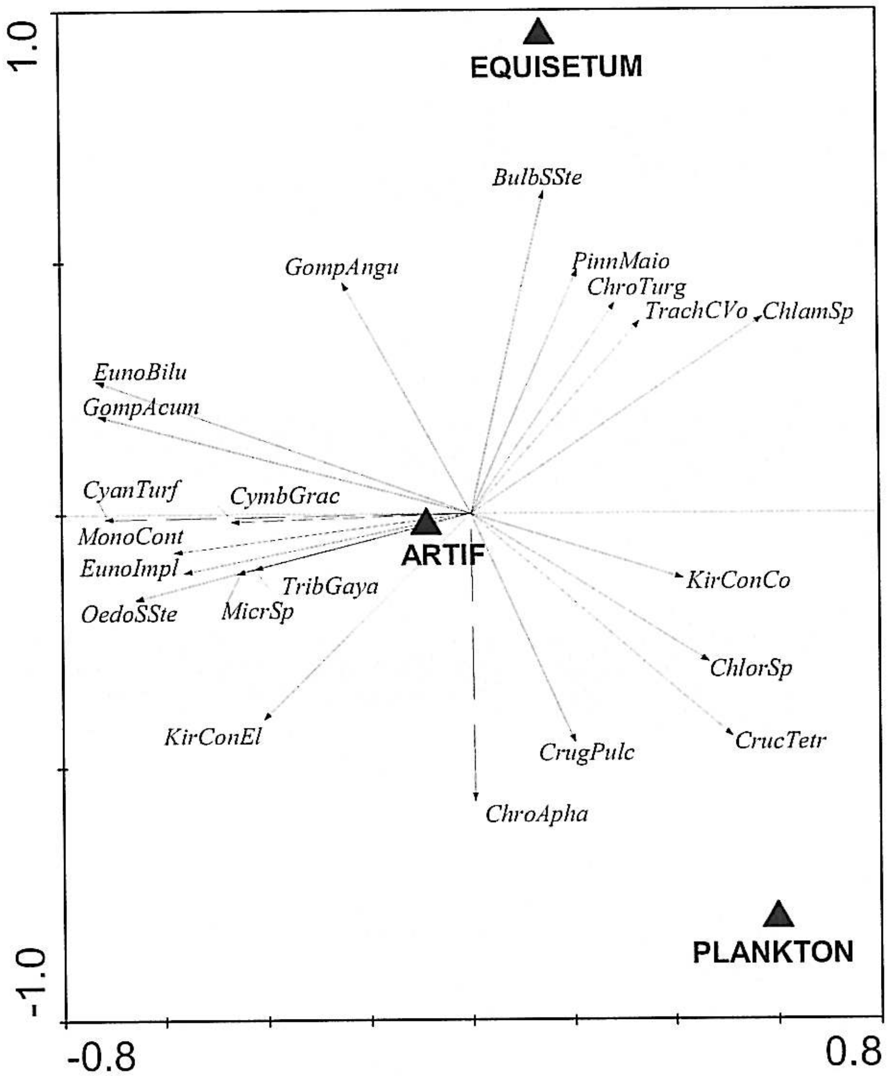

Figure 8. Results of a redundancy analysis (RDA) describing relative occurrences of species in relation to substrate types in late summer 2005.

ARTIF=artificial substrates, EQUISETUM=*Equisetum fluviatile*, BulbSSte=*Bulbochaete* sp. steril., ChlamSp=*Chlamydomonas* sp., ChlorSp=cf. *Diplochloris*, ChroApha=*Chroococcus aphanocapsoides*, ChroTurg=*Chroococcus turgidus*, CrucTetr=*Crucigenia tetrapedia*, CrugPulc=*Crucugeniella pulchra*, CyanTurf=*Cyanodictyon turfosum*, CymbGrac=*Cymbella gracilis*, EunoBilu=*Eunotia bilunaris*, EunoImpl=*Eunotia implicata*, GompAcum=*Gomphonema acuminatum*, GompAngu=*Gomphonema angustatum*, KirConCo=*Kirchneriella contorta* var. *contorta*, KirConEl=*Kirchneriella contorta* var. *elegans*, MicrSp=*Microthamnion* sp., MonoCont=*Monoraphidium contortum*, OedoSSte=*Oedogonium* sp. steril., PinnMaio=*Pinnularia maior*, TrachCVo=*Trachelomonas* cf. *volvocina*, TribGaya=*Tribonema gayanum*

3.2.4. Winter 2006

Since it was only possible to retrieve 3 glass slides and 1 PVC panel from beneath the ice cover because of a double layer structure of the ice, the artificial substrates were not included in the statistical analyses. However, the relative species composition was examined. Surprisingly, numerous living algal and cyanobacterial species were found under the ice cover (Appendix 5). The assemblages on the bottom side of the ice cover composed of diatoms, green algae and the dinoflagellate (Dinophyta) *Cystodinium cornifax* (Appendix 8d). *Synura* sp., *Euglena acus* and *Aulacoseira* sp. were the most abundant planktonic species found in the middle of the pond, whereas the plankton sampled in the littoral zone composed of species almost identical to those which prevailed on the bottom side of the ice cover. Epiphytic species on *Equisetum fluviatile* resembled those on the ice cover as well, but *Eunotia incisa* was the dominant epiphytic species.

3.3. Periphytic communities on glass slides vs. plastic panels (PVC)

3.3.1. Autumn 2004

Significant preferences of the species to either of the two types of artificial substrates (p=0.04) can be seen in the RDA biplot (Figure 9). The substrate type explained only 3.5% of the total variability in the species data. Most of the species seemed to prefer PVC panels to glass slides, e.g. *Pinnularia interrupta* and *Pediastrum boryanum*.

Only small differences in the composition of the most abundant species on glass vs. PVC panels were observed. On both substrates, diatoms, especially *Fragilaria virescens*, were the prevailing group over green algae, e.g. *Monoraphidium contortum* and *Scenedesmus* spp., blue-greens, *Cyanodictyon turfosum* and *Chroococcus aphanocapsoides*, and the dinoflagellates, mainly *Peridinium umbonatum* (data not shown).

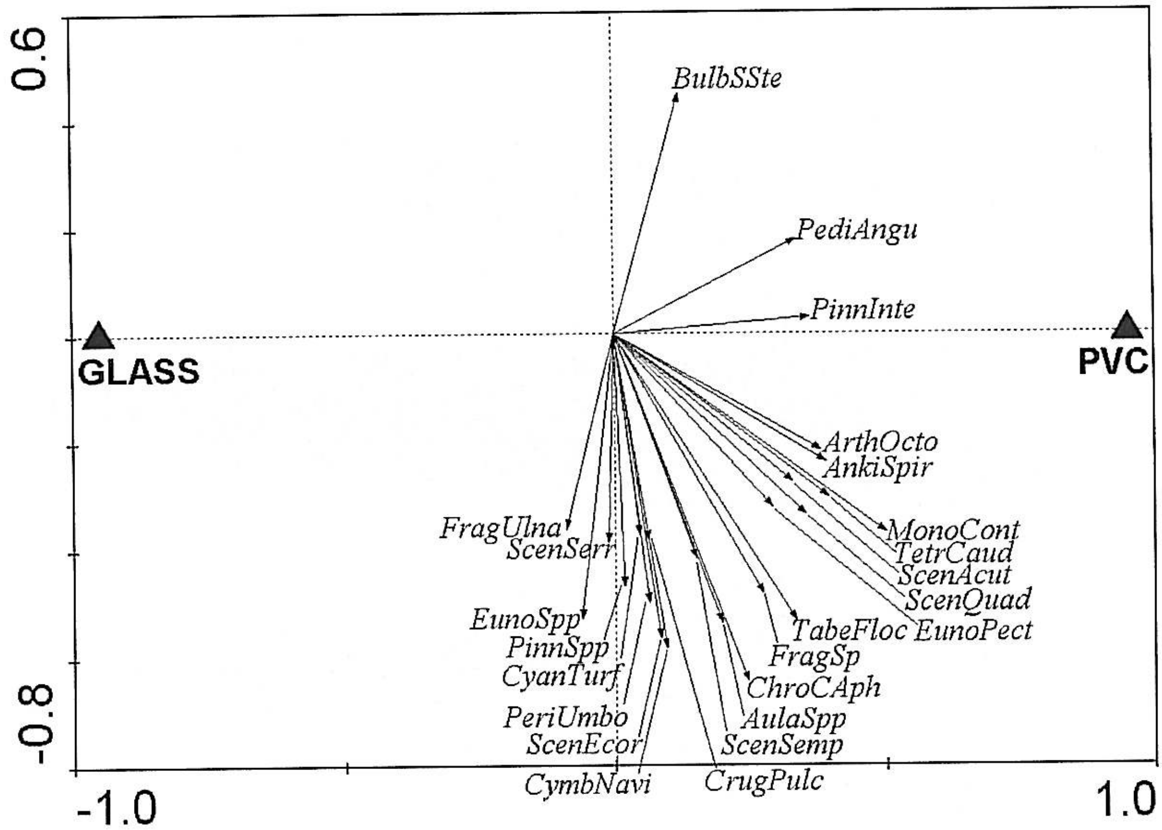

Figure 9. Results of a redundancy analysis (RDA) describing relative occurrences of species in relation to the artificial substrate used in autumn 2004.

GLASS=glass slides, PVC=PVC panels; AnkiSpir=*Ankistrodesmus spiralis*, ArthOcto=*Arthrodesmus octocornis*, AulaSpp=*Aulacoseira* spp., BulbSSte=*Bulbochaete* sp. steril., ChroCAph=*Chroococcus* cf. *aphanocapsoides*, CrugPulc=*Crucugeniella pulchra*, CyanTurf=*Cyanodictyon turfosum*, CymbNavi=*Cymbella naviculiformis*, ScenQuad=*Scenedesmus quadricauda*, EunoPect=*Eunotia pectinalis*, EunoSpp=*Eunotia* spp., FragSp=*Fragilaria* sp., FragUlna=*Fragilaria ulna*, MonoCont=*Monoraphidium contortum*, PediAngu=*Pediastrum angulosum*, PeriUmbo=*Peridinium umbonatum*, PinnInte=*Pinnularia interrupta*, PinnSpp=*Pinnularia* spp., ScenAcut=*Scenedesmus acutus*, ScenCEco=*Scenedesmus* cf. *ecornis*, ScenSemp=*Scenedesmus sempervirens*, ScenSerr=*Scenedesmus serratus*, TabeFloc=*Tabellaria flocculosa*, TetrCaud=*Tetraedron caudatum*

3.3.2. Spring 2005

As mentioned above, all the glass slides got unglued and were lost during the spring experiment. Due to this fact, comparative studies of periphyton growing on PVC and glass panels could not be performed.

3.3.3. Late summer 2005

No significant differences were found in the relative abundances of species in relation to the glass vs. PVC panels (p=0.106). The substrate type explained 2.6% of the total variability in the species data. Nevertheless, some species seemed to prefer either glass or PVC panels, especially *Microthamnion* sp. apparently inclined to glass slides (data not shown).

The composition of the most abundant species on glass slides and PVC panels was almost the same with only minimal differences in the percentage occurrences. Diatoms made up the greatest proportion of species, followed by green algae and blue-green algae. *Eunotia bilunaris*, *Fragilaria virescens* and *Tabellaria flocculosa* were the most frequent diatom species in this period, while *Eutetramorus fottii* and *Scenedesmus quadricauda* were the most abundant green algae. *Cyanodictyon turfosum* and *Chroococcus aphanocapsoides* were the dominant species of cyanobacteria (data not shown).

3.3.4. Winter 2006

As mentioned above, the winter data were not tested by statistical analyses. In addition, attached algae/cyanobacteria were scarce on the artificial substrates and both glass and PVC panels were dominated by heterotrophic bacteria.

3.4. Periphytic communities under naturally illuminated vs. experimentally darkened conditions

3.4.1. Autumn 2004

Because the straw mat on one of the floats (middle of the pond) flipped over, the autumn data from this float were not used for the analyses. An RDA biplot (Figure 10) shows a significant preference of some species to light or dark conditions (p=0.003). The irradiance explained 5% of the total variability in the species data. Green algae such as *Euastrum gayanum*, *Kirchneriella contorta* var. *elegans*, *Ankistrodesmus* sp. etc. tended considerably to the naturally illuminated conditions, whereas the displayed diatom species seemed to be indifferent to irradiance.

A few differences can be distinguished between the relative species occurrences under illuminated and darkened conditions (data not shown). Higher relative abundances of diatom, blue-green and dinoflagellate species were observed under the darkened conditions compared to the naturally illuminated conditions. Although diatoms were prevailing under the illuminated conditions as well, green algae were also of a great importance. On straw mats, considerable differences could be found in the species composition on the top sides compared to the bottom sides (Appendix 9a,b). Diatoms prevailed on the bottom sides of the mats, whereas on the top sides green algae were as abundant as diatoms. *Scenedesmus quadricauda* was the dominant species on the top sides.

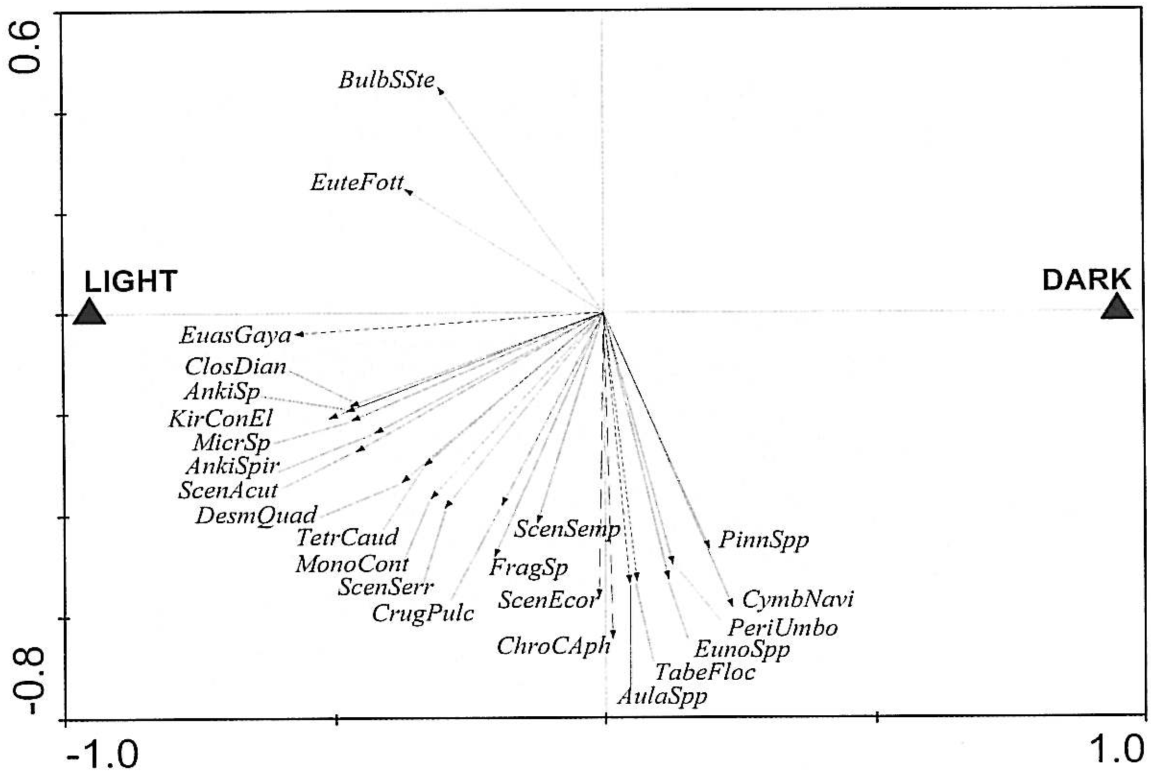

Figure 10. Results of a redundancy analysis (RDA) describing relative occurrences of species in relation to the light conditions in autumn 2004.

LIGHT=naturally illuminated conditions, DARK=experimentally darkened conditions, AnkiSp=*Ankistrodesmus* sp., AnkiSpir=*Ankistrodesmus spiralis*, AulaSpp=*Aulacoseira* spp., BulbSSte=*Bulbochaete* sp. steril., ChroCAph=*Chroococcus* cf. *aphanocapsoides*, ClosDian=*Closterium dianae*, CrugPulc=*Crucugeniella pulchra*, CymbNavi=*Cymbella naviculiformis*, DesmQuad=*Scenedesmus quadricauda*, EuasGaya=*Euastrum gayanum*, EunoSpp=*Eunotia* spp., EuteFott=*Eutetramorus fottii*, FragSp=*Fragilaria* sp., KirConEl=*Kirchneriella contorta* var. *elegans*, MicrSp=*Microthamnion* sp., MonoCont=*Monoraphidium contortum*, PeriUmbo=*Peridinium umbonatum*, PinnSpp=*Pinnularia* spp., ScenAcut=*Scenedesmus acutus*, ScenCEco=*Scenedesmus* cf. *ecornis*, ScenSemp=*Scenedesmus sempervirens*, ScenSerr=*Scenedesmus serratus*, TabeFloc=*Tabellaria flocculosa*, TetrCaud=*Tetraedron caudatum*

3.4.2. Spring 2005

The relative abundances of species showed significant differences in the distribution under naturally illuminated and experimentally darkened conditions (p=0.044) (data not shown). The irradiance explained 10.3% of the total variability in the species data. The occurrence of diatom species, such as *Aulacoseira* spp., *Gomphonema acuminatum* and *Stauroneis anceps*, was positively correlated with the darkened conditions.

Relative occurrences of the most abundant species under naturally illuminated and experimentally darkened conditions differed as well (data not shown). Diatoms were the dominant group under both conditions, however, the composition of the most abundant species and their proportions were different. Diatoms made up a greater proportion of the most abundant species under darkened conditions compared to illuminated ones. In contrast relative abundances of green algae were somewhat higher under illuminated conditions.

3.4.3. Late summer 2005

Significant preferences of some species to light or dark conditions ($p<0.001$) can be seen in the RDA biplot (Figure 11). The irradiance explained 24.7% of the total variability in the species data. The diatoms *Eunotia implicata*, *Eunotia bilunaris*, *Gomphonema acuminatum* and the blue-green alga *Cyanodictyon turfosum* evidently preferred the darkened conditions, whereas the green algae *Kirchneriella contorta* var. *elegans*, *Closterium dianae*, *Teilingia granulata*, *Botryococcus braunii* etc. tended markedly to the illuminated conditions.

It can be clearly seen that the most abundant species differed under light and dark conditions (Appendices 9c,d). Diatom species prevailed under the darkened conditions. The relative abundance of the blue-green alga *Cyanodictyon turfosum* was higher under the darkened conditions as well. The relative occurrence of green algae was markedly lower under the darkened conditions in comparison with the illuminated conditions Small differences in the relative occurrences of the most abundant species on the top vs. bottom sides of the straw mats were recorded (data not shown). Diatoms were predominant on both sides, however, they reached a higher relative abundance under the darkened conditions.

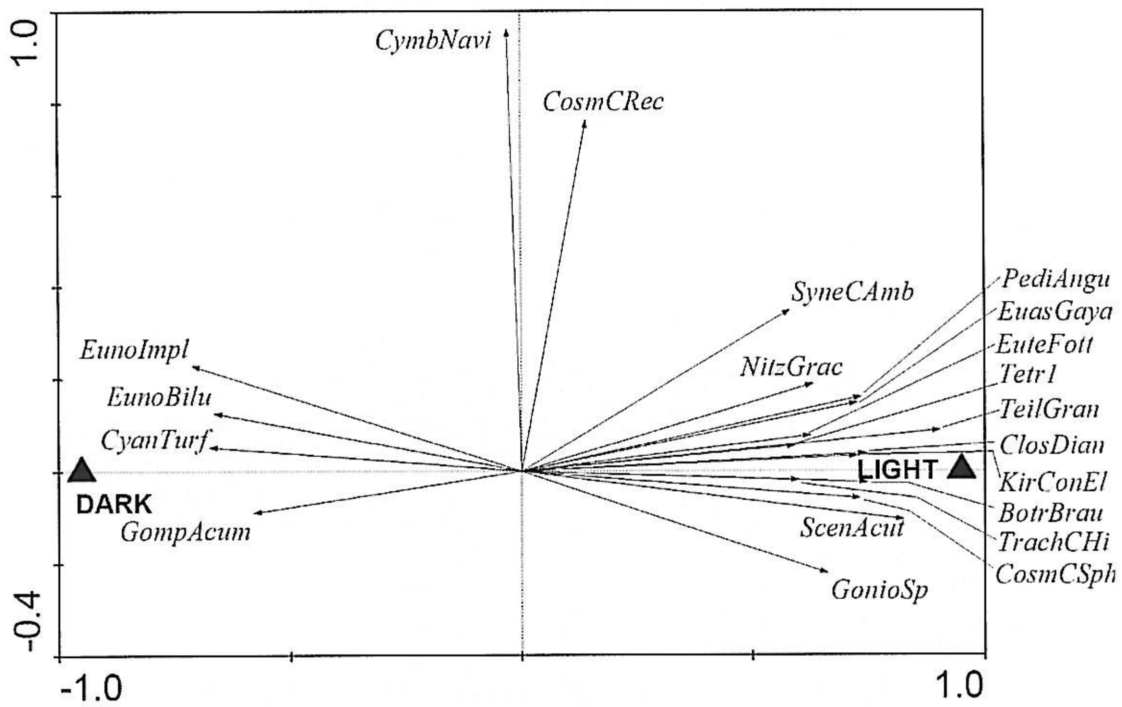

Figure 11. Results of a redundancy analysis (RDA) describing relative occurrences of species in relation to the light conditions in late summer 2005.

Glossary to the RDA figures 10 and 11: LIGHT=naturally illuminated conditions, DARK=experimentally darkened conditions, BotrBrau=*Botryococcus braunii*, ClosDian=*Closterium dianae*, CosmCRec=*Cosmarium* cf. *rectangulare*, CosmCSph=*Cosmarium* cf. *sphagnicolum*, CyanTurf=*Cyanodictyon turfosum*, CymbNavi=*Cymbella naviculiformis*, EuasGaya=*Euastrum gayanum*, EunoBilu=*Eunotia bilunaris*, EunoImpl=*Eunotia implicata*, EuteFott=*Eutetramorus fottii*, GompAcum=*Gomphonema acuminatum*, GonioSp=*Goniochloris* sp., KirConEl=*Kirchneriella contorta* var. *elegans*, NItzGrac=*Nitzschia gracilis*, PediAngu=*Pediastrum angulosum*, ScenAcut=*Scenedesmus acutus*, SyneCAmb=*Synechococcus* cf. *ambiguus*, TeilGran=*Teilingia granulata*, Tetr1=Tetrasporal 1, TrachCHi=*Trachelomonas* cf. *hispida*

4. Discussion

4.1. Species diversity

Algal and cyanobacterial communities in the pond Huntov were rather rich in terms of species diversity (over 150 species in total, Appendix 5). The most abundant groups were diatoms, green algae and cyanobacteria, which is in a good agreement with general knowledge about periphyton assemblages in inland waters (KALFF, 2002).

In both autumn 2004 and late summer 2005, more species were recorded on natural substrates compared to artificial ones, whereas in both spring 2005 and winter 2006 contrary was the case. This difference might be attributed to an unequal availability of natural substrates, along with a lower number of examined artificial substrates in spring and winter seasons.

4.2. Comparison of periphyton on natural and artificial substrates

Results of studies dealing with periphyton differ as regards the similarity of attached species on natural and artificial substrates. CATTANEO & KALFF (1979) and LANE et al. (2003) concluded that there was a good agreement in periphyton composition on artificial and natural substrates. In contrast, BURKHOLDER & WETZEL (1989), SCHAGERL & DONABAUM (1998) and BARBIERO (2000) reported that the composition of periphyton on artificial substrates provided a poor representation of the communities on natural substrates. The results of the present study support the latter conclusion.

Overall, no particular algal group preferred either natural or artificial substrates. This is an important conclusion, because particular algal groups use various means of attachment which may be differently efficient on relatively smooth surfaces, such as those of glass and PVC panels. SEKAR et al. (2004) studied adhesion of *Chlorella vulgaris* (Chlorophyceae), *Nitzschia amphibia* (Bacillariophyceae) and *Chroococcus minutus* (Cyanobacteria) to various substrates (glass, Perspex, titanium etc.). They proved that the attachment was highest in *N. amphibia*, followed by *C. minutus* and *C. vulgaris*. They concluded that the variation of attachment may have been related to attachment mechanisms and their differential ability to produce extracellular polymeric substances (EPSs). WOODS & FLETCHER (1991), while studying the attachment strength and adhesion of four marine diatoms, found out that the attachment rate varied among different species. In the present study, preferences of some species to natural or artificial substrates were recorded. The green alga *Pediastrum angulosum* inclined considerably to the natural substrates, whereas the green alga *Microthamnion* sp.

tended markedly to the artificial substrates. Thus, the ability to attach solid surfaces is probably not a characteristic of a whole group but of a single species.

Generally, diatoms were the dominant group on almost all examined substrates with only a few exceptions. Firstly, green algae were the most abundant group on *Equisetum fluviatile* and *Carex rostrata* in the autumn 2004, with the predominating species *Bulbochaete* sp. steril. In addition, the overall preference of *Bulbochaete* sp. steril. to *Equisetum fluviatile* can be clearly seen in the RDA plots (Figures 4, 6, 8). *Bulbochaete* is a filamentous green alga which attach to substrates by means of holdfast-like rhizoids (WEHR & SHEATH, 2003). It is possible that once attached, *Bulbochaete* overgrows the smaller species and thereby shades them. Lower irradiance may reduce the numbers of the smaller epiphytic species and the filamentous alga becomes the dominant. Only those species which are able to grow on the filaments of *Bulbochaete* or to adapt themselves to lower irradiance between the filaments might survive (e.g. diatoms, desmids). This theory is supported by the findings of PILLSBURY & LOWE (1999) who observed filamentous green algal dominance under higher light conditions, while diatoms and desmids dominated under lower light conditions in acidic lakes.

In contrast, *Bulbochaete* was found scarcely on the artificial substrates, which is in accord with the findings of GODWARD (1934, in BROWN 1976). He observed that algae with a branched, filamentous habit were rather rare on glass slides as compared with nearby vegetation in English ponds. It is probably difficult for some green algae, such as *Bulbochaete*, to form an initial colonisation stage and/or to remain attached to smooth surfaces (e.g. glass and PVC panels). On the other hand, the horsetail *Equisetum fluviatile* has a relatively smooth surface as well, thus the roughness of the substrates may not have been the crucial factor. The colonisation patterns on the horsetail also might have been affected by the production of biologically active substances by the plant. Much evidence exists about aquatic plants releasing such chemicals, which influence algal growth. Chemical structures and the effects of the substances on algae are various (e.g. nutrient enrichment, allelopathy). There are many reports about the transfer of nutrients from macrophytes to their epiphytes (HARLIN, 1973; MCROY & GOERING, 1974). PIP & STEWART (1976 in JONES et al., 2000) proposed that plants possibly release carbohydrates, which are subsequently taken up by periphyton. This could give an advantage to directly attached species (e.g. *Bulbochaete*) over the species which are not in direct contact with the plant. It seems that there could be a relation between viable *Equisetum fluviatile* and its epiphytic *Bulbochaete*, because the relative occurrence of the latter was much lower on dead floating *Equisetum* compared to the living one (Appendices 7 a,b). Allelopathy is another mechanism by which the plants might influence their periphyton.

GROSS et al. (1996) proved that extracts of *Myriophyllum spicatum* exhibited a strong inhibitory action against various coccoid and filamentous cyanobacteria and to a less extent against chlorophytes and diatoms. In the present study, green algae and diatoms prevailed over coccoid and filamentous cyanobacteria, which could have been caused by stronger allelopathy of the macrophytes against prokaryotic species. However, algicidal and cyanobactericidal substances have not been recorded in *Equisetum fluviatile* to date.

Secondly, *Zygnema* sp. steril. was the prevailing species between the submersed shore vegetation in the autumn 2004. *Zygnema* is a filamentous conjugating green alga (Zygnematales) mainly occurring in clumps entangled among macrophytes or trapped near shore. Filamentous species, such as *Zygnema*, often form 'monocultures' and are scarcely overgrown by smaller epiphytic algae unlike other filamentous green algae, such as *Bulbochaete* and *Oedogonium* (Chlorophyceae). Cell walls of the filamentous Zygnematales are covered by smooth mucilaginous layer, which makes the filamentous forms slippery to touch (VAN DEN HOOK, 1995). Probably, the slime layer on the surface of the filaments could inhibit the attachment of smaller epiphytic species.

Lastly, in the late summer 2005, the blue-green alga *Chroococcus aphanocapsoides* was the most abundant species in plankton. This was the only case when cyanobacteria were the prevailing group. Otherwise, diatoms prevailed substantially in all the investigated communities. Diatoms (Bacillariophyceae) are able to occupy a variety of habitats and are often the most abundant photosynthetic organisms in marine and fresh waters. They are able to exist in sessile forms by production of EPSs in the form of stalks, tubes, apical pads, adhering films, fibrils and cell coatings (HOAGLAND et al., 1993). DRUM (1969, in HOAGLAND et al., 1993) pointed out that polysaccharide secretion by many diatoms is a crucial part of their biological success.

Surprising was the finding of viable periphyton on the bottom side of the ice cover. The algal and cyanobacterial assemblages (about 2×2 cm) were firmly attached, and they had to be scraped from the ice surface by scissors. This proves that the assemblages could not have been attached to the ice cover just by chance during the removal of the ice blocks from the water.

Occurrence of algae in and under the ice cover as well as at the ice-water interface is a well-known phenomenon from seas and large freshwater reservoirs (HEGSETH, 1997; KÜHL et al., 2001; WATSON et al., 2001; GRANSKOG et al., 2003). However, most of the studies have been focused on the Arctic and Antarctic areas (GARISSON, 1991; GOSSELIN et al., 1997; MCMINN et al., 2003; THOMSON et al., 2006), and there are only a few studies about the 'ice algae' in smaller reservoirs (e.g. lakes, ponds) in the temperate zone (AGBETI & SMOL, 1995; FELIP et al., 1995; 1999). Nevertheless, there are no records about firmly attached periphytic assemblages on the bottom side of the ice cover even in these rare studies. Moreover, the ice assemblage in the present study included algal species of Bacillariophyceae, Chlorophyceae, Zygnemophyceae, Dinophyta and Cyanobacteria which were also present in periphyton during warm periods of the year. Such finding has not been described yet.

Another interesting fact is that no similar assemblages developed on artificial substrates although the colonisation time on artificial and ice substrates must have been the same (the ice cover must have developed after the floats were put into the holes). On the other hand, the artificial substrates were submerged about 35 cm under the water surface, whereas the assemblages developed on an approximately 6 cm thick ice cover. Hence, the irradiance might have been the limiting factor in the case of artificial substrates.

4.3. Comparison of periphyton on glass and PVC panels

Since the results of the two statistical analyses in this study are contradictory, it is impossible to say which artificial substrate is the most favourable for periphyton in the investigated pond. DANILOV & EKELUND (2001) used glass slides, glass tubes, pieces of PVC and pieces of wood for comparison of their usefulness when studying periphyton in lakes of different trophic status. In their study, glass tubes turned out to be the most favourable substrate, whereas no algae were detected on the PVC pieces. The plastics were covered entirely by a slime layer of bacteria. Thus, the authors proposed that the nature of plastic might have had some inhibitory effects on algal growth. These conclusions are out of accord with the findings of the present study because many species were able to attach to both glass slides and PVC panels.

The attachment ability of algae and cyanobacteria to plastic and glass might be useful in food industry, especially in processing of bottled water, because the bottles are commonly made of plastic and glass. There have been some records on green biofilms in bottles with mineral water (HUNTER, 1993; PENLAND & WILHELMUS, 1999). Green algae, especially flagellate species of Chlamydophyceae, predominated in these assemblages. Since green algae are not known as producers of poisonous substances, negative effects on consumers' health are unlikely. Nevertheless, green biofilm in a bottle will certainly discourage consumers from purchase of such mineral water (PUMANN, 2005).

A possible way for algae to get into the mineral water is insufficient rinse of returnable bottles in which algae had grown (PUMANN, 2005). Recently, many studies have examined the microbial quality of bottled water, since there has been a considerable increase in its consumption during the past decade. However, most studies have been focused on bacteria which occur in bottled mineral water far more frequently than algae (HUNTER, 1993; WARBURTON et al., 1998; ARMAS & SUTHERLAND, 1999; VENIERI et al., 2006). JAYASEKARA et al. (1999) tested the attachment of bacteria to the inner surface of bottles using rinsing techniques and electron microscopy. They concluded that although most of the attached bacteria were rinsed out from the PVC bottles during the first two rinses, about 10-20% was not removed. No similar studies were performed on algae although they might answer the question whether algal assemblages occur in the bottles because of insufficient rinse. In addition, the efficiency of attachment of algae to either plastic or glass could be tested in order to determine whether algae prefer either of the substrates.

4.4. Comparison of periphyton under naturally illuminated and darkened conditions

Shading was found to be a very important factor affecting the periphyton composition. Generally, green algae preferred the illuminated conditions, whereas diatoms seemed to be rather indifferent. Nevertheless, some diatom species inclined to the darkened conditions (Figure 11). The blue-green alga *Cyanodictyon turfosum* preferred the darkened conditions as well. This blue-green alga typically occurs in the metaphyton between submerged macrophytes (KOMÁREK & ANAGNOSTIDIS, 1999) and so it is shaded by plants. Thus, the darkened artificial substrates might have provided more natural conditions for this blue-green alga compared to the illuminated substrates.

Diatoms were the prevailing group under both illuminated and darkened conditions in terms of percentage occurrence, whereas green algae were of a great importance only when illuminated. Cyanobacteria and dinoflagellates were found under both conditions. These findings agree with those of SEKAR et al. (2002) who observed that the light-grown biofilms consisted of diatoms, green algae and cyanobacteria, whereas the dark-grown biofilms included mostly diatoms, a few green algae (mainly desmids) and cyanobacteria. In addition, both the algal density and the amount of chlorophyll a were significantly higher in light-grown biofilms than in dark-grown ones. These findings are in contrast with those of MARKS & LOWE (1993), who studied the interactive effect of nutrients and light levels on algal species composition in an oligotrophic lake. They showed that the periphyton biovolume and cell densities increased significantly with both nitrogen and phosphorus enrichment but were not affected by shading. Nevertheless, more evidence exists that the irradiance markedly influences the periphyton compositon (STEINMAN & MCINTIRE, 1986; HOAGLAND & PETERSON, 1990; MARKS & LOWE, 1993; SEKAR et al., 2002; ROBERTS et al., 2004).

5. Conclusions

1) The relative occurrences of cyanobacteria and algae differed considerably on natural and artificial substrates in all investigated periods. Preferences of some species to natural or artificial substrates were recorded. The green alga *Bulbochaete* sp. steril. inclined to the natural substrates, whereas the green alga *Microthamnion* sp. tended markedly to the artificial substrates. Generally, diatoms were the dominant group on almost all examined substrates.

2) It is impossible to decide whether the periphytic communities preferred either of the two artificial substrates (PVC/glass) because the results of the statistical analyses are contradictory. Nevertheless, some species seemed to prefer either glass or PVC panels.

3) The relative species abundances differed significantly under naturally illuminated and experimentally darkened conditions in all investigated periods. Generally, green algae inclined to the illuminated conditions, whereas diatoms tended rather to the darkened ones. However, diatoms were the prevailing group under both illuminated and darkened conditions in terms of percentage occurrence.

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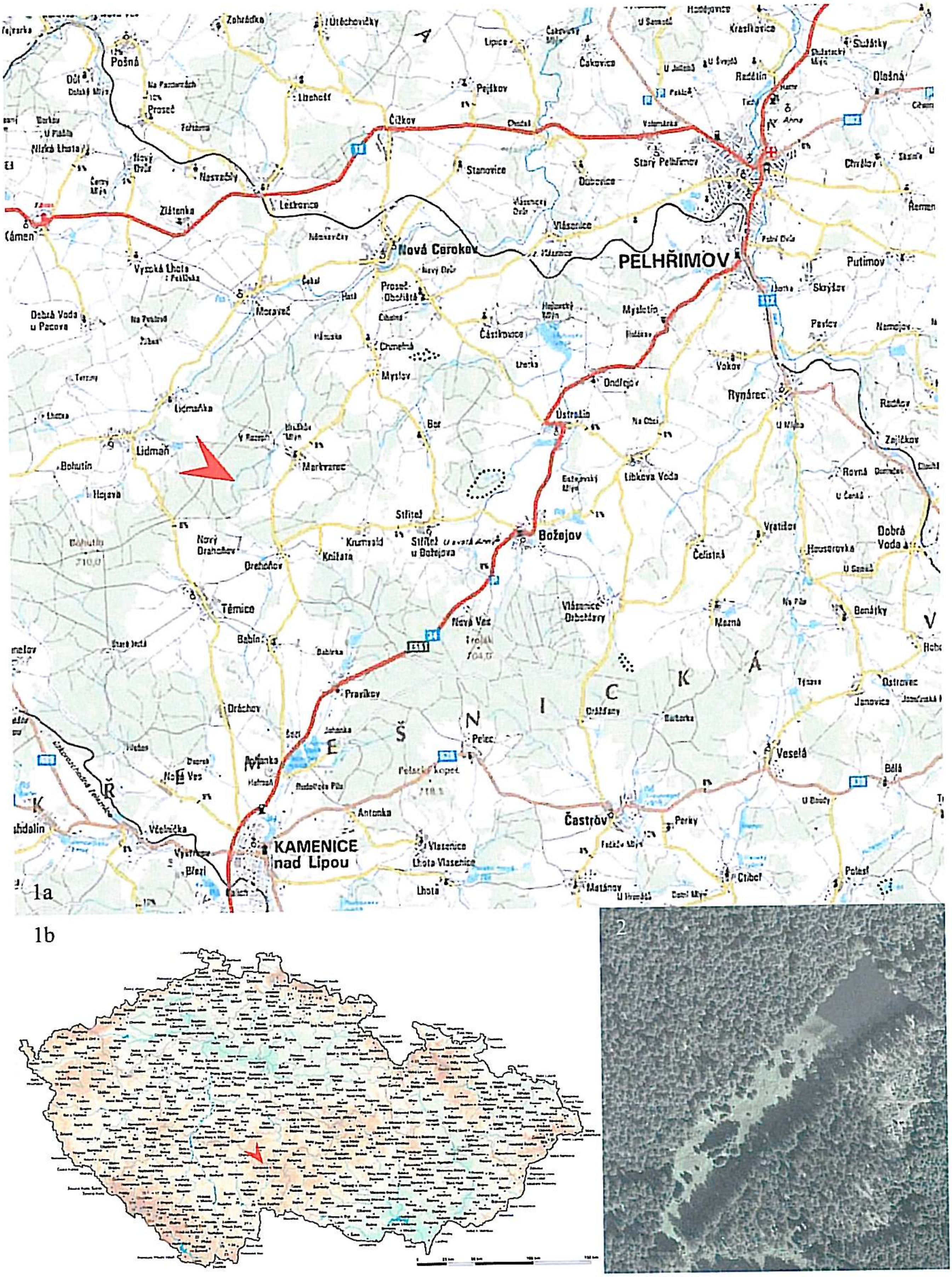
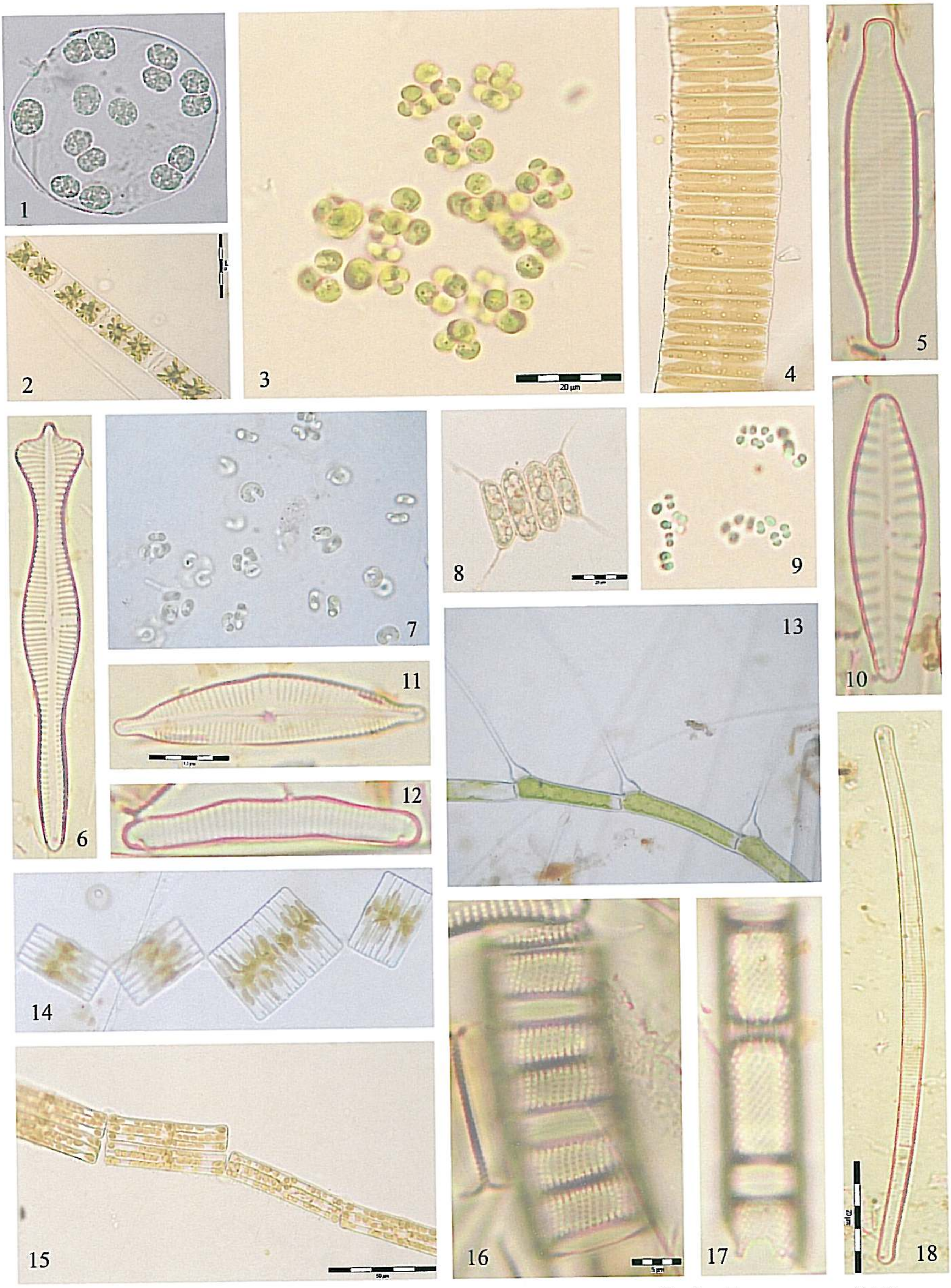
7. Appendices

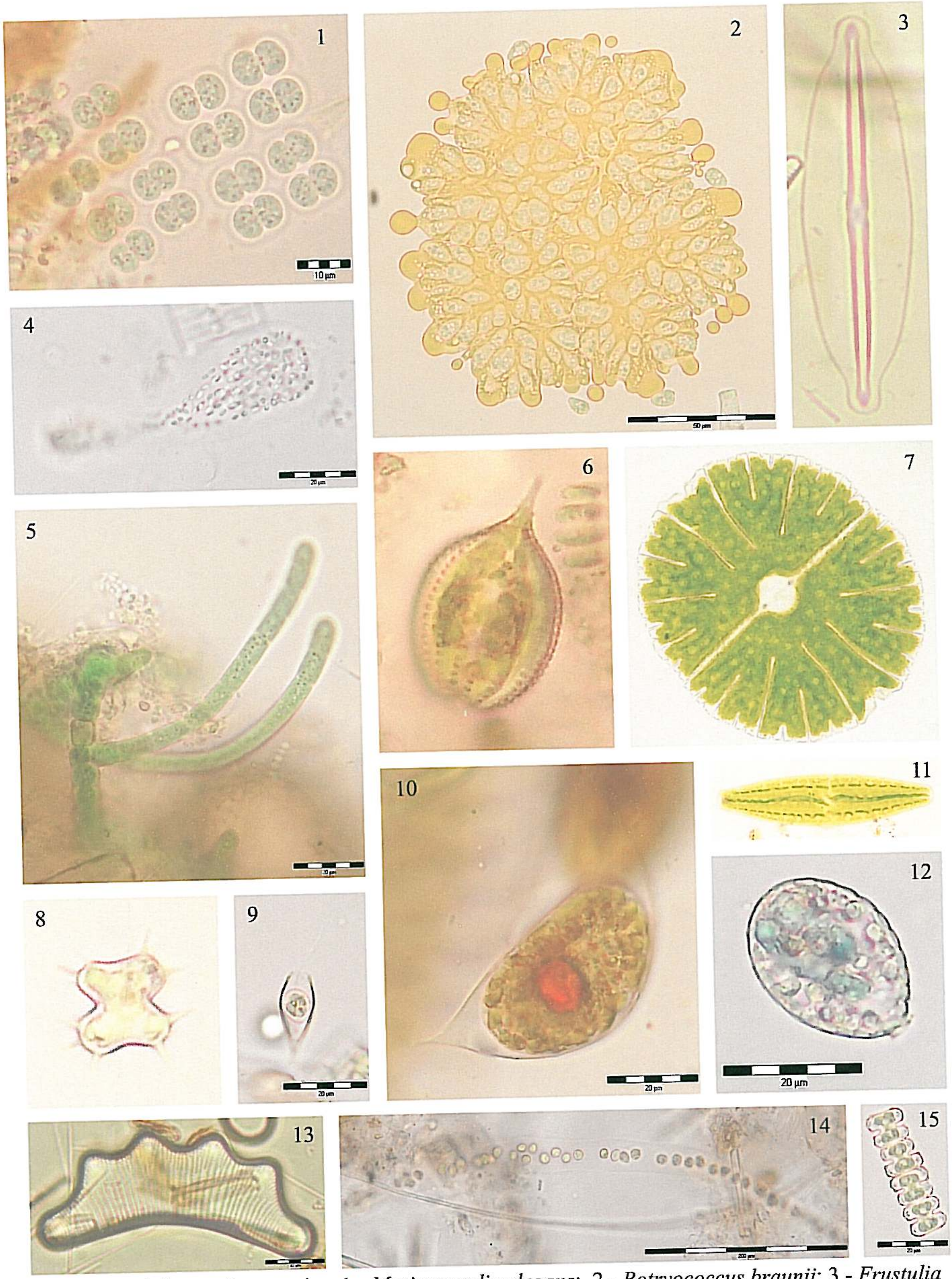


Appendix 1. 1a,b - Location of the pond Huntov, 2 - Aerial photograph of the pond

Appendix 2. Pond Huntov: 1 - General view of the pond from the dam; 2 - *Equisetum fluviatile*, dominant species of the littoral zone; 3 - Periphytic communities on artificial substrates; 4 - Epiphytic communities on *Equisetum fluviatile*; 5 - Position of the floats in the pond



Appendix 3. Frequent species: 1 - Tetrasporal 1; 2 - *Zygnema* sp. steril.; 3 - *Eutetramorus fottii*; 4, 5 - *Fragilaria virescens*; 6 - *Gomphonema acuminatum*; 7 - *Kirchneriella contorta* var. *elegans*; 8 - *Scenedesmus quadricauda*; 9 - *Chroococcus aphanocapsoides*; 10 - *Gomphonema angustatum*; 11 - *Cymbella naviculiformis*; 12 - *Eunotia implicata*; 13 - *Bulbochaete* sp. steril.; 14 - *Tabellaria flocculosa*; 15 - *Tabellaria fenestrata*; 16 - *Aulacoseira alpigena*; 17 - *Aulacoseira valida*; 18 - *Eunotia bilunaris*



Appendix 4. Interesting species: 1 - *Merismopedia elegans*; 2 - *Botryococcus braunii*; 3 - *Frustulia rhomboides*; 4 - *Cyanodictyon turfosum*; 5 - *Hapalosiphon hibernicus*; 6 - *Phacus monilatus* var. *suecicus*; 7 - *Micrasterias thomasiana*; 8 - *Isthmochloron trispinatum*; 9 - *Epipyxis* sp.; 10 - *Cystodinium cornifax*; 11 - *Netrium digitus*; 12 - *Paulinella chromatophora*; 13 - *Eunotia serra*; 14 - *Palmodictyon viride*; 15 - *Teilingia granulata*

Appendix 5. List of periphyton taxa found on different substrates during 4 periods.

	Artificial substrates				Natural substrates			
	A04	S05	LS05	W06	A04	S05	LS05	W06
CYANOBACTERIA/15								
Aphanocapsa grevillei (HASSALL) RABENHORST	-	-	-	-	-	+	-	-
Aphanocapsa sp.	-	-	-	-	+	-	-	-
Cyanodictyon turfosum LEDERER	+	+	+	-	+	+	+	+
Geitlerinema splendidum (GREVILLE) ANAGNOSTIDIS	-	-	+	-	-	-	-	-
Hapalosiphon hibernicus W. & G. S. WEST	+	-	+	-	-	-	-	+
Chroococcus aphanocapsoides SKUJA	+	+	+	-	+	+	+	-
Chroococcus turgidus (NÄGELI) HANSGIRG	+	-	+	-	-	-	+	-
Leptolyngbya cf. *pseudovaleriana*	-	-	-	-	-	+	-	-
Leptolyngbya sp.	-	-	-	-	-	-	-	+
Lyngbya sp.	-	-	-	-	-	+	-	-
Merismopedia elegans A. BRAUN ex KÜTZING	+	+	+	-	+	+	+	-
Phormidium insigne SKUJA	+	-	+	-	+	+	+	-
cf. *Pseudanabaena*	+	+	+	-	-	+	-	-
cf. *Synechococcus ambiguus*	-	-	+	-	-	-	+	-
Cyanobacterium sp.	-	-	-	-	-	+	-	-
GLAUCOPHYTA/1								
Paulinella chromatophora LAUTERBORN	-	-	+	-	-	-	+	-
CHROMOPHYTA/55								
Bacillariophyceae/45								
Achnanthes cf. *subatomoides*	+	+	-	-	-	-	-	-
Amphora libyca EHRENBERG	x	-	-	-	-	-	-	-
Anomoeoneis brachysira (BRÉBISSON) GRUNOW	+	+	+	-	-	+	-	-
Anomoeoneis vitrea (GRUNOW) ROSS	+	+	+	-	+	+	+	-
Aulacoseira alpigena (GRUNOW) KRAMMER	x	-	-	-	-	-	-	-
Aulacoseira valida (GRUNOW) KRAMMER	x	-	-	-	-	-	-	-
Aulacoseira spp.	+	+	+	-	+	+	+	+
cf. *Caloneis silicula*	+	-	-	-	-	-	-	-
Cymbella gracilis (EHRENBERG) KÜTZING	+	+	+	-	+	+	+	-
Cymbella naviculiformis AUERSWALD	+	+	+	-	+	+	+	+
Cymbella silesiaca BLEISCH	-	+	+	-	-	+	-	-
Eunotia bilunaris (EHRENBERG) MILLS	+	+	+	-	+	+	+	+
Eunotia exigua RABENHORST	+	+	+	-	-	+	-	-
Eunotia formica EHRENBERG	+	-	-	-	+	-	-	-
Eunotia implicata NÖRPEL et al.	-	+	+	-	+	+	+	+
Eunotia incisa GREGORY	+	+	+	+	+	+	+	+
Eunotia muscicola var. *tridentula* NÖRPEL & LANGE-BERTALOT	x	-	-	-	-	-	-	-
Eunotia pectinalis (DILLWYN) RABENHORST	+	+	+	-	+	+	+	+
Eunotia cf. *praerupta*	+	+	-	-	-	+	-	-
Eunotia serra EHRENBERG	+	+	+	-	+	+	-	+
Fragilaria ulna (NITZSCH) LANGE-BERTALOT	+	+	-	-	-	+	-	-
Fragilaria virescens (EHRENBERG) GRUNOW	+	+	+	-	+	+	+	+
Fragilaria sp.	+	+	+	-	+	+	+	-
Frustulia rhomboides (EHRENBERG) DE TONI	+	+	+	-	+	+	+	+
Gomphonema acuminatum EHRENBERG	+	+	+	+	+	+	+	+
Gomphonema angustatum (KÜTZING) RABENHORST	+	+	+	-	+	+	+	+
Gomphonema gracile EHRENBERG	+	+	+	-	+	+	+	-
Gomphonema sp.	+	+	-	-	-	+	-	-
Navicula cocconeiformis GREGORY	x	-	-	-	-	-	-	-
Navicula pupula KÜTZING	-	+	+	-	+	+	+	-
Neidium ampliatum (EHRENBERG) KRAMMER	+	+	+	-	+	+	+	-
Nitzschia gracilis HANTZSCH	+	+	+	+	+	-	+	-
Nitzschia cf. *intermedia*	x	-	-	-	-	-	-	-
Pinnularia hemiptera (KÜTZING) RABENHORST	x	-	-	-	-	-	-	-
Pinnularia gibba EHRENBERG	+	+	+	-	+	+	+	-

Appendix 5. Continued.

	Artificial substrates				Natural substrates			
	A04	S05	LS05	W06	A04	S05	LS05	W06
Pinnularia interrupta W. SMITH	+	+	+	-	+	+	-	-
Pinnularia maior (KÜTZING) RABENHORST	+	+	+	-	+	+	+	+
Pinnularia microstauron (EHRENBERG) CLEVE	x	-	-	-	-	-	-	-
Pinnularia nodosa EHRENBERG	+	+	-	-	-	-	-	-
Pinnularia polyonca (BRÉBISSON) O. MÜLLER	+	+	+	-	+	+	+	+
Pinnularia sp.	-	+	-	-	-	+	-	-
Pinnularia spp.	+	-	-	-	-	-	-	-
Stauroneis anceps EHRENBERG	+	+	+	-	-	+	+	-
Tabellaria fenestrata (LYNGBYE) KÜTZING	+	+	+	+	-	+	+	+
Tabellaria flocculosa (ROTH) KÜTZING	+	+	+	+	+	+	+	+
Chrysophyceae/5								
Synura sp.	+	-	-	-	-	-	-	+
Epipyxis sp.	+	-	+	-	-	-	-	-
Dinobryon sp.	-	-	+	-	-	-	-	-
Mallomonas sp.	-	+	-	-	-	+	-	-
cf. *Lagynion*	-	-	-	-	-	+	-	-
Xanthophyceae/5								
Isthmochloron trispinatum (W. & G. S. WEST) SKUJA	+	+	-	-	-	-	-	-
Tribonema gayanum PASCHER	-	-	+	-	-	-	-	+
Goniochloris sp.	+	+	+	-	+	+	+	-
cf. *Goniochloris*	-	-	-	+	-	-	-	+
Ophiocytium cf. *gracilipes*	-	+	-	-	-	-	-	-
CHLOROPHYTA/74								
Conjugatophyceae/30								
Arthrodesmus octocornis EHRENBERG ex ARCHER	+	-	-	-	+	-	-	-
Closterium costatum CORDA ex RALFS	-	-	*	-	-	-	+	-
Closterium dianae EHRENBERG	+	+	+	-	+	+	+	+
Closterium kuetzingii BRÉBISSON	+	+	+	-	+	+	+	+
Closterium striolatum EHRENBERG ex RALFS	-	-	+	-	-	+	+	-
Cosmarium cf. *cucumis*	-	-	-	-	-	+	-	-
Cosmarium contractum KIRCHNER	+	+	+	-	+	+	-	+
Cosmarium cf. *rectangulare*	+	-	+	-	-	-	-	-
Cosmarium regnellii WILLE	+	+	+	-	-	+	-	-
Cosmarium cf. *sphagnicolum*	+	-	+	-	-	+	-	-
Cosmarium sp.	-	+	-	-	-	-	-	-
Euastrum ansatum RALFS	-	-	+	-	-	-	-	-
Euastrum gayanum DE TONI	+	+	+	-	+	+	+	-
Gonatozygon brebissonii DE BARY	-	+	+	-	-	+	+	-
Micrasterias thomasiana ARCHER	-	-	-	-	-	+	-	-
Mougeotia sp. steril.	+	+	+	+	+	+	+	+
Netrium digitus (EHRENBERG) ITZIGS & ROTHE	+	-	+	-	+	+	+	-
Pleurotaenium ehrenbergii (BRÉBISSON) DE BARY	-	+	+	+	+	+	+	+
Pleurotaenium trabecula (EHRENBERG) NÄGELI	-	-	+	-	-	+	+	+
Staurastrum avicula BRÉBISSON ex RALFS	+	+	-	-	+	+	-	-
Staurastrum dispar BRÉBISSON	-	-	-	-	-	+	-	-
Staurastrum cf. *planctonicum*	-	-	-	-	-	+	+	-
Staurastrum polymorphum BRÉBISSON (ex RALFS)	-	-	+	-	-	+	-	-
Staurastrum punctulatum (BRÉBISSON) RALFS	+	+	+	-	+	+	+	+
Staurastrum sp.	+	-	-	-	-	-	-	-
Staurodesmus cuspidatus (BRÉBISSON ex RALFS) TEILING	-	-	+	-	-	-	+	-
Staurodesmus dejctus (BRÉBISSON ex RALFS) TEILING	+	-	-	-	-	+	-	-
Teilingia granulata (ROY & BISSET) BOURRELLY	+	+	+	-	+	+	+	-
Zygnema sp. steril	+	-	+	-	+	-	+	-
Desmid sp.	-	+	-	-	-	-	-	-

Appendix 5. Continued.

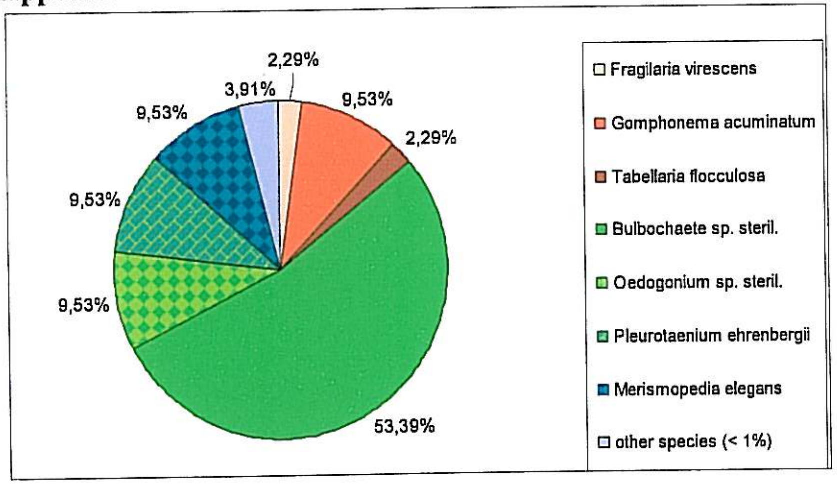
	Artificial substrates				Natural substrates			
	A04	S05	LS05	W06	A04	S05	LS05	W06
Chlorophyceae/44								
Actinastrum hatzschii LAGERHEIM	+	-	-	-	-	-	-	-
Ankistrodesmus cf. *densus*	+	+	+	-	-	-	-	-
Ankistrodesmus fusiformis CORDA	+	-	+	-	-	-	+	-
Ankistrodesmus gracilis (REINSCH) KORŠIKOV	+	-	+	-	+	+	-	-
Ankistrodesmus spiralis (TURNER) LEMMERMANN	+	-	+	-	+	+	+	-
Ankistrodesmus sp.	+	+	-	-	+	+	-	-
Botryococcus braunii KÜTZING	-	+	+	-	+	+	-	-
Bulbochaete sp. steril.	+	-	+	-	-	+	+	+
Chlamydomonas sp.	+	+	+	-	-	+	+	-
cf. *Closteriopsis*	-	-	+	-	-	-	-	-
Crucigenia fenestrata (SCHMIDLE) SCHMIDLE	+	-	-	-	-	-	-	-
Crucigenia tetrapedia (KIRCHNER) W. & G. S. WEST	+	+	+	-	+	+	+	-
Crucigeniella pulchra (W. & G. S. WEST) KOMÁREK	+	+	+	-	+	+	+	-
Dictyosphaerium sphagnale HINDÁK	+	-	+	-	+	+	-	-
cf. *Diplochloris*	-	-	-	-	-	-	+	-
Echinosphaeridium sp.	-	-	-	-	+	-	-	-
Eutetramorus fottii (HINDÁK) KOMÁREK	-	-	+	-	-	-	+	+
Kirchneriella contorta var. *elegans* PLAYFAIR	+	+	+	-	+	+	+	-
Kirchneriella contorta var. *contorta* (SCHMIDLE) BOHL	+	-	-	-	-	-	+	-
Microthamnion sp.	+	+	+	-	-	+	-	-
Monoraphidium contortum (THURET) KOMÁRKOVÁ-LEGNEROVÁ	+	+	+	-	+	+	+	-
Oedogonium sp. steril.	+	+	+	+	+	+	+	+
Oedogonium pringsheimii CRAMER	-	-	-	-	-	-	-	+
Podohedra sp.	-	+	+	+	-	-	-	-
Pediastrum angulosum (EHRENBERG) ex MENEGHINI	+	-	+	-	+	+	+	+
Pediastrum tetras (EHRENBERG) RALFS	-	-	+	-	-	+	-	-
Palmodictyon viride KÜTZING	-	-	-	-	-	+	-	-
Quadrigula sp.	+	-	+	-	-	-	+	-
Scenedesmus acuminatus (LAGERHEIM) CHODAT	+	+	+	-	+	+	+	-
Scenedesmus acutus MEYEN	+	+	+	-	+	+	+	-
Scenedesmus cf. *ecornis*	+	+	+	-	+	+	+	+
Scenedesmus quadricauda (TURPIN) BRÉBISSON	+	+	+	-	+	+	+	+
Scenedesmus sempervirens CHODAT	+	+	+	-	+	+	+	-
Scenedesmus serratus (CORDA) BOHLIN	+	+	+	-	+	+	+	-
Tetraedron caudatum (CORDA) HANSGIRG	+	+	+	-	-	+	+	-
Tetraedron incus (TEILING) G. M. SMITH	-	-	+	-	-	-	-	-
Willea irregularis (WILLE) SCHMIDLE	*	-	-	-	-	-	-	-
Tetrasporal1	+	+	+	-	+	+	+	+
Tetrasporal2	-	-	-	-	-	-	-	+
UGO1	+	-	-	-	-	-	-	-
UGO2	+	-	-	-	-	-	-	-
UGO3	+	-	-	-	-	-	-	-
UGO4	+	-	-	-	-	-	-	-
EUGLENOPHYTA/9								
Euglena acus EHRENBERG	-	-	-	-	-	-	-	+
Euglena spirogyra EHRENBERG	-	+	+	-	-	+	-	-
Phacus monilatus var. *suecicus* LEMMERMANN	+	+	-	-	-	+	-	-
Trachelomonas abrupta SWIRENKO	-	-	*	-	-	-	+	+
Trachelomonas cf. *hispida*	-	-	+	-	+	-	+	-
Trachelomonas rugulosa STEIN	+	-	+	-	-	-	+	+
Trachelomonas sp.	-	-	-	-	-	+	-	-
Trachelomonas spp.	+	+	-	-	-	-	-	-

Appendix 5. Continued.

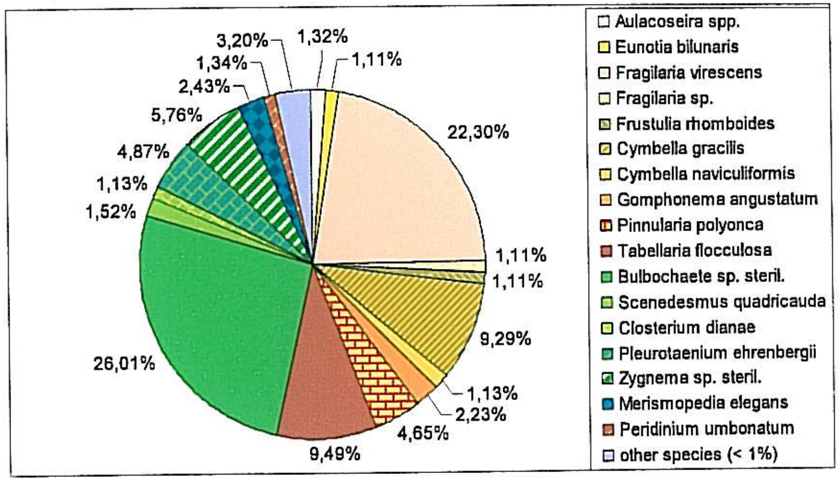
	Artificial substrates				Natural substrates			
	A04	S05	LS05	W06	A04	S05	LS05	W06
Trachelomonas cf. *volvocina*	+	-	+	-	+	-	+	+
DINOPHYTA/3								
Cystodinium cornifax (SCHILLING) KLEBS	+	+	+	+	-	-	+	+
Peridinium umbonatum STEIN	+	+	+	-	+	+	+	+
Peridinium sp.	+	+	+	-	-	-	-	+

A04=Autumn 2004, S05=Spring 2005, LS05=Late summer 2005, W06=Winter 2006;
Species: + present, - absent, * found only on mats, x determined only on permanent slides; UGO=undefined green object
Artificial substrates:
A04, LS05 – glass/PVC panels, bamboo sticks, straw mats
S05 – only PVC panels
W06 – glass/PVC panels
Natural substrates:
A04 – *Equisetum fluviatile*, *Carex rostrata*, wood, shore, plankton
S05 – *Equisetum fluviatile*, dead floating *Equisetum fluviatile*, bryophytes, wood, shore, plankton
LS05 – *Equisetum fluviatile*, plankton
W06 – *Equisetum fluviatile*, plankton (middle of the pond, littoral zone), ice cover

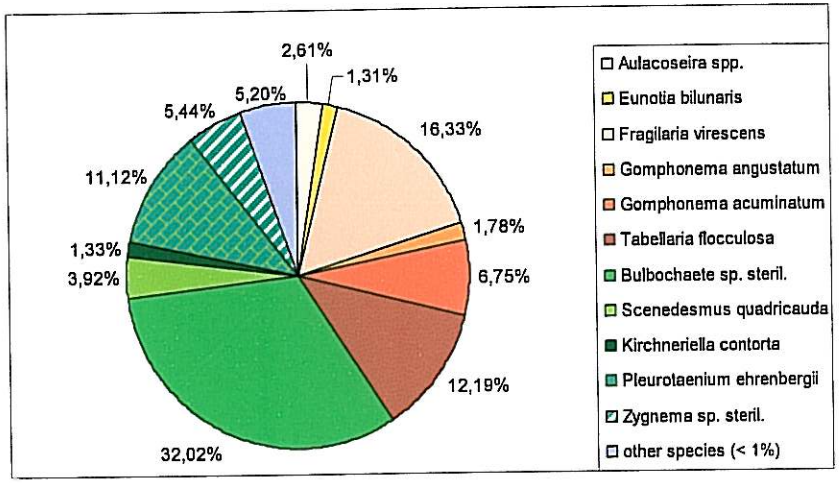
Appendix 6.



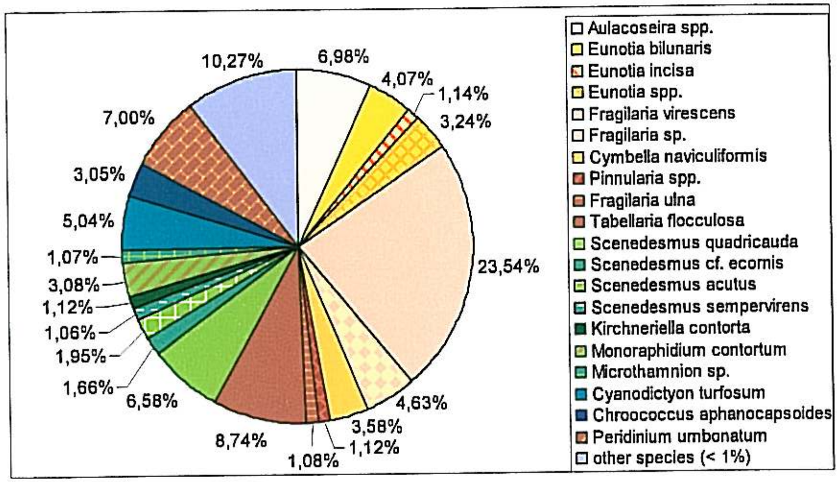
a) Relative occurrence of most abundant species on *Equisetum fluviatile* - autumn 2004



b) Relative occurrence of most abundant species on wood (twigs) - autumn 2004

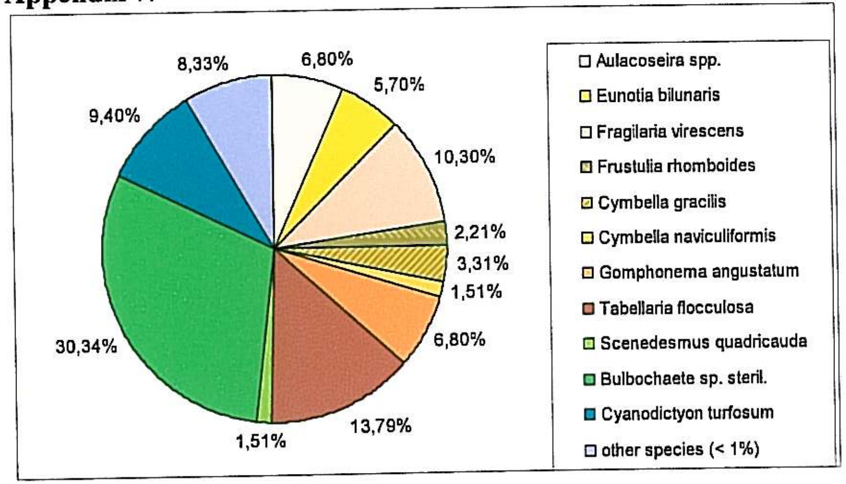


c) Relative occurrence of most abundant species on *Carex rostrata* - autumn 2004

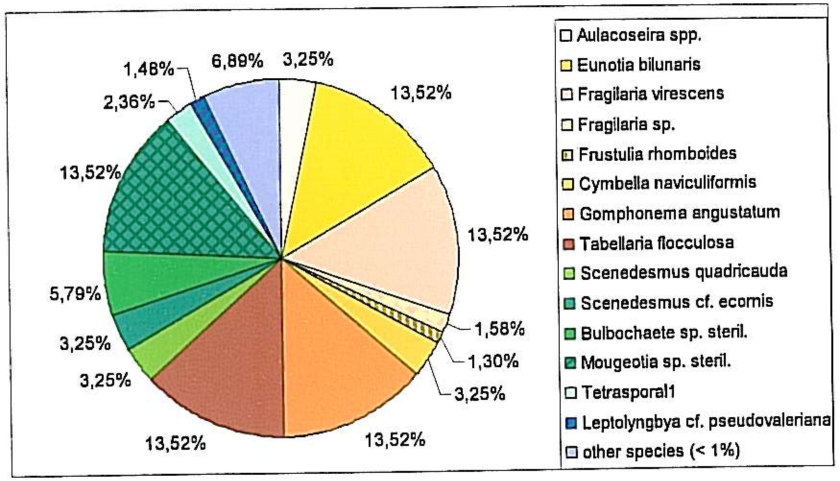


d) Relative occurrence of most abundant species on artificial substrates (glass+PVC) - autumn 2004

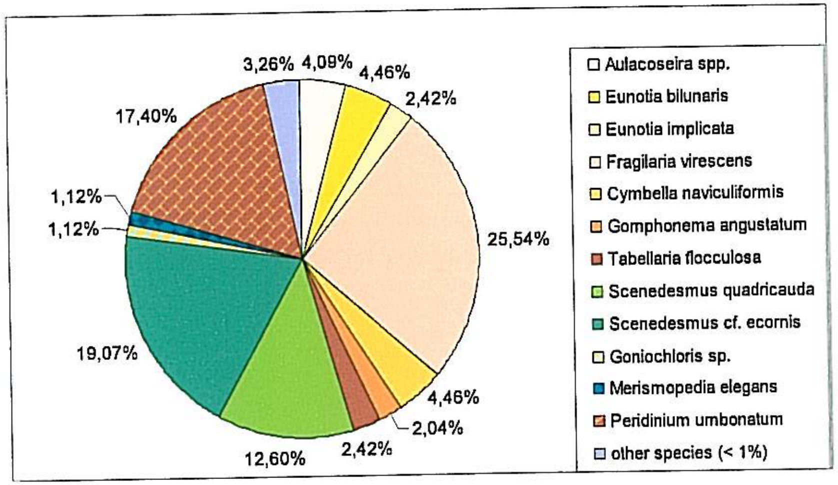
Appendix 7.



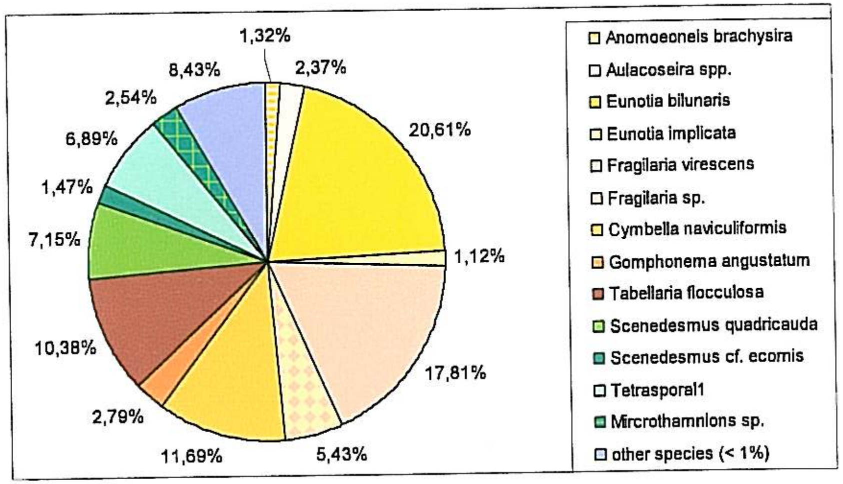
a) Relative occurrence of most abundant species on *Equisetum fluviatile* - spring 2005



b) Relative occurrence of most abundant species on dead floating *Equisetum fluviatile* - spring 2005

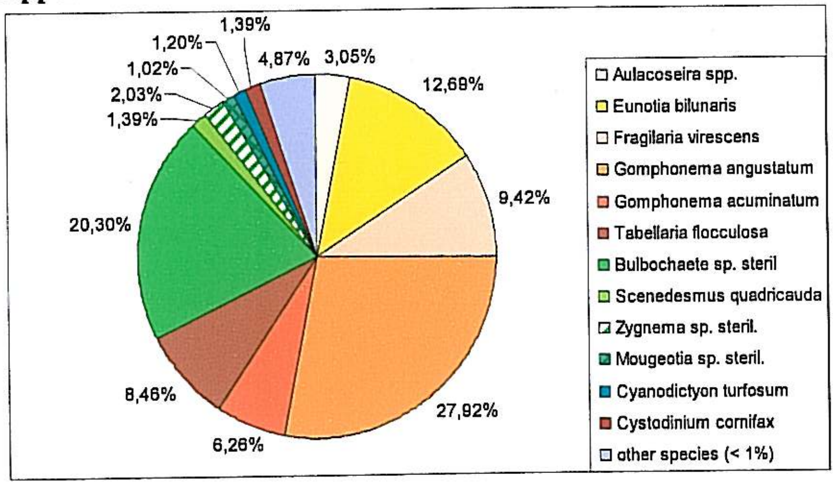


c) Relative occurrence of most abundant species in plankton - spring 2005

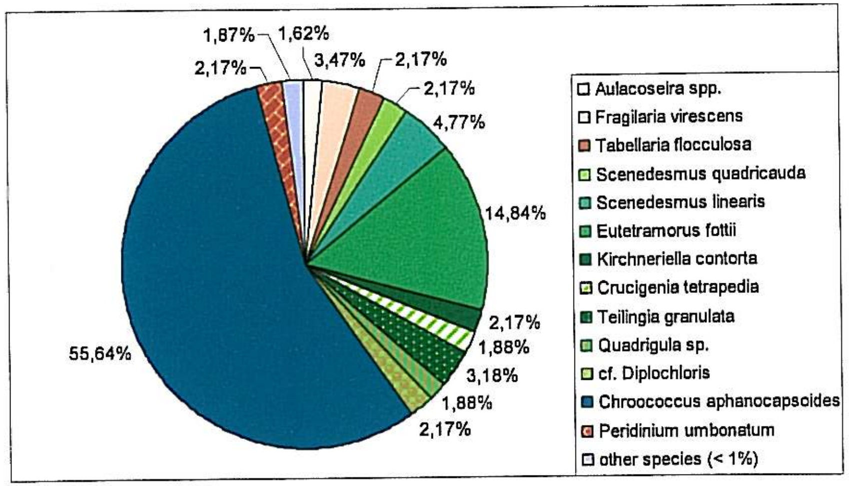


d) Relative occurrence of most abundant species on artificial substrates (PVC) - spring 2005

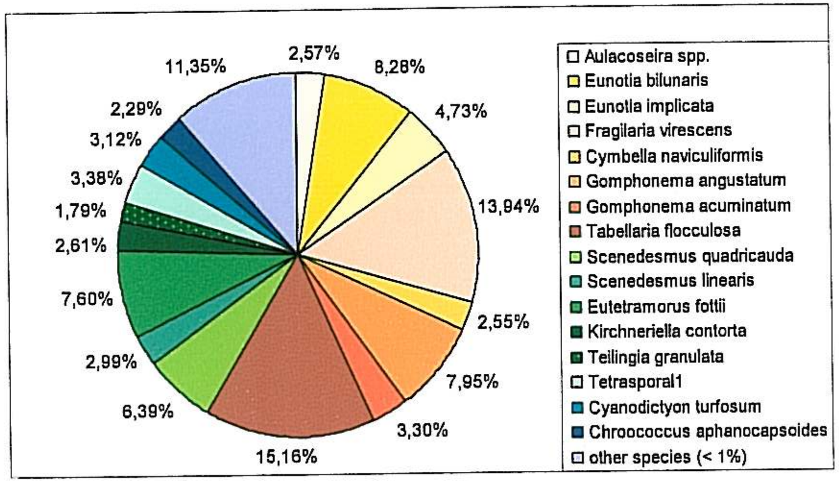
Appendix 8.



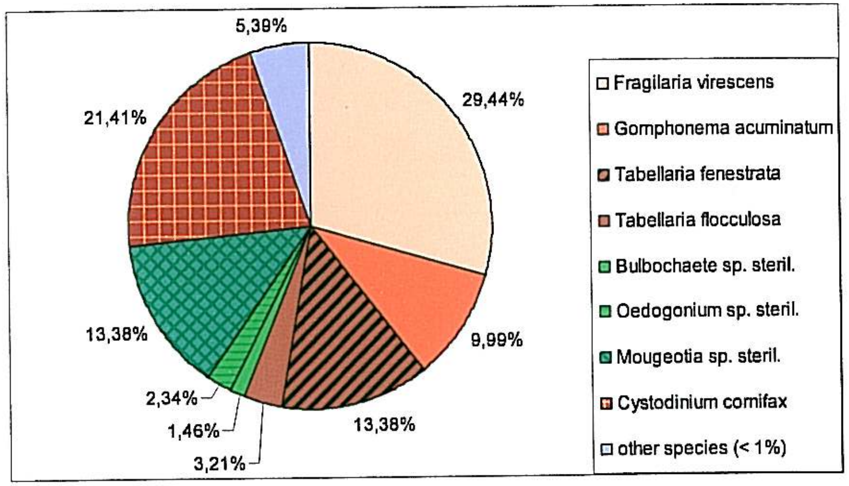
a) Relative occurrence of most abundant species on *Equisetum fluviatile* - late summer 2005



b) Relative occurrence of most abundant species in plankton - late summer 2005

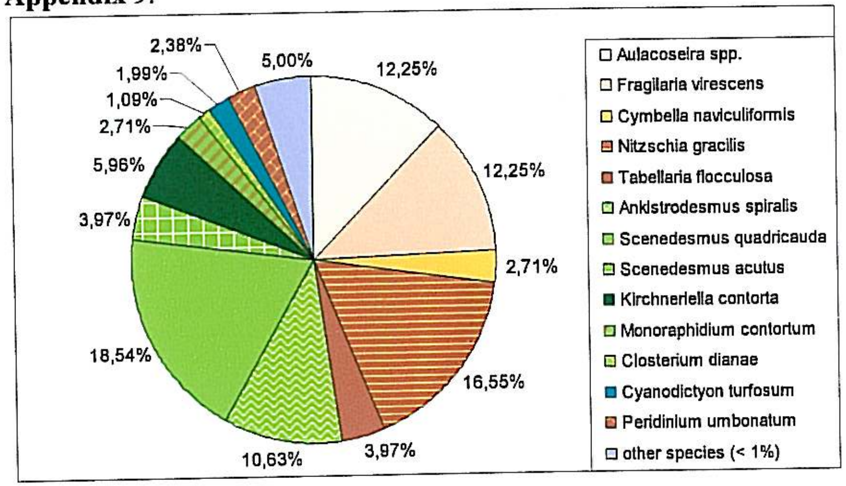


c) Relative occurrence of most abundant species on artificial substrates (glass+PVC) - late summer 2005

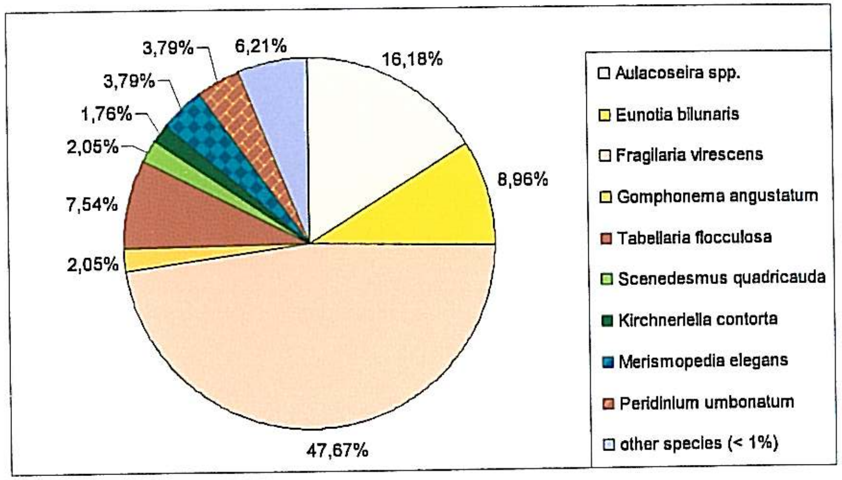


d) Relative occurrence of most abundant species on the bottom side of the ice cover - winter 2006

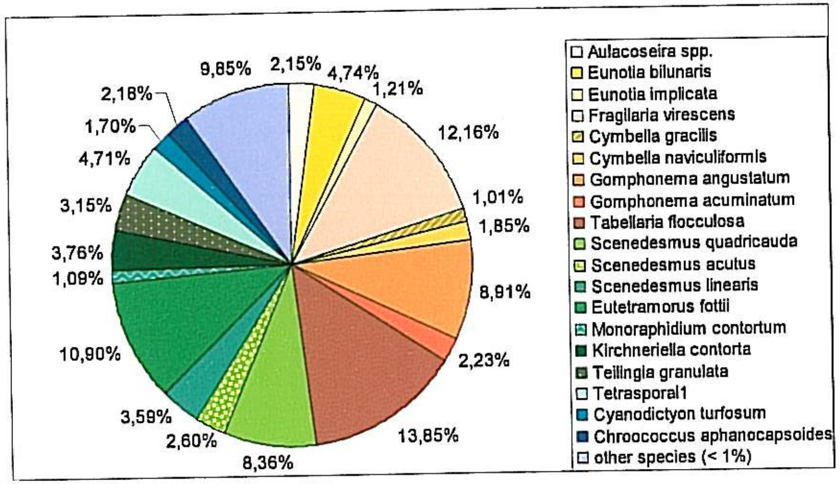
Appendix 9.



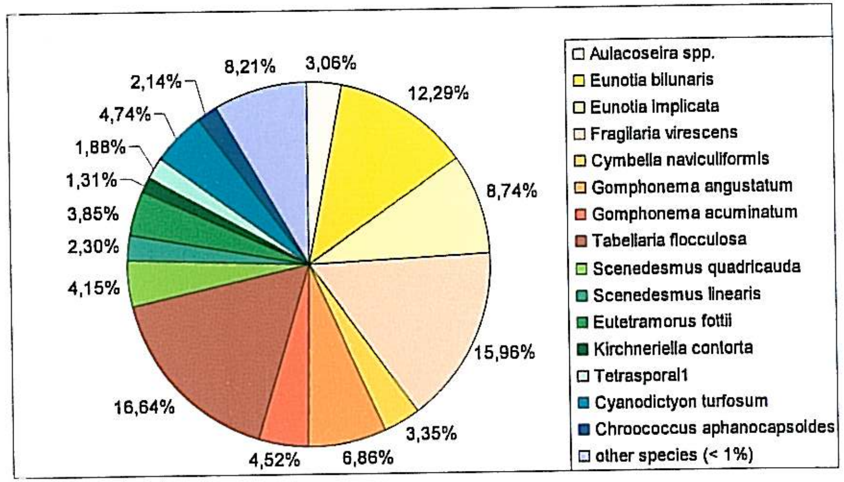
a) Relative occurrence of most abundant species on mats (top side) - autumn 2004



b) Relative occurrence of most abundant species on mats (bottom side) - autumn 2004



c) Relative occurrence of most abundant species under naturally illuminated conditions - late summer 2005



d) Relative occurrence of most abundant species under experimentally darkened conditions - late summer 2005