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TEMPORAL AND SPATIAL TURNOVER OF FRESHWATER DIATOMS

IMPLICATIONS FOR BIOASSESSMENT

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ACADEMIC DISSERTATION

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ABSTRACT

Water quality in streams usually changes fast, and sensitive biological indicators are essential for monitoring these changes. Diatoms are widely used in biological stream quality assessments. However, there are temporal and spatial variations in diatom communities that may result in unreliable quality assessments. Under different kinds of environmental stressors, diatoms can produce deformed frustules. Heavy metals are among the most common causes of these teratological forms. In addition to eutrophication and organic pollution, diatoms could potentially be used for indicators of heavy metal enrichment.

The aim here was to examine the interannual and intra-annual turnover of stream diatom communities. The focus of this study was on whether specific species traits and species local abundance and regional distribution affected temporal occurrence of diatom species. The study also included an examination of how stable diatom inferred stream classifications are in time and if pure spatial variation in diatoms should be considered when assessing stream water quality based on diatoms. The final aspect of the study was to determine which heavy metals, and to what extent, trigger the occurrence of deformations in diatom *Achnanthidium minutissimum*.

There was a positive correlation between the temporal occurrence of diatoms and their local abundance and regional distribution. Species that occurred more frequently also had larger niche breadths and niche positions than temporally rare species. Cell size and attachment ability were also positively correlated with species temporal occurrence. These results imply that abundant and widely distributed species with larger niches and the ability to attach to the substratum sustain persistent populations in varying environmental conditions typical for streams. The most persistent species are thus perhaps the most reliable species to be used as indicators of water quality. The stream classification based on diatoms resulted in temporally stable and statistically distinct community types. Thus, the results suggest that sampling of diatoms in every three years seems to be a reliable procedure to assess biological water quality.

The investigation also revealed that the study regions differ in their diatom species composition more than in their environmental features indicating that diatoms are structured not only by the local environment but also by large-scale processes, possibly related to climate, dispersal and history. As diatom species composition varies between regions, future bioassessments would benefit from regional stratification. Otherwise, relationships with environmental variables may

be masked by trans-regional differences in species pools caused by the large – scale processes.

Finally, study results indicated that two metals, copper and zinc, and a metalloid, antimony, were the most likely triggers of *A. minutissimum* deformations. From a quantitative point of view, it is worth noting that the lowest concentration triggering the deformations can be fairly low, particularly in the case of copper contamination. The definition of their morphological and quantitative characteristics, along with a better taxonomic circumscription of the affected species, should allow the use of deformations as a reliable indicator of heavy metal enrichment in freshwater habitats.

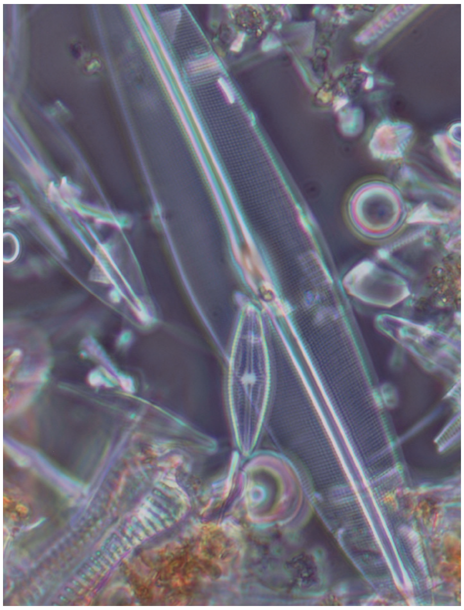


Photo 1. *Diatoms in a microscope (1000X magnification).*

Photo 2. *Sampling site.*

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their Roman numerals:

I Virtanen, L.K., P. Köngäs, S. Aitto-Oja and J. Soininen. 2011. Is temporal occurrence of diatoms related to species traits, local abundance, and regional distribution? *Journal of Phycology* 47: 1445-1453.

II Virtanen, L.K. and J. Soininen. 2014. Temporal variation of community-environment relationships and stream classifications in benthic diatoms: implications for bioassessment. *Submitted manuscript*.

III Virtanen, L.K. and J. Soininen. 2012. The roles of environment and space in shaping stream diatom communities. *European Journal of Phycology* 47: 160-168.

IV Cantonati, M., N. Angeli, L.K. Virtanen, A.Z. Wojtal, J. Gabrieli, E. Falasco, I. Lavoie, S. Morin, A. Marchetto, C. Fortin and S. Smirnova. 2014. *Achnanthidium minutissimum* (Bacillariophyta) valve deformities as indicators of metal enrichment in diverse widely-distributed freshwater habitats. *Science of the Total Environment* 475: 201-215.

AUTHORS´ CONTRIBUTION TO THE PUBLICATIONS

I	J. Soininen and L.K. Virtanen were responsible for the original idea for the study as well as the study design. Data was collected by P. Köngäs and S. Aitto-Oja. L.K. Virtanen performed the data analysis and wrote the main part of the paper.
II	The study was planned jointly. L.K. Virtanen collected the data and was primarily responsible for the data analysis and manuscript preparation.
III	The study was planned jointly. L.K. Virtanen collected the data and was primarily responsible for the data analysis and manuscript preparation.
IV	M. Cantonati was responsible for the original idea for the study and was also primarily responsible for study design and data analyses. L.K. Virtanen and the other authors participated in data collection and manuscript preparation. J. Gabrieli performed the main water chemistry analyses.

ABBREVIATIONS

ADMI	*Achnanthidium minutissimum*
ADPY	*Achnanthidium pyrenaicum*
AIC	Akaike´s information criterion
ANOSIM	analysis of similarities
ANOVA	analysis of variance
CCA	canonical correspondence analysis
CLT	cymbelliclinum-like teratology
DCA	detrended correspondence analysis
EF	enrichment factor
GLM	general linear models
IndVal	indicator value method
LM	light microscope
PCA	principal component analysis
SEM	scanning electron microscope

1 INTRODUCTION

1.1 STREAM HABITATS AND THEIR ORGANISMS

Freshwater is characterized by having low concentrations of dissolved salts and occurs in rivers, streams, lakes, ponds, ice, and underground as groundwater. Freshwater habitats can be divided into lentic and lotic systems. Running waters, i.e., lotic ecosystems are remarkably fluctuating, water flow being one of the key factors influencing their ecology (Allan & Castillo 2008). Current velocity typically varies widely in streams, ranging from roaring rapids to slow backwaters. Streams, together with lakes, are unique ecosystems on Earth and their rich biodiversity is currently severely threatened by anthropogenic influences (Heino et al. 2009).

Water chemistry between streams varies tremendously and the chemistry is foremost determined by geology and natural land cover of the surrounding environment, but can also be influenced by precipitation, runoff and the addition of nutrients and other pollutants from anthropogenic sources (e.g. agriculture, forestry and other industries and population) (Allan & Castillo 2008). Water temperature in streams also varies on seasonal and daily time scales and spatially due to climate, extent of stream canopy cover, and the relative importance of groundwater inputs (Cluis 1972; Allan & Castillo 2008). Temperature is important in determining the metabolic rates of stream organisms, their distribution and also their success in interacting with other stream organisms.

Light is an important factor in streams, because it, among other things, provides the energy necessary to drive primary production via photosynthesis. The amount of light that a stream receives can be related to canopy cover. A small stream, for example, might be shaded by surrounding forests. In wider river systems, the sun reaches the stream surface more easily as there is no thick vegetation that covers the river. Light intensity can also be related to the amount of aquatic humic substances (humus) or fine inorganic suspensoids (clay) (Thurman 1985; Davies-Colley et al. 1992; Pace & Cole 2002). By measuring water color or turbidity one can estimate the amount of humus or clay in the water that can also affect light penetration and thus light conditions in the water (Davies-Colley et al. 1992; Estlander et al. 2009; Horppila et al. 2011).

The type, the shape and the size of the substrate are also important for aquatic biota in streams (Stevenson et al. 1996). The inorganic substrate in streams is composed of the geological material present in the catchment that is eroded, transported, sorted, and deposited by the current (Cattaneo et al. 1997). Substrate can also consist of organic material such as autumn shed leaves, submerged wood, moss, and plants. Typically, particle size decreases downstreams (Vannote et al. 1980).

Algae, consisting of benthic algae and in the largest streams also phytoplankton, are the most significant primary producers in streams (Stevenson et al. 1996; Allan & Castillo 2008). Some genera of benthic algae (in diatoms, e.g. *Achnanthidium* and *Gomphonema*) can attach themselves to objects, e.g. stones or plants, to avoid being washed away by the current. Alternatively, some algae live on the substrate, but do not attach (e.g. in diatoms, genera *Anomoeoneis* and *Frustulia*). Phytoplankton in streams originate from lakes or backwaters and drift freely in the water column, but cannot make stable populations in smaller streams. The other primary producers, macrophytes, exhibit limited adaptations to fast flow and are

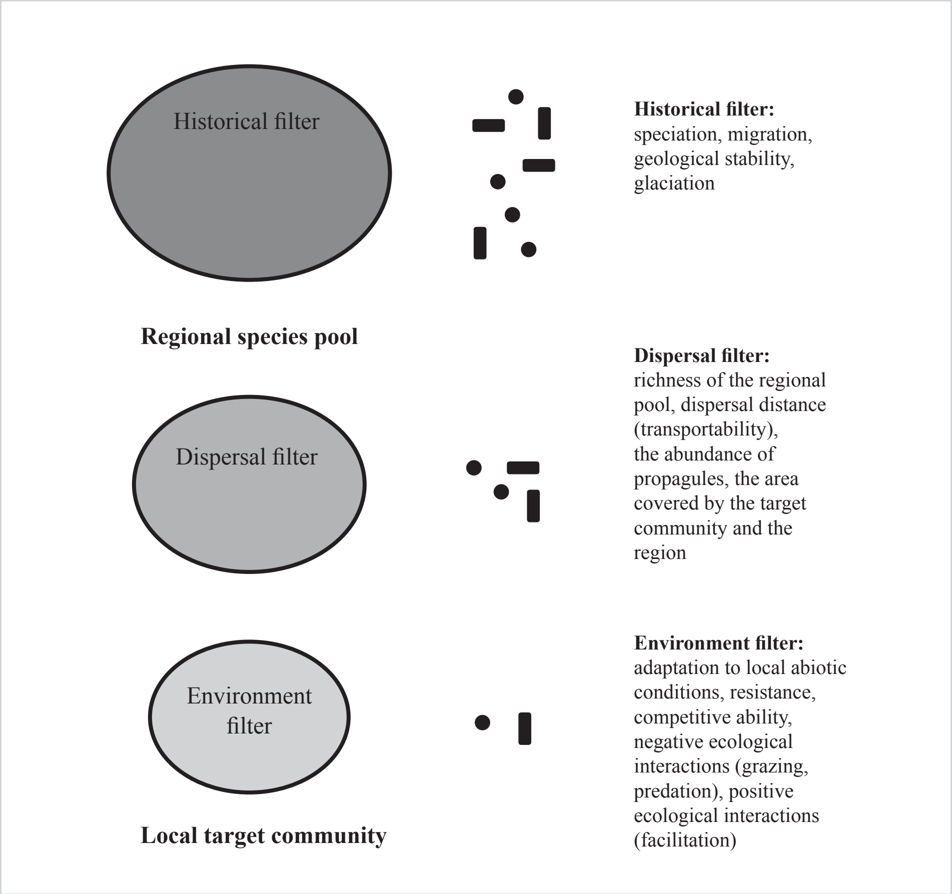


Fig. 1. *Conceptual model visualising the assembly of local communities from regional species pool. Modified from Hillebrand & Blenckner (2002).*

most successful in low currents (Allan & Castillo 2008). The most important primary consumers in streams are macroinvertebrates and fish, which graze on primary producers (Feminella et al. 1989; McIntosh & Townsend 1996).

In streams, benthic algae have an important role in water quality assessment and monitoring, as they often form the most abundant, diverse and stable community of primary producers. Diatoms especially are widely used as water quality indicators, because they are locally abundant and widespread in various kinds of aquatic ecosystems (Stevenson 2014). Ecology of diatoms is relatively well known and diatoms respond predictably to many water chemistry variables (Soininen 2007; Stevenson 2014). The community structure of diatoms is the outcome of abiotic factors, such as hydrological and chemical variables, and biotic factors (Stevenson et al. 1996; Lange et al. 2011). Hence, environmental variables that influence diatom communities include water chemistry (nutrients, pH, and conductivity), current velocity, light, grazing, temperature and the type and size of the substrate. In addition, local diatom communities are shaped by dispersal processes and other historical factors, such as glaciation (Fig. 1) (Biggs et al. 1990; Stevenson et al. 1996). Streams and their biodiversity are highly vulnerable to climate change, which can cause changes in both proximate and ultimate variables, and thus in diatom species occurrence (Heino et al. 2009).

1.2 TEMPORAL AND SPATIAL TURNOVER OF STREAM DIATOM COMMUNITIES

1.2.1 FACTORS AFFECTING TEMPORAL TURNOVER OF DIATOMS AT SPECIES AND COMMUNITY LEVEL

Spatial variation in diatom community composition at multiple scales in streams is relatively widely understood, but major patterns in temporal turnover are still understudied. (1) At species level, diatom species` intrinsic traits may affect their temporal occurrence. Temporal occurrence is expected to be positively related to the growth rate of an organism, dispersal rate and population size but negatively to disturbances (Marquet et al. 1990; Mccormick & Cairns 1994; Hillebrand et al. 2001; Finlay 2002; Rosenfield 2002; Brown et al. 2004; Reynolds 2006). Therefore, organism size is expected to have a strong influence on its occurrence (**I**). Also, if a diatom species is able to attach to substratum, it may occur more frequently in time as cells are less prone to be detached from the bottom by the water current (Horne & Goldman 1994; Stevenson et al. 1996).

Substrate does not seem to have a strong effect on temporal occurrence of diatom species. When Soininen and Eloranta (2004) studied diatoms in three different growth habitats, they did not find any clear differences in community stabilities among epipelic, epiphytic and epilithic habitats.

In addition, the niche characteristics of diatom species may correlate with their temporal occurrence, as species that have a broad and less marginal niche are more capable of living in continuously variable conditions than species with a narrow or marginal niche (Brown 1984; Sultan et al. 1998). If a species is able to sustain a large population size in variable environmental conditions, it should also occur frequently in time as high abundance may prevent local extinction (Soininen & Heino 2005). Also, species that have wide distributions regionally should occur regularly in time as widely distributed species may easily disperse to a site via many dispersing events from a greater number of source locations, thus increasing the longevity of their populations (Soininen & Heino 2005).

(2) At the community level, the flood disturbance regime (reflecting climate, geology and topography) and associated changes in water quality are perhaps the most fundamental factors determining temporal turnover of diatoms (Stevenson et al. 1996). Apart from being a major loss mechanism, the frequency and intensity of floods can influence a range of variables important to diatom colonization and growth processes (e.g. nutrient concentrations, water clarity, temperature, grazing, current velocity and substratum size) (Stevenson et al. 1996). The frequency of floods also dictates the time available for benthic algal accrual. Soil and hydrological factors such as precipitation, flow and runoff among other things affect stream water quality and thus diatom communities, but human driven factors influence them too (Allan & Castillo 2008). The variation in land use, agriculture and wastewaters from industry and residential areas can also explain the variation in water quality and thus also in diatom communities.

In addition, the degree of temporal turnover in diatom communities may be related to community diversity. Thus, in highly diverse communities, abundances of some of the species may vary strongly but total biomass and community composition may vary less (Tilman 1996; Lehman & Tilman 2000; Mykrä et al. 2011). High diversity may buffer the system against perturbation, as in species-poor communities, changes in abundance of only a few species can have a strong relative effect on community composition.

Temporal turnover may also be affected by the productivity of the ecosystem and ecosystem size. In highly productive ecosystems, community may vary more due to multiple stable states in communities (Chase 2003). Korhonen et al. (2013) remind, however, that temporal turnover may not overwhelm the strong distinction in diatom communities between oligotrophic and mesotrophic to eutrophic streams. Within less productive streams, temporal turnover may add less noise to between-site variation in communities compared to highly productive streams, where communities at different sites may often resemble each other more (Lavoie et al. 2008; Korhonen et al. 2013). Finally, the studies of Adler & Lauenroth (2003) and Adler et al. (2005) have shown that temporal turnover increases as the ecosystem size decreases and vice versa. Yet, other studies have suggested that this relationship may not be valid generally and species turnover can be actually faster in larger ecosystems (e.g. Korhonen et al. 2010).

1.2.2 SPATIAL TURNOVER OF STREAM DIATOMS AT DIFFERENT SCALES

Spatial turnover in stream diatom communities can be studied at different scales. At the finest level of resolution, algal taxa can show preferences for the substrate e.g. for stones or moss (Cantonati et al. 2012). Some stalked diatoms, such as *Fragilaria* and *Gomphonema* species, may prefer epilithon and epiphyton, and in contrast some prostrate diatoms, such as *Achnanthidium minutissimum* and *Cymbella minuta*, dominate the epipsammon, where they obtain more protection from abrasion (Passy 2007). Most diatom taxa are able to colonize most substrata, but can develop into mature communities only if habitats are stable (Blinn et al. 1980). Differences in species composition can also occur between different habitats in a stream (e.g. pools and rapids), and these reflect spatial differences in shear stress, nutrient transfer, light conditions and substratum type (Stevenson et al. 1996).

If viewed at larger scale, it is still controversial if diatom species are cosmopolites (e.g. Baytut 2013). Some studies on microbial distributions, including diatoms, have suggested that unicellular organisms have unlimited access to all sites within a region, particularly over long time period (Fenchel & Finlay 2004). It has been documented that unicellular organisms are well dispersed, with geographical isolation occurring only rarely due to high dispersal rates driven by huge population sizes (Finlay 2002). If diatom species were dispersed everywhere, then more or less the same species should be found in all continents in similar environmental conditions – thus, whether a species occur at a given site or not would be determined only by local environmental conditions. However, e.g. Soininen et al. (2004), Vanormelingen et al. (2008), Verleyen et al. (2009) and Heino et al. (2010) have shown that this is not often the case and a significant proportion of variation in diatom communities is explained by spatial factors, indicating the role of large-scale processes such as evolutionary history and dispersal limitation, geology, topography, climate and large-scale variation in human activities. Historical factors may not often influence diatoms through water quality, but affect diatom community composition at large scales through speciation and species dispersal. Given that diatoms are potentially influenced by many large-scale processes, the diatom communities of boreal streams also incorporate a strong spatial component at regional scales (Soininen et al. 2004).

1.3 DIATOMS AS BIOINDICATORS

Algae have been used for a century in environmental assessments of water bodies and nowadays their use has spread in countries around the world (Stevenson 2014). In 2000, the European Union passed the Water Framework Directive (WFD; European Parliament, Council 2000), mandating the use of different organismal groups to monitor the conditions of surface waters. Because of diatoms` fast response to environmental change, they are useful as indicators of stream water quality (Stevenson et al. 1996). The sensitivity or tolerance of algae to eutrophication and other forms of pollution has led to the development of many indicator systems and indices to assess water quality in rivers. In particular, methods have been developed for the biological monitoring of European rivers using algae (Whitton et al. 1991; Whitton & Rott 1996), and several diatom-based indices have been developed to estimate river water quality (Descy 1979; Sládeček 1986; Coste & Ayphassorho 1991; Descy & Coste 1991; Schiefele & Schreiner 1991; Prygiel & Coste 1993; Eloranta 1995; Kelly & Whitton 1995; Kelly et al 1995).

Intra-annual and interannual variation of stream diatom communities can render the assessment of water quality difficult, however. Classification or grouping of sampling sites can vary easily in time if clear changes in community composition take place. Yet, the importance of diatom species` traits, species` local abundance and regional distribution on temporal occurrence is understudied. Annual turnover of communities may affect the values of biological monitoring variables, e.g. biological indices. According to WFD prescription, diatom sampling has usually taken place every three years, but only little is known how representative such sampling is. Due to dispersal limitation, it can also be difficult to predict community composition based on environmental variables. What has not yet been properly determined is whether the variation in diatom communities between regions is larger than the variation in environmental conditions.

So far, the diatom based monitoring tools have mainly focused on eutrophication and organic pollution (e.g. CEMAGREF 1982; Kelly et al. 2008). In addition to nutrient enrichment, species composition of diatoms, as well as cell deformations of diatoms (see Box 1), can be used in studying environmental changes related to climate, acidification, and other pollution issues such as heavy metal enrichment (Stevenson et al. 2010). In the presence of different kind of environmental stressors, diatoms may produce frustules that are deformed in different ways. Mostly they have anomalies in symmetry, striation pattern, raphe course and structure (Falasco 2009a). The exact physiological processes causing these deformations are not yet known (Morin et al. 2012), but it is known that the causes behind them can be diverse. The most common cause appears to be toxic concentrations of heavy metals. Cell size reduction (e.g. Cattaneo et al. 1998) and frustule deformations (e.g. Ruggiu et al. 1998) have often been connected with high heavy metals concentrations, especially of copper and zinc (Morin et al. 2008b; Falasco et al. 2009b; Lavoie et al. 2012). In spite of the well-known response of diatom communities to heavy metals (e.g. Hirst et al. 2002), no diatom index for the assessment of metal pollution has been developed (Jüttner et al. 2012) and the potential of diatom deformations as a monitoring tool remains mostly unexplored.

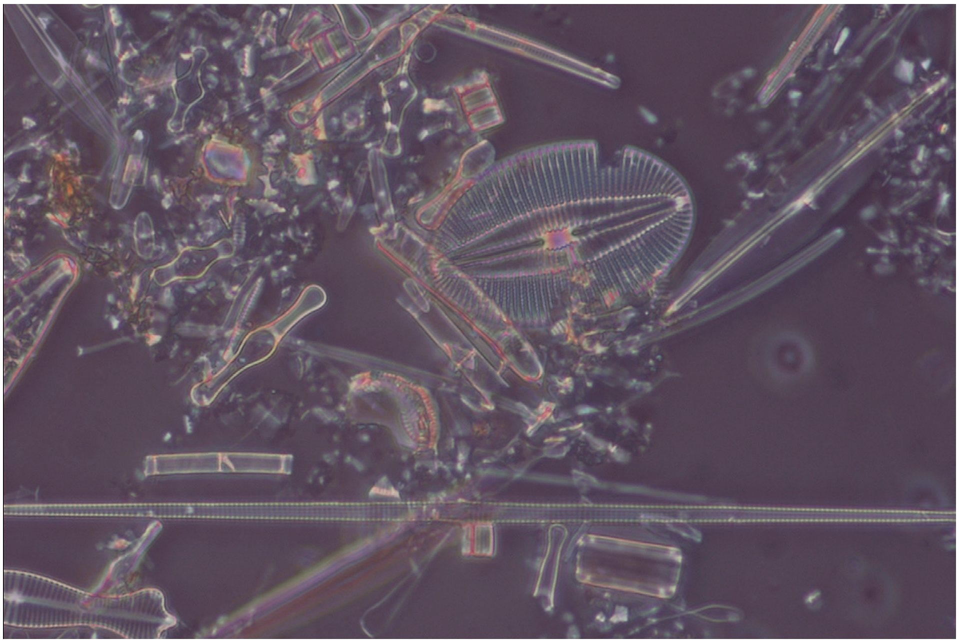


Photo 3. *Diatoms in a microscope (1000X magnification).*

Box 1.

Teratological forms of diatoms are non-adaptive phenotypic deformities, typically affecting the outline or striation pattern. Deformations involving valve outlines are considered to be transferred during reproduction to the next generation, thus creating a population with a different morphology from that of the previous generations (Falasco et al. 2009b). Other types of deformations, for example those affecting structure and distribution of striations, appear to be limited to a few generations. Therefore, a distorted valve outline is the most common type of deformation (Granetti 1968). These deformities have been reported in several diatom species, e.g. *Cocconeis placentula*, *Fragilaria capucina*, *Gomphonema parvulum* and *Nitzschia palea*. However, some types of deformations seem to be more prevalent in certain species (Falasco et al. 2009b).

Several different kinds of environmental stressors can cause teratological cell development, and the assessment of deformed cells in a diatom population or community can give both temporal and quantitative indication of the stress. Several studies indicate a significant positive correlation between the abundance of deformed cells and environmental stressors, such as low current velocity and flow, drought conditions, light intensity or decrease in water quality (Gómez & Licursi 2003; Falasco et al. 2009b). In addition, nutrients, salinity, and herbicide contamination can cause the valve deformations (Cholnoky-Pfannkuche 1971; Locker 1950; Schmid 1980; Håkansson & Chepurnov 1999; Debenest et al. 2008).

Yet heavy metal contamination and artificial growth conditions are the most common causes of deformities (Falasco et al. 2009b). Especially Cd, Cu, and Zn seem to be effective heavy metals in the production of deformed cells. Also combined Cd and Zn contamination has attracted attention; these two heavy metals have been responsible for a deformed valve profile and striation pattern, doubled central area and distorted raphe canal in several species (McFarland et al. 1997; Nunes et al. 2003; Morin et al. 2008a). Additionally, deformed cells stimulated by intense artificial light easily undergo vegetative production, thus conveying deformations through the generations (Granetti 1968).

The main problem in the use of deformed diatom valves as bioindicators is the lack of a morphological classification of teratological forms (Falasco et al. 2009b). If we would like to use the deformations in environmental assessments, a classification system must first be created to provide a common foundation.

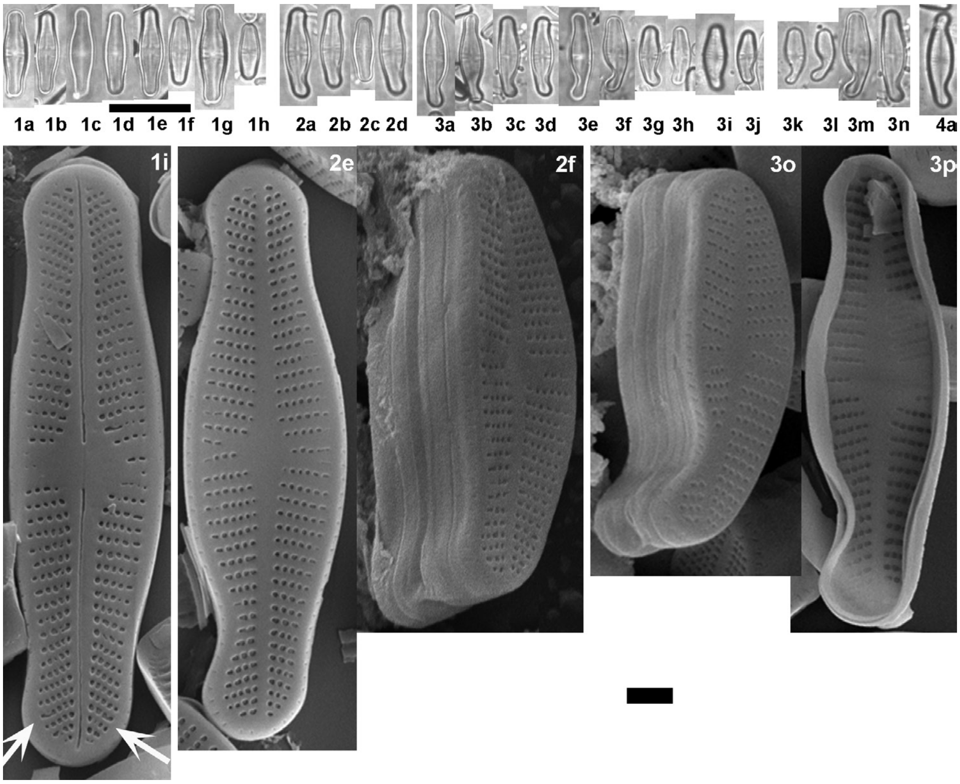


Fig. *LM (1a-h, 2a-d, 3a-n, 4a) and SEM (1i, 2e-f, 3o-p) micrographs of the ADMI CLT: 1a-i normal specimens shown for comparison, 2a-f slightly-deformed CLT, 3a-p markedly deformed CLT, 4a specimen with both ends bent off. The white arrows in 1i show that slight irregularities of the areole can also be present on normal valves. LM, scale bar, 10 µm. SEM, scale bar, 1 µm. See abbreviations on page 8, and 3.2.2 and* **IV** *for more information about ADMI CLT. Photo: Nicola Angeli.*

2 OBJECTIVES OF THE THESIS

The primary aim of this thesis was to examine the major patterns in temporal and spatial turnover of stream diatoms. The secondary aim was to consider how the knowledge about the turnover of diatom communities would facilitate their use as bioindicators for water quality.

The thesis is composed of four studies (**I-IV**). The first two papers (**I & II**) focus on the temporal patterns of diatom species and communities. In the first paper (**I**), the aim was to untangle the patterns and discuss underlying processes in temporal occurrence of lotic diatom species. The main objective in paper **II** was to study how stable diatom inferred stream classifications are in time. In paper **III**, the role of spatial and environmental factors in shaping stream diatom communities was examined. In the fourth study (**IV**), it was examined if diatom valve deformities could be used as an indicator of metal enrichment in diverse widely-distributed freshwater habitats.

The thesis focuses thus on the following questions:

1. Is temporal occurrence of diatom species related to species traits (i.e. body size, attachment ability, and niche characteristics), local abundance and regional distribution (**I**)?

2. Is there temporal variation in diatom community-environment relationships and in stream classifications (**II**)?

3. Is the variation in diatom communities between regions larger than the variation in measured environmental variables (**III**)?

4. Which and to what extent heavy metals trigger the occurrence of diatom deformations, especially in *Achnanthidium minutissimum* (**IV**)?

3 MATERIAL AND METHODS

3.1 STUDY AREA

3.1.1 PAPERS I, II, III

In paper **I**, diatoms were sampled at eight stream sites located in southern Finland. Four of the sampling sites were located in eutrophic River Vantaanjoki in basin Vantaanjoki and the four others were located in oligotrophic Rivers Evojoki and Luutajoki in basin Kokemäenjoki. In papers **II** and **III**, diatoms were sampled at 40 stream sites located in four separate basins in Finland. In addition to the basins Vantaanjoki and Kokemäenjoki we also sampled the basins Kymijoki and Vuoksi. For more detailed description of the sampling sites, see the maps in papers **I-III**. These basins were selected because it was possible to sample them all in a relatively short period of time (2-3 weeks), yet they cover relatively long environmental and geographical gradients (**III**). Nine stream sites were sampled in the basins Kokemäenjoki, Kymijoki and Vuoksi and eight stream sites in the basin Vantaanjoki (**II, III**). Three stream sites were also sampled in the basin Ingarskilanjoki and two in the basin Porvoonjoki. We combined the data of these two basins to the data of the basin Vantaanjoki (**II, III**), because all these basins were located in the densely populated and intensively cultivated region in southern Finland, and are notably more eutrophic than the other basins.

3.1.2 PAPER IV

The eight main study sites were widely distributed in Europe and Canada, and differed considerably in their characteristics (see paper **IV** for details). The sites were Miniera St. Valentino spring (Italy), Lumijoki River (Finland), Gromolo stream (Italy), Riera d´Osor (Spain), Charest River (Canada), Skalka spring (Poland), Riou-Viou (France), and the paleolimnologically investigated site Lake Orta (Italy). Also a comparative analysis using 15 springs selected from the CRENODAT dataset was conducted. The CRENODAT dataset consists of 110 springs, and it represents the outcome of a comprehensive multi-taxa and multidisciplinary project focusing on springs in the south-eastern Alps (Cantonati et al. 2010). In addition, we considered two geographically distant sites where deformations in *Achnanthidium pyrenaicum* (ADPY) were found (the spring in the south-eastern Alps, Italy, and the spring in the surroundings of St. Petersburg, Russia).

3.2 SAMPLING AND LABORATORY ANALYSIS

3.2.1 PAPERS I, II, III

In paper **I**, the samples were collected at each site nine times per summer between years 2006 – 2008. Sampling days were chosen so that starting from the 1st of June the summer was divided in periods of 20 days. Between every 20 days sampling period there was a gap of 20 days in the sampling. For every three periods, three sampling days were randomly picked (Underwood 1997). In papers **II** and **III**, the samples were collected at each site once per year in June or July. In paper **II** we used the data from three consecutive years (2010-2012) and in paper **III** the data contained only the year 2010. The same sampling period facilitated the comparison among the samples, as there is also intra-annual variation in diatom communities (see Korhonen et al. 2013) (**III**).

For sampling, a riffle was divided into ten transects across the stream at intervals of one meter (**I-III**). Ten stones were picked from these sections, one per section. Diatom samples were collected semi-quantitatively from the stones by using a toothbrush and a soft rubber plate, in which a hole of a size of 9 cm^2 was cut. For each stone, a same-sized sample was brushed, and finally all the ten subsamples were pooled into one sample container. Simultaneously to diatom sampling, studied environmental variables were measured at the stream sites (**II, III**). At each sampling occasion, a 500 ml water sample for total P and N analyses was collected as was a 250 ml sample for the water color analyses, and also pH, conductivity and water temperature were measured (**II, III**). For the year 2010, the values of current velocity, water depth, shading by the canopy and particle size in the study sites are found in paper **III**.

In papers **I-III**, organic material was removed from the diatom samples by boiling with hydrogen peroxide (30% H_2O_2). Cleaned diatoms were mounted in Naphrax® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK). A total of 500 frustules per sample were identified if possible to species level using Krammer & Lange-Bertalot (1986-1991). We followed Krammer & Lange-Bertalot´s nomenclature except that in papers **II** and **III** we used the most recent name for *Achnanthidium minutissimum* (Kützing) Czarnecki (1994). Frustules were counted using phase contrast light microscopy (magnification 1000X). Nutrient concentrations (P and N) were measured using an automated ion analyzer (Lachat Instruments, QuikChem® 8000 Series FIA System (FIA+)) (**II, III**). Water color was estimated visually using a comparator (**II, III**).

3.2.2 PAPER IV

Water samples for metals and trace elements were collected at each site. Generally, analyses were carried out in the same laboratory on 2-5 replicate water samples (IDPA-CNR, University of Venice, Italy), and sampling and analyses were repeated for two consecutive years (2010-2011). The only exception was Lumijoki River (Finland), which was sampled for the first time in 2012.

An Inductively Coupled Plasma Sector Field Mass Spectometry (ICP-SFMS; Element2, Thermo Fisher, Bremen, Germany) equipped with a desolvation system (APEX IR, Elemental Scientific, Omaha, US) was used to determine concentrations of 29 elements (Li, Be, Na, Mg, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Mo, Ag, Cd, Sn, Cs, Sb, Ba, Tl, Pb, Bi, U) in the samples taken from Miniera St. Valentino spring (Italy), Lumijoki River (Finland), Gromolo stream (Italy), and Skalka spring (Poland). The water samples collected from Riera d´Osor (Spain), Charest stream (Canada), Riou-Viou (France), and Orta Lake (Italy) were all analysed differently; see paper **IV** for details in water sampling and analytical methods.

For diatom sampling, preparation, and analyses, European standards (EN 13946 2003; EN 14407 2004; EN 14996, 2006) were followed whenever possible. The epilithic samples were obtained by scraping at least five boulders using a toothbrush, and the epibryon was collected by sampling entire bryophyte plants from at least three different points within the spring head (Cantonati et al. 2007). Diatom samples to be used for chemical digestion and permanent mount production were fixed and preserved in formalin solution (final conc. = 2-3%). An aliquot of the formaldehyde-fixed sample was cleaned using hydrogen peroxide (30%) and dichromate ($K_2Cr_2O_7$). Permanent slides were

Photo 4. *Graphical abstract of the study (***IV***). Photo: Nicola Angeli.*

prepared using Naphrax®. Diatom samples from Riera d´Osor (Spain), Charest stream (Canada), and Riou-Viou (France) were collected from different artificial substrates and treated differently (see paper **IV** for details). Material from Skalka spring (Poland) was collected by scraping from concrete. The sediment core in Lake Orta (Italy) (ORTA 07/2A) was collected with a gravity corer (inner diameter = 6.3 cm) and the sediments were dated by ^{210}Pb and ^{137}Cs (explained in more detail in paper **IV**).

Paper **IV** primarily focused on deformed ADMI frustules characterized by one (sometimes even two) more or less bent off endings, conferring to the specimens a cymbelloid outline ("*cymbelliclinum*"- like shape in the case of one single end). The deformities are sometimes very subtle (very-slight or slight asymmetry) or they can be marked deformities.

During quantification, ADMI CLT were carefully distinguished and categorized as "slightly" and "markedly" deformed. We considered "slight" deformities those teratologies in which the bending off of one ending could be perceived only by accurate inspection, while "marked" teratologies were those in which the bending off caused an evident distortion of the outline rendering the identification of the teratology obvious. Proportions of deformities were obtained counting 200-500 valves (total) in 1-4 replicates.

3.3 DATA ANALYSES

3.3.1 PAPER I

The General Linear Model (GLM) was used to analyse the relationships between temporal occurrence and species` niche characteristics, local abundance, regional distribution, body size and attachment ability. Temporal occurrence of diatom species was quantified as the number of sampling occasions that species was detected at sites and it ranged from 0 (indicating a particular species was, according to countings, not present at all) to 9 (a particular species was present at all countings within a single sampling season). Temporal occurrence was calculated as the mean value using the data from the three consecutive years.

We conducted two separate GLMs using two different data sets. In the first GLM we studied the relationship between temporal occurrence of species and its niche breadth and position. Values for diatom species` niche breadth and position were obtained from Heino and Soininen (2006). In the second GLM we examined the relationships between species` temporal occurrence and regional distribution, local abundance and species traits (i.e., body size and attachment ability). We collected the values for the regional distribution in Finland and maximum local abundance mainly from the diatom data sets used in Soininen et al. (2004) and Soininen (2008). Maximum local abundance was defined as a maximum relative abundance (%) that species gained in the same data set. Values for the species body size (cell minimum length, maximum length, minimum breadth and maximum breadth) were obtained from the literature (Krammer & Lange-Bertalot 1986-1991).

For the attachment ability of cells, we used two different classifications. First, we divided the diatom species into attached and non-attached species mainly according to Krammer and Lange-Bertalot (1986-1991). Secondly, we divided diatoms into three functional groups according to Passy (2007). Our purpose was also to review whether the classification of Passy (2007) is useful when considering the temporal occurrence of diatoms. In both GLM, the most parsimonious GLM models were identified using Akaike´s information criterion (AIC; Burnham & Anderson 2000). Finally, we conducted an Analysis of Variance (ANOVA) for testing the possible differences in occurrence among the functional groups and between attached and non-attached diatoms. For more detailed description about the analyses, see paper **I**.

3.3.2 PAPERS II, III

In papers **II** and **III**, Detrended Correspondence Analyses (DCA; Hill & Gauch 1980) were first run to assess the gradient lengths of the first axes in the diatom data from the sampling years. Gradient lengths along the first DCA axes were 3.5 SD or more, indicating that methods assuming a unimodal response model are best fitted for analysing these data. Then, we used Canonical Correspondence Analysis (CCA) to study the relationship between the diatoms and studied different local environmental variables (**II, III**) and in paper **III** also the coordinates of the sampling locations (which we used to represent the ultimate variables). We conducted CCA separately for each sampling year (**II**). CCA is a direct gradient analysis that uses both species and environmental data by combining ordination and regression techniques (ter Braak 1986). It is very widely used to relate the variation in biotic samples to long environmental gradients.

We then conducted Mantel tests to study the relationships among community dissimilarities between the sampling years and, also, the relationships between community dissimilarities and environmental and geographical distances (**II, III**). In paper **II**, we conducted Mantel tests separately for each year. Moreover, in paper **III** we ran partial Mantel tests using three matrices. The first matrix was the biotic matrix; the second matrix was either the environmental or geographical distance matrix (explanatory matrix), and the third matrix was the matrix whose effect was controlled for (either environmental or geographical distance matrix). Hence, with partial Mantel tests we examined the influence of environmental distance on community dissimilarity while controlling for geographical distance and vice versa. This was done to distinguish the pure effects of environmental variables and location on community composition. A partial Mantel test is thus equivalent to partial correlation. In short, Mantel test is a correlation of two dissimilarity or distance matrices. The significances of the relationships between community dissimilarities and distance matrices were assessed using 1000 randomizations.

In paper **II**, we also used Hierarchical Cluster Analyses to examine how the sampling sites cluster based on their diatom composition using the data from the first sampling year 2010. This particular clustering method defines the cluster distance between two clusters to be the maximum distance between their individual components (Yau 2013). At every stage of the clustering process, the two nearest clusters are merged into a new cluster. The process is repeated until the whole data set is agglomerated into one single cluster. We used this clustering method for the diatom data from year 2010 as a benchmark grouping where statistical significance of the species composition among the clusters was tested using Analysis of Similarities (ANOSIM; Clarke 1993). The ANOSIM statistic R is based on the difference of mean ranks between groups (r_B) and within groups (r_W). Then, ANOSIM analysis was conducted separately for the diatom data from years 2011 and 2012, using the benchmark groups derived from the year 2010 diatom data for the sites. If species composition does not vary temporally consistently between sites, we expected that ANOSIM results would show lower significance and lower statistic R for the years 2011 and 2012 than for the year 2010, which represented the year of the benchmark grouping. With ANOSIM, we also tested how temporally consistent are the differences in diatom community composition between four drainage basins.

In paper **II**, we calculated the IPS and TDI indices for each sampling site and for each sampling year using Omnidia 3.6 software (Lecointe et al. 1993). To study if the water quality inferred based on diatom indices changes annually within a basin, we conducted Analysis of Variance (ANOVA) separately for every basin to test for statistical differences among the sampling years. For more information about IPS and TDI indices, see paper **II**.

In paper **III**, to study overall patterns in the biotic and environmental data, we first conducted Detrended Correspondence Analysis (DCA) for diatom community composition and Principal Component Analysis (PCA) for the five significant local environmental variables identified by CCA (conductivity, depth, current velocity, total N and total P). We then conducted Analysis of Similarities (ANOSIM) to test statistically whether there were significant differences in diatom species composition and environmental variables between regions (Clarke 1993). In ANOSIM, we used only the five significant environmental variables identified by CCA. To test how strongly the individual regions differ from each other in their diatom species composition and in their environmental variables, we also conducted ANOVA with Tukey´s *post hoc* tests.

In paper **III**, we further used the indicator value method (IndVal) (Dufrene & Legendre 1997) to identify species that discriminated between the four basins. The indicator value of a taxon varies from 0 to 1, and it attains its maximum value when all individuals of a taxon occur at all sites of a single group. The method selects for indicator species based on both high specificity and high fidelity to a specific group (here a region). The significance of the indicator value for each species was tested with a Monte Carlo randomization procedure with 1000 permutations.

3.3.3 PAPER IV

In order to evaluate the relative contribution from natural (e.g. rock and soil dust) versus anthropogenic sources, trace element concentrations were expressed in the form of crustal enrichment factors. (EF_{Ba}). EF_{Ba} is defined as the concentration ratio of a given element to that of Ba (or any other conservative element which derives mainly from rock and soil dust) normalized to the same concentration ratio characteristic of the upper continental crust (Wedepohl 1995). However, given the large variations in the composition of rock and soil, enrichment factors within ~ ± times the mean crustal abundance (i.e. EF_{Ba} values ranging from ~ 0.1 to 10) do not likely demonstrate an input from sources other than rock and soil dust. See paper **IV** for more details concerning EF. The EFs approach was especially relevant for our study (**IV**) in cases where apparently low concentrations (e.g., in relation to human health issues) were nevertheless corresponding to situations above or well above the natural (crustal) occurrence. Unfortunately, barium was not measured in France and Spain, and EF_{Ba} values could thus not be calculated for Riou-Viou (France) and Riera d´Osor (Spain). However, several attempts to weigh and normalize the concentrations of trace elements (which occurred with high EF in several of the eight widely distributed sites) and to correlate them with the proportions of ADMI CLT failed. The attempts to explain the occurrence of these deformed frustules using only one (Cu) or two (Cu + Zn) metals were also unsuccessful.

Comparative analyses using the CRENODAT dataset and spatio-temporal observations for the Italian spring sites including Miniera St. Valentino spring were also conducted. See paper **IV** for more details.

3.3.4 SUMMARY OF DATA ANALYSES

All analyses in papers **I-IV**, except ANOVA and Tukey´s *post hoc* tests in paper **III**, were conducted using the R version 2.13.0 (**I, III**), 3.0.1 (**IV**) or 3.0.2 (**II**) (R Development Core Team 2009, 2010, 2013) applying the vegan package (Oksanen et al. 2007) (**II-III**) and also the labdsv package (Roberts 2005) (**III**). In paper **III**, ANOVA and Tukey´s *post hoc* tests were conducted using SPSS 15.0 (SPSS Inc., an IBM Company, Armonk, New York, USA). Average values of environmental variables, except pH, were log-transformed due to their skewed distributions if needed (**I-III**). See Table 1 for the summary of the main statistical analyses used in different papers.

Table 1. *Summary of the main statistical analyses and the number of study sites in different papers. See page 8 for abbreviations.*

PAPER	STUDY SITES	MAIN STATISTICAL METHODS
I	8	GLM, ANOVA
II	40	CCA, Mantel test, Hierarchical Cluster Analyses, ANOSIM, ANOVA
III	40	CCA, Mantel test, DCA, PCA, ANOSIM, ANOVA, IndVal
IV	8	EF

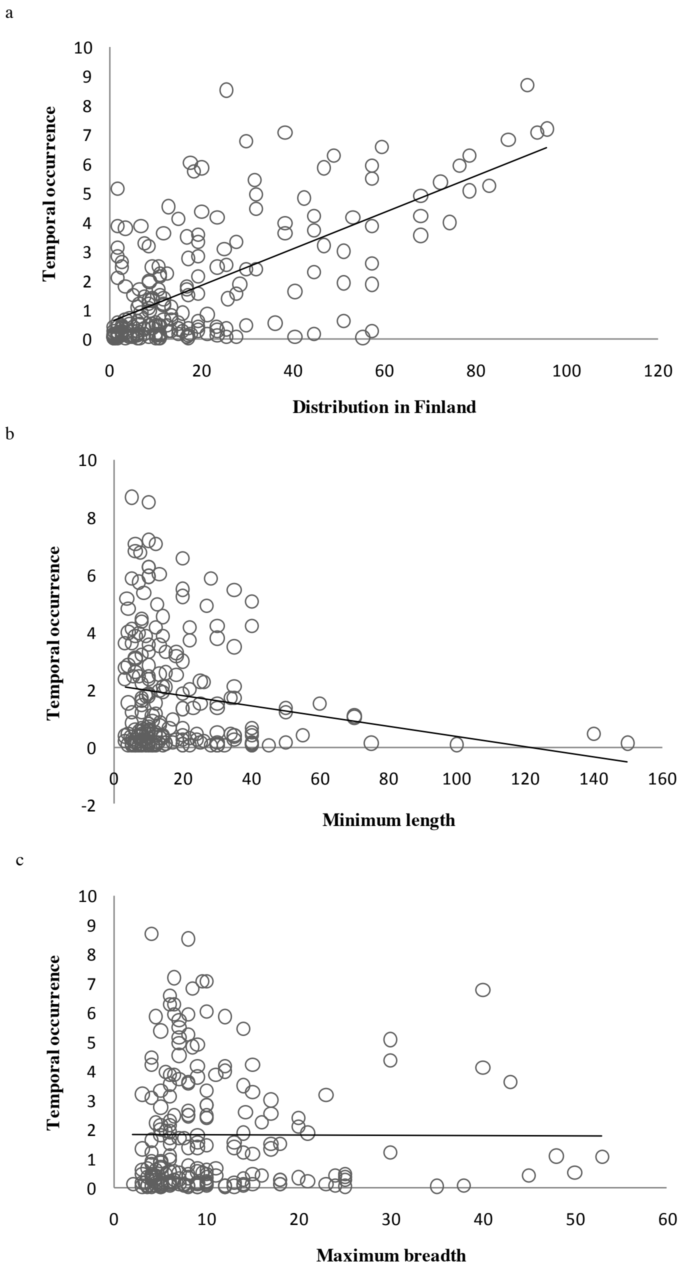
4 RESULTS

4.1 TEMPORAL VARIATION OF STREAM DIATOMS AND COMMUNITY-ENVIRONMENT RELATIONSHIPS (I, II)

We found a significant positive correlation between diatom species` temporal occurrence and species` niche breadth, whereas the correlation between species` temporal occurrence and niche position was significantly negative (**I**). Both of these factors were included in the most parsimonious model of the first GLM, and they jointly explained 21.3% of the variation in temporal occurrence.

The most parsimonius model of the second GLM analysis explaining the temporal occurrence included five factors (cell minimum length, attachment, cell maximum breadth, maximum local abundance and distribution) and they jointly explained 50% of the variation in temporal occurrence (Fig. 2) (**I**). Distribution, maximum local abundance and cell maximum breadth were all significant ($P< 0.05$) factors and they all correlated positively with the temporal occurrence. In contrast, attachment and cell minimum length were only marginally significant ($P < 0.1$). According to T-test, temporal occurrence of diatom species was larger if they were able to attach to substratum ($P= 0.02$).

When we used Passy`s (2007) classification, cell attachment did not enter into the best model (**I**). According to ANOVA, there were also no significant differences in temporal occurrence among the functional groups based on species attachment. We detected 243 diatom species in the data. Most of the species occurred at sites only sporadically. See paper **I** for details.


a
Temporal occurrence
10
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
Distribution in Finland
b
Temporal occurrence
10
8
6
4
2
0
-2
0
20
40
60
80
100
120
140
160
Minimum length
c
Temporal occurrence
10
9
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Maximum breadth

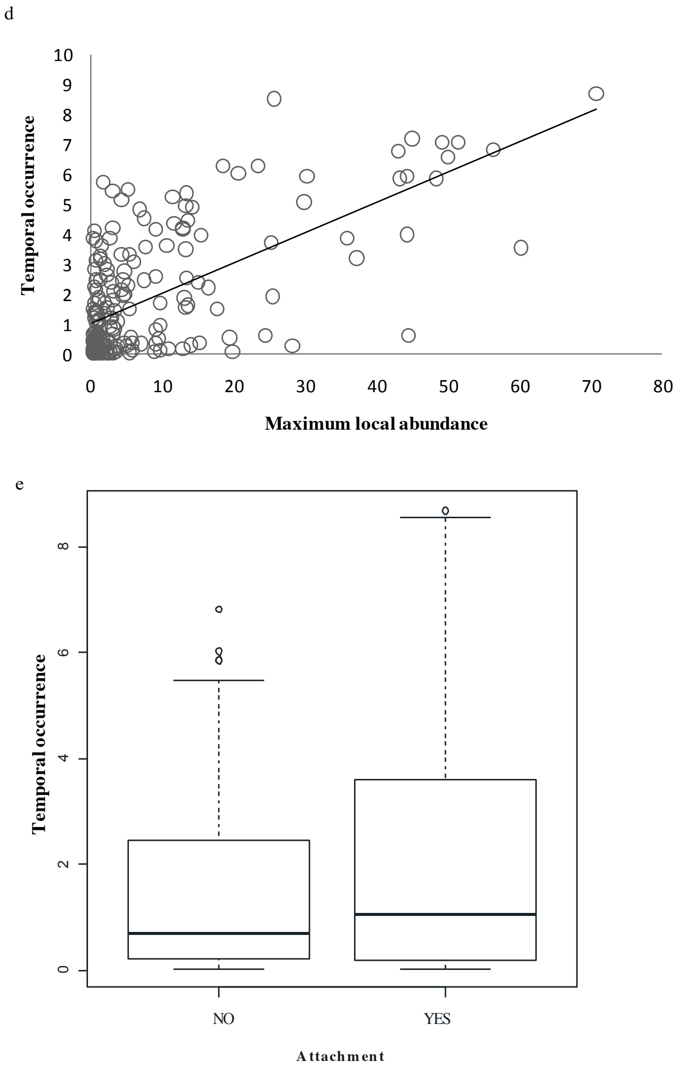

Fig. 2. *The relationships between species temporal occurrence and a) regional distribution, b) maximum local abundance, c) minimum length and d) maximum breadth. The box plot-figure (e) with median and maximum and minimum values presents the temporal occurrence for attached and non-attached species.*

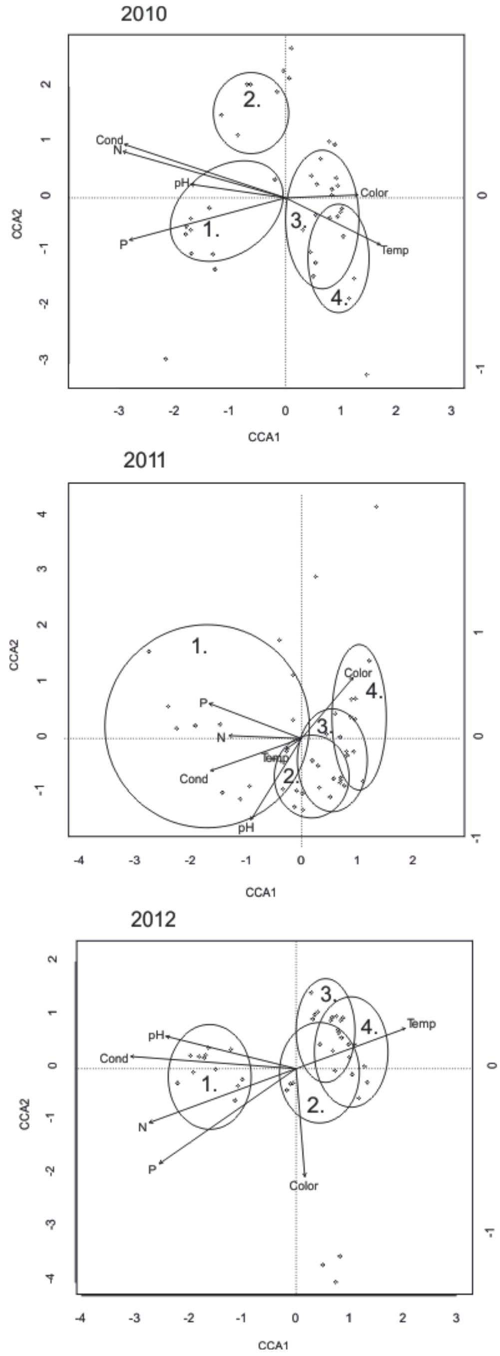

Fig. 3. *Ordination diagram showing the distributions of the basins, 1. Vantaanjoki basin, 2. Kokemäenjoki basin, 3. Kymijoki basin and 4. Vuoksi basin, and relative contributions of environmental variables in the Canonical Correspondence Analysis for each year. Ellipses encircle a minimum of 55% of sites belonging to a given group.*

At community level, variations in environmental conditions affect the patterns in temporal turnover (**II**). Total N and P, and water conductivity were the most significant contributors to the first CCA axis, when CCA was conducted separately for each sampling year (Fig. 3). In 2010, the first two axes explained jointly 18% of the variation in diatom data, in 2011 21% and in 2012 19%. The results of CCA are given in more detail in paper **II**.

According to the Mantel tests (**II**), the correlation coefficient (r) between community dissimilarities for the years 2010 and 2011 and for 2010 and 2012 was 0.660. Between the years 2011 and 2012, the correlation coefficient was 0.568. All these Mantel r values were statistically significant ($p < 0.001$) indicating that community dissimilarities remained similar between years.

The correlation coefficients between environmental distances and community dissimilarities were 0.589 for the year 2010, 0.520 for the year 2011 and 0.402 for the year 2012 and all correlations were statistically significant ($p < 0.001$) suggesting that environment has a consistent influence on communities through time. The correlation coefficients between community dissimilarities and geographical distance were 0.286 (year 2010), 0.210 (2011) and 0.192 (2012), all r values being statistically significant (2010 $p < 0.001$, 2011 $p = 0.009$ and 2012 $p = 0.005$).

4.2 STABILITY OF STREAM CLASSIFICATIONS AND DIATOM INDICES (II)

The Hierarchical Cluster Analysis divided sites into four different clusters (A—D) according to diatom data from the year 2010 (Fig. 4) (**II**). According to ANOSIM, the sites belonging to four clusters comprised significantly different species composition in 2010 (R = 0.7528, p < 0.001), which was expected (**II**). In the years 2011 and 2012, species compositions differed less than in 2010 among the clusters, yet differences were significant (2011: R = 0.5087, p < 0.001, 2012: R = 0.6498, p < 0.001). With ANOSIM, we also studied whether the basins differed in their diatom community composition in different years (**II**). In the year 2012, there were more substantial differences between regions (R = 0.5887, p < 0.001) than in the years 2010 (R = 0.498, p < 0.001) and 2011 (R = 0.2911, p < 0.001).

In paper **II**, to test in which basins biological water quality differed between years, we performed two ANOVAs using IPS and TDI indices. According to the ANOVAs, only in the Kymijoki basin the IPS and TDI

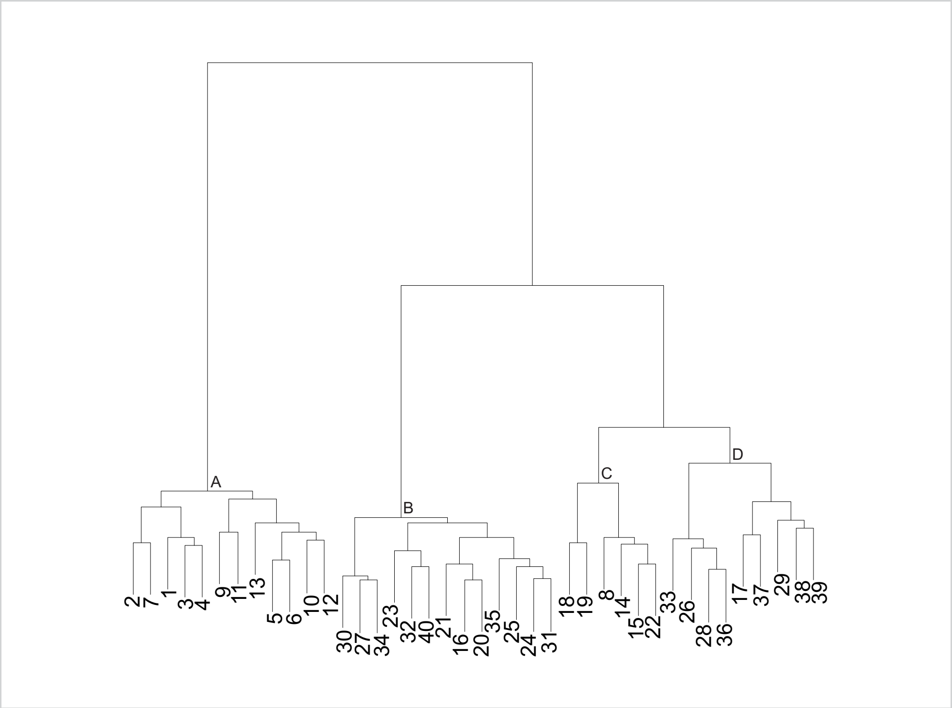

Fig. 4. *Hierarchical Cluster Analysis divided the sampling sites, using the diatom species data from the year 2010, in four clusters (A-D). The numbers refer to sampling sites as follows: 1-13 Vantaanjoki basin, 14-22 Kokemäenjoki basin, 23-31 Kymijoki basin and 32-40 Vuoksi basin.*

indices differed considerably among the sampling years. For both IPS and TDI indices, the results were marginally significant (p = 0.09 for IPS and p = 0.06 for TDI). There were no significant differences in water quality in the other basins between the sampling years.

4.3 SPATIAL TURNOVER OF STREAM DIATOM COMMUNITIES (III)

The regional differences in diatom community composition and regional differences in environmental variables were compared to see if diatom communities vary regionally more than the environmental variables (**III**). The results of ANOSIM revealed that the regions differ slightly more in their diatom community composition than in their environmental variables ($R = 0.5566$, $P < 0.001$ for the diatom community and $R = 0.5276$, $P < 0.001$ for the environmental variables). To test which regions differ from each other significantly, we performed ANOVA with Tukey´s *post hoc* tests for the diatom communities and environmental variables of the regions (**III**). Along DCA and PCA axes 1, the Vantaanjoki basin differed significantly ($P < 0.05$) from the other basins. For more detailed results, see paper **III**.

According to Mantel tests (**III**), the correlation coefficient (r) between geographical distance and community dissimilarity was 0.289 and statistically significant ($P < 0.001$) (Fig. 5). Similarly, the correlation coefficient between environmental distance and community dissimilarity was 0.341 and statistically significant ($P < 0.001$). The correlation coefficient between geographical and environmental distance was 0.102 and non-significant ($P = 0.091$). According to partial Mantel tests (**III**), the pure effect of geographical distance was significant ($P < 0.001$), the correlation coefficient being 0.272. Secondly, the pure effect of environmental distance was also significant ($P < 0.001$), the correlation coefficient being 0.327.

Altogether, 191 different diatom taxa were present in the data. Using IndVal, we identified 13 indicator species for the Vantaanjoki basin, 6 for the Kokemäenjoki basin, 4 for the Kymijoki basin and 20 for the Vuoksi basin. For more detailed description, see paper **III**.

4.4 DIATOM VALVE DEFORMITIES AS INDICATORS OF METAL ENRICHMENT (IV)

Based on the EF values (Table 2, **IV**), certain groups of sites were characterised by especially high concentrations of a metal (or metalloid). At three of the studied sites, Miniera St. Valentino spring (Italy), Lumijoki River (Finland) and Gromolo stream (Italy), the EF value for copper

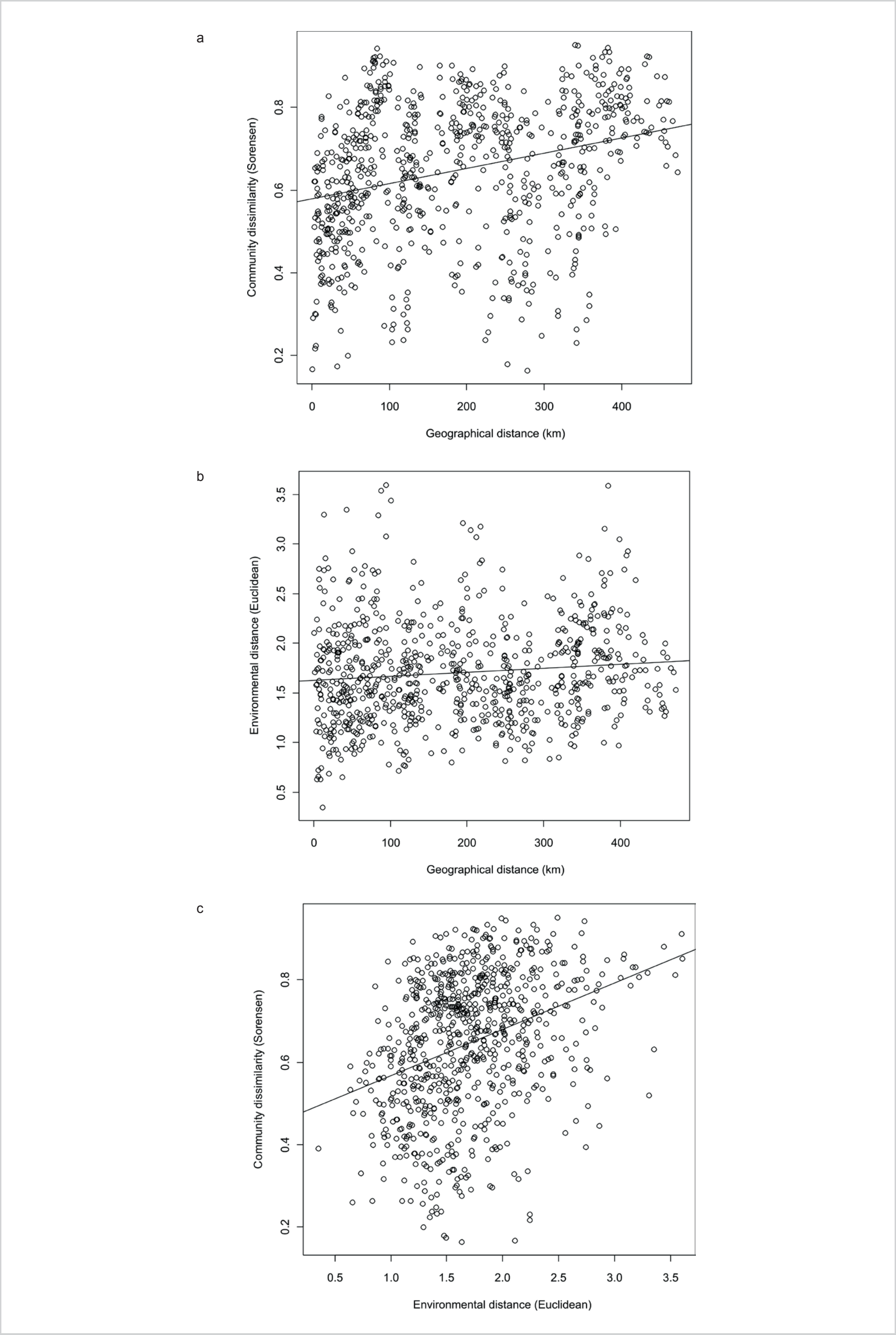

Fig. 5. *The relationships between a) geographical distance and community dissimilarity, b) geographical distance and environmental distance and c) environmental distance and community dissimilarity for the whole data set (n = 40 sites).*

was notably high. In turn, at two of the studied sites, Riera d´Osor (Spain) and Charest River (Canada), the EF value for zinc was notably high. In Skalka spring (Poland), antimony seemed to be the main pollutant. At Lake Orta (Italy), we also observed enrichment in copper, zinc, and chromium. Riou-Viou (France) presented heavy metal concentrations among the lowest found in the study (**IV**).

Characteristic CLT were found at the high copper and zinc sites (**IV**). At the antimony enriched site Skalka spring (Poland), marked CLT occurred as often as the slight teratologies, and the variant form characterised by both ends being bent off was very common. At Riou-Viou (France) and Lake Orta (Italy), teratologies were scarce and were categorised as slight. Quantitatively, the proportion of marked CLT ranged between 0 and 17%. The highest proportion of marked CLT was found at Skalka spring (Poland). The proportion of slight CLT ranged between 0.9 and 21.6%, while the overall proportion of CLT (including the both types of CLT) ranged between 0.9 (Riou-Viou (France)) and 38.6% (Skalka spring (Poland)).

The values at which deformities start to occur could be estimated at about 5 µg L^{-1} for Cu, several thousands (about 3000) µg L^{-1} for Zn, and about 1 µg L^{-1} for Sb (**IV**). The proportions of ADMI CLT were about 15–25% for Cu, 10–15% for Zn, and about 40% for Sb. Marked teratologies represented about 15–50% of the slight teratologies in the copper enriched sites, 0–25% in the zinc enriched sites, and as many as almost 80% in the antimony enriched site.

See the results of comparative analyses using the CRENODAT dataset, sites with ADPY CLT, and spatial and temporal observations at the Italian spring sites in paper **IV**. These results supported our findings from the eight main sites.

5 DISCUSSION

5.1 FACTORS AFFECTING TEMPORAL OCCURRENCE OF STREAM DIATOMS

In paper **I**, we showed that temporal occurrence was a correlate of diatom species traits. Our results indicated a significant positive correlation between temporal occurrence and cell maximum breadth, and a negative, marginally significant correlation between temporal occurrence and cell minimum length. The results support the fact that the smallest algae are more prone to physical disturbances and larger cells benefit from being not that easily detached from the bottom (Reynolds 2006). Although it remains unexplained why these species traits were the best reflecting the body size of diatoms, the cell size is undoubtedly important for species temporal occurrence.

In addition to size, occurrence was related to diatom life form, as the attachment ability generally increased the temporal occurrence of a species (**I**). This seems to support the hypothesis that if a diatom species is able to attach to substratum, it should occur more often in time because the cell is less prone to be detached from the bottom by the water current (Stevenson et al. 1996; Allan & Castillo 2008). We also considered how well the temporal occurrence of diatom cells corresponded to the functional classification of Passy (2007) (**I**). The low motile species were expected to be the most persistent because of their ability to attach and their prostrate growth habit (Mccormick & Stevenson 1991). The results, however, showed that the growth form of diatom cells is likely to be irrelevant for their temporal occurrence. Regardless, we admit the functional value of different diatom growth morphologies when studying species-environment interactions and their potential use in ecological assessments (see Passy 2007).

The diatom species that had wide niche breadths also occurred frequently in time (**I**). This indicates that in fluctuating environmental conditions, such as in streams, a species that is capable of tolerating variable conditions has viable and long-lasting populations (Brown 1984; Sultan et al. 1998). We further found that the species that have marginal niche position also occur only rarely in time, which was expected (**I**). These two results jointly indicate that the species niche characteristics are important for species occurrence in time, agreeing with Heino and Soininen (2006) who showed that species niches strongly affect diatom distribution in space.

In addition to niche characteristics, distribution of diatom species seemed to influence their occurrence in time. We found a positive correlation between species` temporal occurrence at sites and species` maximum local abundance in the diatom data (**I**). This supports the hypothesis that if a species is able to sustain large population sizes, it also occurs frequently in time; possibly indicating that high abundance prevents local extinctions (Soininen & Heino 2005). There was also a positive correlation between species´ temporal occurrence and regional distribution (**I**). Although, there is no empirical evidence, the result may signify that widely distributed species may easily disperse to a site via many dispersal events from several source locations, thus resulting in long-lasting populations. It agrees with some early ideas on rescue effects on populations in patchy habitats (Brown & Kodric-Brown 1977). We highlight though that the local abundance and regional distribution were positively related in the data (**I**). Therefore, their relative roles are not easy to disentangle. Yet, they seemed to have their independent roles as both were selected independently into the best subset GLM (**I**)

.

Current rates of climate change are unprecedented, and biological responses to these changes have also been fast at the level of lotic ecosystems, species and communities (Heino et al. 2009). Climate change can cause changes in both proximate and ultimate variables, which are the most prominent factors affecting diatom species occurrence. E.g. predicted changes in precipitation patterns affect water chemistry, current velocity and water depth that were noted among the most important environmental factors in addition to location in paper **III**.

5.2 TEMPORAL VARIATION IN DIATOM COMMUNITY-ENVIRONMENT RELATIONSHIPS

According to the ordination results, nutrient concentrations and conductivity are the most important environmental factors affecting diatom communities in all sampling years (**II**). This is well in line with the findings of Bere & Tundisi (2011), Smucker & Vis (2011), and Stevenson (2014), showing that diatoms respond consistently to nutrient concentrations and overall level of ionic concentration (conductivity) in the water and, thus, are good indicators for chemical water quality. However, other environmental variables, such as current velocity, water depth and shading by the canopy might also affect diatom turnover. In paper **III**, we showed that current velocity and water depth were also significant factors for the diatom community structure in boreal streams while shading had only minor importance. According to the Finnish Meteorological Institute, the precipitation in the study area was lower in 2010 than in the years 2011 and 2012. In 2012, the precipitation was exceptionally high in the basins Vantaanjoki and Vuoksi. However, these differences in hydrological conditions do not appear to have had a major impact on community patterns as diatom community patterns remained largely the same across the streams in all three years based on Mantel tests (for more detailed description, see paper **II**).

Climate change is causing water temperature to increase. It may be that temperature is somewhat less important of a factor for diatoms than water chemistry, disturbance and light (but see Patrick 1971). Yet, the major factors controlling diatom distribution in nature are often highly correlated (Anderson 2000). Climate change is going to affect diatom community-environment relationships mostly indirectly but also directly through increasing rates of cell metabolism (Raven & Geider 1988). Species that respond with faster growth rates to increased temperatures should be more abundant in the community, although temperature is rarely the main factor controlling the diatom growth (Anderson 2000).

5.3 STABILITY OF STREAM CLASSIFICATIONS AND DIATOM INDICES

Using the diatom species data from the year 2010, the Hierarchical Cluster Analysis divided the sampling sites into four statistically distinct clusters (**II**). The site clusters comprised significantly different species composition for all sampling years, even if differences were more substantial for the benchmark data from the year 2010 based on ANOSIM. According to these results diatom based stream classifications are stable in time, at least for three years that our sampling covered.

ANOSIM analyses further showed that the four sampling basins differed in their diatom community composition at all sampling years and the differences among the R values were notable. This outcome was expected, as the basins differed in environmental conditions and were also located geographically relatively far away from each other. For more details, see paper **II**.

According to ANOVA, the values of IPS and TDI indices in sampled basins remained almost the same among the years (**II**). There was a marginally significant difference in the indices in the Kymijoki basin, but in the other basins no significant annual variation in diatom indices was found. The causes of variation of the indices at the Kymijoki basin remained unsolved, but it may be related to the more variable hydrological conditions in this region. The Vantaanjoki basin was the only basin studied that is exposed to severe human impact, showing also the lowest values of IPS index and the highest values of TDI index. However, in the Vantaanjoki basin, the values of diatom indices varied annually as little as in the other basins. Thus, during our sampling period, human impact did not seem to have an effect on temporal stability of diatom indices. However, on a longer time scale, global warming caused by humans is going to affect diatom communities and thus stream classifications. It is yet unclear whether these longterm changes in diatoms reflect changes in climate *per se*, or water quality changes resulting from vegetation-soil interactions mediated by climate (Engstrom & Hansen 1985).

5.4 SPATIAL TURNOVER OF STREAM DIATOM COMMUNITIES

In addition to water chemistry and physics, geographical location appeared as a significant factor for diatom communities, both in CCA and in Mantel tests (paper **III**). As geographical distance did not correlate significantly with environmental distance, it seems evident that, at least at this scale, environment does not change consistently with geographical gradients. Moreover, a highly significant pure effect of spatial variables on diatom communities was found, suggesting that there may be factors related to history or dispersal also driving the diatom communities, even at the relatively small geographical scale that was considered here. Environment and space seemed to be almost equally strong in affecting the communities based on the results of the partial Mantel tests. This provides evidence that diatom communities are not only structured by local water chemistry, but also by large-scale processes (Soininen et al. 2004; Vyverman et al. 2007; Smucker &Vis 2011). Yet, even if diatoms could respond to climate change by colonizing new regions, the community structure also reflects water chemistry and physical disturbance and not only rapidly rising temperatures (Anderson 2000).

When ANOSIM was used to test whether there are statistically significant differences in diatom species composition and environmental variables between regions, it was found that diatom species composition differed more among regions than the measured environmental variables (**III**). Considering this regional variation is essential in diatom monitoring. For example, two streams that are in a pristine state, both having excellent water quality, may have different species composition if streams are located in different regions, presuming that the composition is not determined only by local environmental factors. Therefore, regional stratification based, for example, on ecoregions that may have unique and non-overlapping diatom flora for each water quality class, could be beneficial for bioassessments (Soininen et al. 2004).

5.5 DIATOM VALVE DEFORMITIES AS INDICATORS OF METAL ENRICHMENT

In fact, ADMI is a species complex, consisting of several morphologically similar species (Potapova & Hamilton

2007). Considering all the species contributing to the ADMI complex, CLT were observed to affect mainly (although not only) *Achnanthidium minutissimum sensu stricto*. ADMI s.s. was by far the most common and abundant species of the ADMI complex in the eight main sites (**IV**). The taxonomy of ADMI is complicated but constant progress is being made (e.g. Ector 2011) and future work will allow for an appropriate and circumscribed application of the CLT concept as an indicator of selected metals or metalloids. Cattaneo et al. (2004) found that abundance of *Achnanthidium minutissimum* was positively related to all metals, but especially with Cu, Fe, and Zn. Valve deformations in ADMI are also associated in particular with Cu, whereas cell size reduction appears to be a general response of the species to elevated metal concentrations. While numerous studies relate ADMI deformities and metal contamination, no data from the literature linked physical stressors such as osmotic stress, desiccation, and disturbance with teratological forms of the species (Francoeur & Biggs 2006; Passy 2007; Falasco et al. 2009b).

The results from the analyses of EF and concentrations of metals and metalloids in the eight sites studied (**IV**), as well as the observations on the potential of different trace elements to trigger the occurrence of ADMI CLT (in 15 springs carefully selected from the 110-springs CRENODAT data set), led to the hypothesis that two metals (copper and zinc) and one metalloid (antimony) were involved. From a quantitative point of view, it is worth noting that the lowest concentrations triggering ADMI CLT can be fairly low, particularly in the case of copper. ADMI also appears to be well-adapted for colonizing metal-enriched (Cu, Zn, Sb) sites both with normal and CLT specimens. The definition of their morphological (particularly slight deformities) and quantitative characteristics (relative abundance with respect to the total number of ADMI), along with a better taxonomic circumscription of the affected species, should allow for the use of deformities as a reliable indicator of Cu, Zn or Sb enrichment in a wide variety of freshwater habitats. Typically, the rates of metal uptake and accumulation increase with increasing temperature (Cairns et al.1975; McLusky et al. 1986; Hutchins et al. 1996; Heugens et al. 2002). Environmental temperature can also affect the bioavailability of metals due to the higher solubility of metal compounds, and thus higher concentrations of free metal ions (Sokolova & Lannig 2008). As temperatures are rising due to climatic change, heavy metals effects on diatoms need more studying.

5.6 SOME METHODOLOGICAL ASPECTS

Traditional diatom samples, such as those that were used in this study, consist of living cells containing chloroplast and dead empty cells, which are not distinguished from each other (Gillett et al. 2009). Acid or hydrogen peroxide digestion is used to remove the organic material and to allow precise diatom identification. The method is based on the assumption that the abundance of live diatoms is proportional to the abundance of dead diatoms (e.g., the most abundant live species is also the most abundant dead species), so the distinction between the living and dead cells is not important. However, dead diatoms might comprise local species, euplankton, and dislodged individuals from upstream.

It is yet unclear whether differentiating live and dead diatoms in a sample would contribute significantly to the accuracy and precision of diatom-based bioassessment. Gillett et al. (2009) documented that counts that considered alive cells only vs. the counts that considered both alive and dead cells resulted in similar environment-community relationships. Several researchers have, however, suggested that assemblages generated by the traditional method may better integrate spatial and temporal variability of the sampled stream and its watershed because the accumulation of live and dead diatoms over space and time may provide extensive environmental information (Cox 1998; Stevenson & Pan 1999). Though, Pryfogle & Lowe (1979) showed that the traditional counting technique might mask the specific response of the local taxa to the local environmental conditions and introduce more noise to the data by its inability to distinguish dead diatoms. Also Round (1998) has suggested that the dead diatoms from adjacent habitats might be a significant source of error when assessing diatom communities and their local environment.

Differences in diatom identification can also be a source of error when comparing diatom communities and ecological status class boundaries (Prygiel et al. 2002, Kahlert et al. 2009). In paper **I**, the samples had several analysts. However, Kahlert et al. (2012) concluded that the error introduced by different species identification has little effect on indices and the resulting interpretation of ecological state, when countings by different analysts were compared. Yet, care should be taken especially when identifying broken valves or small taxa and when cells are seen in girdle view. In addition, comparable samples should be collected in the same period of time at different years, as was done in paper **II**, because there is significant intra-annual variation in diatom communities (Korhonen et al. 2013).

6 CONCLUSIONS AND IMPLICATIONS FOR BIOMONITORING

In conclusion, diatoms are good indicators of stream water quality over longer time scales, which is beneficial for stream assessment programs. *Based on the results, sampling of diatoms, e.g., in every three years seems to be a relatively reliable procedure to assess biological water quality at least in boreal streams, as diatom inferred stream classifications remained temporally stable and statistically distinct throughout the years (***II***). Furthermore, the values of IPS and TDI indices remained relatively stable at the basins and mainly showed non-significant between-year changes.* No significant annual variation in community-environment relationships was found in diatoms, water chemistry consistently being the main factor structuring communities (**II**). Regardless, caution is needed as in streams where variation of water quality is known to be especially high, peaks in nutrient load might remain unnoticed. On the whole, it is important for decision-makers to evaluate the sensitivity of their decisions to possible classification inaccuracies and continually review classifications. *It also must be borne in mind that local diatom community composition also reflects large-scale factors, such as dispersal processes, climate and history (***III***). Characterizing spatial variation in communities may help to estimate the effects of regional species pools on local communities.* In the future, researchers should be encouraged to further study temporal and spatial turnover in freshwater diatom communities as such information is highly needed to support decision making. Climatic change and its impact on diatom communities' turnover is expected to increase in the future, thus modifying the patterns.

*Additionally, the results showed that temporal occurrence of diatom species in streams is associated with species ecological niche size and position (***I***). The species distribution in Finland, their abundance, cell size and ability to attach also affect species occurrence in time. The most persistent diatom species are thus perhaps the most reliable species to be used as indicators of water quality*. In general, the choice of the metric used to assess water quality is of prime importance. Usually both biological and physicochemical metrics are needed to produce sufficient information, because biological and chemical factors reflect change at different temporal scales.

Based on our results as well as those of others, we also conclude that changes in both community composition and morphology of species, can be used as indicators of heavy metal loading (**IV**). The occurrence of deformed ADMIs are a distinct indication of metal contamination. Moreover, ADMI seems to never deform from stressors other than heavy metal enrichment.

The specific type of deformities and their proportions observed in very diverse types of freshwater habitats emphasizes their potential for being used as a heavy metal indicator (in particular for Cu, Zn and Sb contamination). The other advantage of focusing mainly on the ADMI complex is its very wide geographic distribution. However, ADMI is very tolerant to metals as the proportions of markedly deformed valves are low, and the deformations originating from metal contamination consist mainly in a majority of slight, easily overlooked deformations. Reliable quantification of especially slight ADMI CLTs thus requires high expert knowledge.

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REFERENCES

Adler, P.B. & W.K. Lauenroth. 2003. The power of time: spatio-temporal scaling of species diversity. Ecol. Lett. 6: 749-756.

Adler, P.B., E.P. White, W.K. Lauenroth, D.M. Kaufman, A. Rassweiler & J.A. Rusak. 2005. Evidence for a general species-time-area relationship. Ecology 86: 2032-2039.

Allan, J.D. & M.M. Castillo. 2008. Stream ecology. Springer, Dordrecht, Netherlands, 372 pp.

Anderson, N.J. 2000. Miniview: Diatoms, temperature and climatic change. Eur. J. Phycol. 35: 307-314.

Baytut, Ö. 2013. A study on the phylogeny and phylogeography of a marine cosmopolite diatom from the southern Black Sea. Oceanol. Hydrobiol. St. 42: 406-411.

Bere, T. & J.G. Tundisi. 2011. Influence of ionic strength and conductivity on benthic diatom communities in a tropical river (Monjolinho), São Carlos-SP, Brazil. Hydrobiologia 661: 261-276.

Biggs, B.J.F., M.J. Duncan, I.G. Jowett, J.M. Quinn, C.W. Hickey, R.J. Davies-Colley & M.E. Close. 1990. Ecological characterization, classification, and modeling of New-Zealand rivers – An introduction and synthesis. New Zeal. J. Mar. Fresh. 24: 277-304.

Blinn, D.W., A. Fredericksen & V. Korte. 1980. Colonization rates and community structure of diatoms on three different rock substrata in a lotic system. Br. phycol. J. 15: 303-310.

Brown, J.H. 1984. On the relationship between abundance and distribution of species. Am. Nat. 124: 255-279.

Brown, J.H., J.F. Gillooly, A.P. Allen, V.M. Savage & G.B. West. 2004. Toward a metabolic theory of ecology. Ecology 85: 1771-1789.

Brown, J.H. & A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration on extinction. Ecology 58: 445-449.

Burnham, K.P. & D.R. Anderson. 2000. Model Selection and Inference: A Practical Information-Theoric Approach. Springer, New York, USA, 353 pp.

Cairns, J. Jr., A.G. Heath & P.C. Parker. 1975. The effects of temperature upon the toxicity of chemicals to aquatic organisms. Hydrobiologia 47: 135-171.

Cantonati, M., E. Bertuzzi & A. Scalfi. 2010. CRENODAT (Biodiversity assessment and integrity evaluation of springs of Trentino - Italian Alps – and long-term ecological research): project design and preliminary results. In: E. Beheim, G.S. Rajwar, M. Haigh & J. Krecek (eds.). Integrated Watershed Management: Perspectives and Problems. Proceedings of the Headwater ´05 Conference, Bergen, Norway, June 2005. Springer Publishers, Netherlands, p.121-132.

Cantonati, M., L. Füreder, R. Gerecke, I. Jüttner & E.J. Cox. 2012. Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. Freshwater Science 31:463–480.

Cattaneo, A., A. Asioli, P. Comoli & M. Manca. 1998. Organisms´ response in a chronically polluted lake supports hypothesized link between stress and size. Limnol. Oceanogr. 43: 1938-1943.

Cattaneo, A., Y. Couillard, S. Wunsam & M. Courcelle. 2004. Diatom taxonomic and morphological changes as indicators of metal pollution and recovery in Lac Dufault (Québec, Canada). J. Paleolimnol. 32: 163-175.

Cattaneo, A., T. Kerimian, M. Roberge & J. Marty. 1997. Periphyton distribution and abundance on substrata of different size along a gradient of stream trophy. Hydrobiologia 354: 101-110.

CEMAGREF. 1982. Etude des méthodes biologiques quantitatives d'appréciation de la qualité des eaux. Rapport Division Qualité des Eaux Lyon, Agence financière de Bassin Rhone - Méditerranée – Corse, Pierre-Bénite.

Chase, J.M. 2003. Community assembly: when should history matter? Oecologia 136: 489-498.

Cholnoky-Pfannkuche, K. 1971. Abnormaler Formenwechsel von *Nitzschia palea* in Kultur. Nova Hedwigia 21: 883-886.

Clarke, K.R. 1993. Non-parametric multivariate analysis of changes in community structure. Aust. J. Ecol. 18: 117-143.

Cluis, D.A. 1972. Relationship between stream water temperature and ambient air temperature. Nord. hydrol. 3: 65-71.

Coste, M. & H. Ayphassorho. 1991. Etude de la qualité des eaux du Bassin Artois-Picardie à l`aide des communautés de diatomées benthiques (Application des indices diatomiques). Rapport Cemagref, Bordeaux , Agence de l`Eau Artois-Picardie, Douai, 227 pp.

Cox, E.J. 1998. A rationale and some suggestions for developing rapid biomonitoring techniques using identification of live diatoms. In Proceedings of the 15th International Diatom Symposium: 43-50.

Czarnecki, D.B. 1994. The freshwater diatoms culture collection at Loras College, Dubuque, Iowa. In: Kociolke, J.P. (ed.). Proceedings of the 11 International Diatom Symposium. Memoirs of the California Academy of Sciences, p. 155-174.

Davies-Colley, R.J., C.W. Hickey, J.M. Quinn & P.A. Ryan. 1992. Effects of clay discharges on streams. Hydrobiologia 248: 215-234.

Debenest, T., J. Silvestre, M. Coste, F. Delmas & E. Pinelli. 2008. Herbicide effects on freshwater benthic diatoms: induction of nucleus alterations and silica wall abnormalities. Aquat. Toxicol. 88: 88-94.

Descy, J.P. 1979. A new approach to water quality estimation using diatoms. Nova Hedwigia/ Beiheft 64: 305–323.

Descy, J.P. & M. Coste. 1991. A test of methods for assessing water quality based on diatoms. Verh. Internat. Verein. Limnol. 24: 2112–2116.

Dufréne, M. & P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67: 345-366.

Eloranta, P. 1995. Type and quality of river waters in central Finland described using diatom indices. In: D. Marino & M. Montresor (eds.). Proceedings of the 13th International Diatom Symposium, 1–7 September 1994, Aquafredda di Maratea, Italy. Koeltz Scientific Books, Koeningstein, p. 271–280.

Ector, L. 2011. 1st European Workshop on Diatom Taxonomy (1st EWDT). Algol. Stud.136/137: 1-4.

EN 13946. 2003. Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms for rivers. Comité Européen de Normalisation (CEN).

EN 14407. 2004. Water quality. Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. Comité Européen de Normalisation (CEN).

EN 14996. 2006. Water quality. Guidance on assuring the quality of biological and ecological assessments in the aquatic environment. Comité Européen de Normalisation (CEN).

Engstrom, D.R. & B.C.S. Hansen. 1985. Postglacial vegetational change and soil development in southeastern Labrador as inferred from pollen and chemical stratigraphy. Can. J. Bot. 63: 543-561.

Estlander, S., L. Nurminen, M. Olin, M. Vinni & J. Horppila. 2009. Seasonal fluctuations in macrophyte cover and water transparency of four brown-water lakes – implications for crustacean zooplankton in littoral and pelagic habitats. Hydrobiologia 620: 109-120.

European Parliament, Council. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy (The EU Waterframework Directive).

Falasco, E., F. Bona, G. Badino, L. Hoffmann & L. Ector. 2009a. Diatom teratological forms and environmental alterations: a review. Hydrobiologia 623: 1-35.

Falasco, E., F. Bona, M. Ginepro, D. Hlúbiková. L. Hoffmann & L. Ector. 2009b. Morphological abnormalities of diatom silica walls in relation to heavy metal contamination and artificial growth conditions. Water SA 35(5): 595-606.

Feminella, J.W., M. E. Power & V.H. Resh. 1989. Periphyton responses to invertebrate grazing and riparian canopy in three northern California coastal streams. Freshwater Biol. 22: 445-457.

Fenchel, T. & B. J. Finlay. 2004. The ubiquity of small species: patterns of local and global diversity. Bioscience 54: 777-784.

Finlay, B.J. 2002. Global dispersal of free-living microbial eukaryote species. Science 296: 1061-1063.

Francoeur, S.N. & B.J.F. Biggs. 2006. Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. Hydrobiologia 561: 59-69.

Gillett, N., Y. Pan & C. Parker. 2009. Should only live diatoms be used in the bioassessment of small mountain streams? Hydrobiologia 620: 135-147.

Gómez, N. & M. Licursi. 2003. Abnormal forms in *Pinnularia gibba* (Bacillariophyceae) in a polluted lowland stream from Argentina. Nova Hedwigia 77: 389-398.

Granetti, B. 1968. Alcune forme teratologiche comparse in colture di Navicula minima Grun. e Navicula seminulum Grun. Giorn. Bot. Ital. 102: 469-484.

Heino, J., L.M. Bini, S.M. Karjalainen, H. Mykrä, J. Soininen, L.C.G. Vieira & J.A.F. Diniz. 2010. Geographical patterns of micro-organismal community structure: are diatoms ubiquitously distributed across boreal streams? Oikos 119: 129-137.

Heino, J. & J. Soininen. 2006. Regional occupancy in unicellular eukaryotes: a reflection of niche breadth, habitat availability or size-related dispersal capacity? Freshwater Biol. 51: 672-685.

Heino, J., R. Virkkala & H. Toivonen. 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biol. Rev. 84: 39-54.

Heugens, E.H.W., A.J. Hendriks, T. Dekker, N.M. van Straalen & W. Admiraal. 2002. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Crit. Rev. Toxicol. 31(3): 247–284.

Hill, M.O. & H.G. Gauch. 1980. Detrended Correspondence-Analysis – an improved ordination technique. Vegetatio 42: 47-58.

Hillebrand, H. & T. Blenckner. 2002. Regional and local impact on species diversity – from pattern to processes. Oecologia 132: 479-491.

Hillebrand, H., F. Watermann, R. Karez & U.G. Berninger. 2001. Differences in species richness patterns between unicellular and multicellular organisms. Oecologia 126: 114-124.

Hirst, H., I. Jüttner & S.J. Ormerod. 2002. Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. Freshwater Biol. 47: 1752-1765.

Horne, A.J. & Goldman, C.R. 1994. Limnology, 2nd edt. McGraw-Hill, New York, xii, 576 pp.

Horppila, J., S. Estlander, M. Olin, J. Pihlajamäki, M. Vinni & L. Nurminen. 2011. Gender-dependent effects of water quality and conspecific density on the feeding rate of fish – factors behind sexual growth dimorphism. Oikos 120: 855-861.

Hutchins, D.A., J.L. Teyssié, F. Boisson, S.W. Fowler & N.S. Fisher. 1996. Temperature effects on uptake and retention of contaminant radionuclides and trace metals by the brittle star Ophiothrix fragilis. Mar. Environ. Res. 41: 363–378.

Håkansson, H. & V. Chepurnov. 1999. A study of variation in valve morphology of the diatom Cyclotella meneghiniana in monoclonal cultures: effect of auxospore formation and different salinity conditions. Diatom Res. 14: 251-272.

Jüttner, I., P.J. Chimonides & S.J. Ormerod. 2012. Developing a diatom monitoring network in an urban river-basin: initial assessment and site selection. Hydrobiologia 695: 137-151.

Kahlert, M., R-L. Albert, E-L. Anttila, R. Bengtsson, C. Bigler, T. Eskola, V. Gälman, S. Gottschalk, E. Herlitz, A. Jarlman, J. Kasperoviciene, M. Kokociński, H. Luup, J. Miettinen, I. Paunksnyte, K. Piirsoo, I. Quintana, J. Raunio, B. Sandell, H. Simola, I. Sundberg , S. Vilbaste & J. Weckström. 2009. Harmonization is more important than experience – results of the first Nordic-Baltic diatom intercalibration exercise 2007 (stream monitoring). J. Appl. Phycol. 21: 471-482.

Kahlert, M., M. Kelly, R-L. Albert, S. F.P. Almeida, T. Bešta, S. Blanco, M. Coste, L. Denys, L. Ector, M. Fránková, D. Hlúbiková, P. Ivanov, B. Kennedy, P. Marvan, A. Mertens, J. Miettinen, J. Picinska-Fałtynowicz, J. Rosebery, E. Tornés, S. Vilbaste & A. Vogel. 2012. Identification versus counting protocols as sources of uncertainity in diatom-based ecological status assessment. Hydrobiologia 695: 109-124.

Kelly, M., S. Juggins, R. Guthrie, S. Pritchard, J. Jamieson, B. Rippey et al. 2008. Assessment of ecological status in U.K. rivers using diatoms. Freshwater Biol. 53: 403-422.

Kelly, M. G., C.J. Penny & B.A. Whitton. 1995. Comparative performance of benthic diatom indices used to assess river water quality. Hydrobiologia 302: 179–88.

Kelly, M.G. & B.A. Whitton. 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. J. Appl. Phycol. 7: 433–444.

Korhonen, J.J., P. Köngäs & J. Soininen. 2013. Temporal variation of diatom assemblages in oligotrophic and eutrophic streams. Eur. J. Phycol. 48: 141-151.

Korhonen, J.J., J. Soininen & H. Hillebrand. 2010. A quantitative analysis of temporal turnover in aquatic species assemblages across ecosystems. Ecology 91: 508-517.

Krammer, K. & H. Lange-Bertalot. 1986-1991. Bacillariophyceae. Süßwasserflora von Mitteleuropa, 2 (1-4). Fischer, Stuttgart, Germany, 521 pp.

Lange, K., A. Liess, J.J. Piggott, C.R. Townsend & C.D. Matthaei. 2011. Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. Freshwater Biol. 56: 264-278.

Lavoie, I., S. Campeau, F. Darchambeau, G. Cabana & P.J. Dillon. 2008. Are diatoms good integrators of temporal variability in stream water quality? Freshwater Biol. 53: 827-841.

Lavoie, I., M. Lavoie & C. Fortin. 2012. A mine of information: benthic algal communities as biomonitors of metal pollution leaching from abandoned tailings. Sci. Total Environ. 425: 231-241.

Lecointe, C., M. Coste & J. Prygiel. 1993. Omnidia: software for taxonomy, calculation of diatom indices and inventories management. Hydrobiologia 269: 509-513.

Lehman, C. & D. Tilman. 2000. Biodiversity, stability, and productivity in competitive communities. Am. Nat. 156: 534-552.

Locker, F. 1950. Beiträge zur Kenntnis des Formwechsels der Diatomeen an Hand von Kulturversuchen. Österr. Bot. Z. 97: 322-332.

Marquet, P.A., S.A. Navarrete & J.C. Castilla. 1990. Scaling population-density to body size in rocky intertidal communities. Science 250: 1125-1127.

McFarland, B.H., B.H. Hill & W.T. Willingham. 1997. Abnormal Fragilaria spp. (Bacillariophyceae) in streams impacted by mine drainage. J. Freshwater Ecol. 12: 141-149.

McLusky, D.S., V. Bryant & R. Campbell. 1986. The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. Oceanogr. Mar. Biol. Annu. Rev. 24: 481–520.

Mccormick, P.V. & J. Cairns. 1994. Algae as indicators of environmental-change. J. Appl. Phycol. 6: 509-526.

Mccormick, P.V. & R.J. Stevenson. 1991. Mechanisms of benthic algal succession in lotic environments. Ecology 72: 1835-1848.

McIntosh, A.R. & Townsend, C.R. 1996. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behaviour? Oecologia 108: 174-181.

Morin, S., M. Coste & F. Delmas. 2008a. A comparison of specific growth rates of periphytic diatoms of varying cell size under laboratory and field conditions. Hydrobiologia 614: 285-297.

Morin, S., T.T. Duong, S. Boutry & M .Coste. 2008b. Modulation de la toxicité des métaux vis-á-vis du développement des biofilms de cours d´eau (bassin versant de Decazeville, France)/Mitigation of metal toxicity to freshwater biofilms development (Decazeville watershed, SW France). Cryptogam. Algol. 29(3): 201-216.

Morin, S., A. Cordonier, I. Lavoie, A. Arini, S. Blanco, T. T. Duong et al. 2012. Consistency in diatom response to metal-contaminated environments. In: H. Guasch et al. (eds.). Emerging and Priority Pollutants in Rivers, 19. The Handbook of Environmental Chemistry. p. 117-146.

Mykrä, H., J. Heino, J. Oksanen & T. Muotka. 2011. The stability-diversity relationship in stream macroinvertebrates: influences of sampling effects and habitat complexity. Freshwater Biol. 56: 1122-1132.

Nunes, M.L., E. Ferreira Da Silva & S.F.P. Almeida. 2003. Assessment of water quality in the Caima and Mau river basins (Portugal) using using geochemical and biological indices. Water, Air, Soil Pollut. 149: 227-250.

Oksanen, J., R. Kindt, P. Legendre & R.B. 0´hara. 2007. Vegan: R functions for vegetation ecologist. Available at: *http://vegan.r-forge.r-project.org/*

Pace, M.L. & J.J. Cole. 2002. Synchronous variation of dissolved organic carbon and color in lakes. Limnol. Oceanogr. 47: 333–342.

Passy, S.I. 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. Aquat. Bot. 86: 171-178.

Patrick, R. 1971. The effects of increasing light and temperature on the structure of diatom communities. Limnol. Oceanogr. 16: 405-421.

Potapova, M. & P. B. Hamilton. 2007. Morphological and ecological variation within the Achnanthidium minutissimum (Bacillariophyceae) species complex. J. Phycol. 43: 561-575.

Pryfogle, P.A. & R.L. Lowe. 1979. Sampling and interpretation of epilithic lotic diatoms. In R. Wetzel (ed.). Methods and Measurements of Periphyton Communities: A review. American Society for Testing and Materials, STP 690: 77-89.

Prygiel, J., P. Carpentier, S. Almeida, M. Coste, J-C. Druart, L. Ector, D. Guillard, M-A. Honoré, R. Iserentant, P. Ledeganck, C. Lalanne-Cassou, C. Lesniak, I. Mercier, P. Moncaut, M. Nazart, N. Nouchet, F. Peres, V. Peeters, F. Rimet, A. Rumeau, S. Sabater, F. Straub, M. Torrisi, L. Tudesque, B. Van de Vijver, H. Vidal, J. Vizinet & N. Zydek. 2002. Determination of the biological diatom index (IBD NF T 90-354): results of an intercomparison exercise. J. Appl. Phycol. 14: 27-39.

Prygiel, J. & M. Coste. 1993: Utilisation des indices diatomiques pour la mesure de la qualité des eaux du basin Artois-Picardie: bilan et perspectives. Annals Limnol. 29(3–4): 255–267.

Raven, J.A. & R.J. Geider. 1988. Temperature and algal growth. New Phytol. 110: 441-461.

Reynolds, C.S. 2006. Ecology of Phytoplankton. Cambridge Univ. Press, Cambridge, UK, 535 pp.

Roberts, D.W. 2005. Laboratory for Dynamic Synthetic Vegephenomenology. Available at: http://cran.r-project.org/web/packages/labdsv/index.html

Rosenfield, J.A. 2002. Pattern and process in the geographical ranges of freshwater fishes. Glob. Ecol. Biogeogr. 11: 323-332.

Round, F.E. 1998. A problem in algal ecology: contamination of habitats from adjacent communities. Cryptogamie Algol. 19: 49-55.

Ruggiu D., A. Luglié, A. Cattaneo & P. Panzani. 1998. Paleoecological evidence for diatom response to metal pollution in Lake Orta (N. Italy). J. Paleolimnol. 20: 333-345.

Schiefele, S. & C. Schreiner. 1991. Use of diatoms for monitoring nutrient enrichment, acidification and impact of salt rivers in Germany and Austria. In: B.A. Whitton, E. Rott & G. Friedrich (eds.). Use of algae for monitoring rivers. Institüt für Botanik, Universität Innsbruck, Innsbruck, p. 25–32.

Schmid, A.M.M. 1980. Valve morphogenesis in diatoms: A pattern-related filamentous system in pennates and the effect of APM, colchicine and osmotic pressure. Nova Hedwigia 33: 811-847.

Sládeček, V. 1986. Diatoms as indicators of organic pollution. Acta Hydrochim. Hydrobiol. 14: 555–566.

Smol, J.P. & E.F. Stoermer (Eds.). 2010. The Diatoms: Applications to the Environmental and Earth Sciences. 2nd edn. Cambridge University Press, Cambridge, pp. 686 pp.

Smucker, N.J. & M.L. Vis. 2011. Spatial factors contribute to benthic diatom structure in streams across spatial scales: Considerations for biomonitoring. Ecol. Indic. 11: 1191-1203.

Soininen, J. 2007. Environmental and spatial control of freshwater diatoms: a review. Diatom Res. 22: 473-490.

Soininen J. 2008. The ecological characteristics of idiosyncratic and nested diatoms. Protist 159: 65-72.

Soininen, J. & P. Eloranta. 2004. Seasonal persistence and stability of diatom communities in rivers: are there habitat specific differences? Eur. J. Phycol. 39: 153-160.

Soininen, J. & J. Heino. 2005. Relationships between local population persistence, local abundance and regional occupancy of species: distribution patterns of diatoms in boreal streams. J. Biogeogr. 32: 1971-1978.

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.

Sokolova, I.M. & G. Lannig. 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. Clim. Res. 37: 181-201.

Stevenson, J. 2014. Ecological assessments with algae: a review and synthesis. J. Phycol. 50: 437-461.

Stevenson, R.J., M.L. Bothwell, & R.L. Lowe. 1996. Algal ecology: Freshwater benthic ecosystems. Academic Press, San Diedo, California, 753 pp.

Stevenson, R.J. & Y. Pan. 1999. Assessing environmental conditions in rivers and streams with diatoms. In E.F. Stoermer & J.P. Smol (eds.). The Diatoms Applications for the Environmental and Earth Sciences. Cambridge University Press, New York, p. 11-40.

Sultan, S.E., A.M. Wilczek, S.D. Hann & B.J. Brosi. 1998. Contrasting ecological breadth of co-occurring annual Polygonum species. J. Ecol. 86: 363-383.

ter Braak, C.J.F. 1986. Canonical correspondence-analysis – a new eigenvector technique for multivariate direct gradient analysis. Ecology 67: 1167-1179.

Thurman, E.M. 1985. Organic Geochemistry of Natural Waters. Springer, Dordrecht, Netherlands, 440 pp.

Tilman, D. 1996. Biodiversity: population versus ecosystem stability. Ecology 77: 350-363.

Underwood, A.J. 1997. Experiments in ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, UK, 504 pp.

Vannote, R.L., G. W. Minshall, K. W. Cummins, J. R. Sedell & C. E. Cushing. 1980. The River Continuum Concept. Can. J. Fish. Aquat. Sci. 37: 130-137.

Vanormelingen, P., E. Verleyen & W. Vyverman. 2008. The diversity and distribution of diatoms: from cosmopolitanism to narrow endemism. Biodivers. Conserv. 17: 393-405.

Verleyen, E., W. Vyverman, M. Sterken, D.A. Hodgson, A. De Wever, S. Juggins, B. Van de Vijver, V.J. Jones, P. Vanormelingen, D. Roberts, R. Flower, C. Kilroy, C. Souffreau & K. Sabbe. 2009. The importance of dispersal related and local factors in shaping the taxonomic structure of diatom metacommunities. Oikos 118: 1239-1249.

Vyverman, W., E. Verleyen, K. Sabbe, K. Vanhouette, M. Sterken, D.A. Hodgson, D.G. Mann, S. Juggins, B. Van de Vijver, V. Jones, R. Flower, D. Roberts, V.A. Chepurnov, C. Kilroy, P. Vanormelingen & A. De Wever. 2007. Historical processes constrain patterns in global diatom diversity. Ecology 88: 1924-1931.

Wedepohl, K.H. 1995. The composition of the continental crust. Geochim. Cosmochim. Ac. 59: 1217-1232.

Whitton, B.A., E. Rott & G. Friedric (eds.). 1991. Use of algae for monitoring rivers. Institut für Botanik, Universität Innsbruck, Innsbruck, 193 pp.

Whitton, B.A. & E. Rott (eds.). 1996. Use of algae for monitoring rivers II. Institute für Botanik, Universität Innsbruck, 124 pp.

Yau, Chi. 2013. R Tutorial with Bayesian Statistics Using OpenBUGS. Available at: *r-tutor.com*