

Similar small-scale variation of diatom assemblages on different substrates in a mesotrophic stream

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Abstract – The aim of the present study was to analyze if small-scale spatial variation of benthic diatom assemblages has consequences for biomonitoring. Benthic diatom samples were collected at one sampling site in a mesotrophic stream in Middle-Sweden from stone and plant (macrophytes and mosses) substrate. Our results showed that spatial variation of both the diatom species composition and the calculated bioindices were similar on both small (distance of centimeter) and medium (distance of decimeters) scales. Spatial variation was also similar on both studied substrates. This implies that it does not matter if a small or a larger area is sampled for biomonitoring as long as no major environmental factors impact certain sites systematically. Diatom assemblages and indices were significantly different between substrates. Spatial variation did not contribute much to this variation, and variation on a slide was unimportant. These results confirm earlier findings that small-scale spatial variation is not a problem when using diatoms to detect anthropogenic impacts to a stream or lake.

Keywords: bioindices, diatoms, diversity, epilithon, epiphyton, freshwater, monitoring, sampling

Introduction

Diatoms are frequently used for monitoring water quality status in streams and also recently in lakes (SIS 2014). Many indices are in use to reflect eutrophication or pollution (BIRK et al. 2012). Usually diatoms reflect water chemistry well (RIMET 2012).

The European standard for diatom sampling in freshwater for biomonitoring (SIS 2014) allows sampling from different substrates. Still, diatom assemblages can differ between substrates, e.g. stones and macrophytes. It is not well studied how these differences impact diatom indices and recommendations about which substrate to sample are contradictory. The European sampling standard (SIS 2014) recommends sampling from stones in opposite to BESSE-LOTOTSKAYA et al. (2006) recommending macrophytes. KRÖPFL et al. (2006) even

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found differences of diatom indices between different artificial substrates. WINTER and DUTHIE (2000) showed that differences between substrates have not been consistent over time. They conclude that more studies are needed to evaluate detailed information about the effect of substrate on freshwater diatom assemblages from different environments.

It is important to keep a comparable sampling strategy when studying the effect of substrate on the attached algal assemblage to avoid differences that may occur due to it being a different sampling area. Differences in index values might otherwise be related to the fact that a diatom assemblage sampled from a restricted area represents local factors whereas a sample pooled from many stones across the stream rather represent a stream's water quality. A diatom sample taken according to EU standard is pooled from at least five subsamples. Macrophytes often grow in relatively restricted places, whereas stones mostly can be sampled across the whole river or lake section. A pooled sample taken from macrophytes would therefore represent the local environment around a spot, whereas a pooled sample taken from stones would reflect the environmental conditions of the watercourse. In other words, observed differences could be due to the fact that the pooled sample from macrophytes has a low spatial variation and might deviate from a whole-stream average value just because it is taken from a relatively restricted area.

The small-scale variation of benthic diatom assemblages on different substrates is not very well studied in freshwater and it is not known what impact it has on the bioindices derived by a different sampling strategy when a substrate is restricted to a small area. Many studies have focused on spatial variation at larger scales (SOININEN 2007). A general view has been established that history is important but local factors are steering diatom communities (SOININEN 2007, VYVERMAN et al. 2007, MANN and VANORMELINGEN 2013). Confirming this, most studies on space have proved that diatom indices are sufficiently robust to reflect an anthropogenic impact despite spatial or temporal variation of the species composition (ROTHFRITZ et al. 1997, KELLY 2002, LAVOIE et al. 2005, KING et al. 2006). The studied »small« spatial scale is usually represented by a distance of meters or rarely decimeters (ROTHFRITZ et al. 1997, PRYGIEL et al. 2002, LAVOIE et al. 2005, O'DRISCOLL et al. 2014, SVOBODA et al. 2014), the scale usually sampled in a standard investigation (SIS 2014). Variation at smaller spatial scales is rarely studied. A rare exception is MACHOVA-CERNA and NEUSTUPA (2009) who investigated small-scale variation in a peat-bog in comparison to larger-scale variation and found significant differences in species composition even at the smallest sampled scale. More information is available about small-scale variation from marine and brackish water studies on soft bottom algal assemblages (e.g. SABUROVA et al. 1995, COLEMAN 2002). These studies found that spatial variation increases with scale, but not linearly. Variation at the laboratory scale has been found to usually be of minor importance compared with variation at spatial and other scales, both regarding slide replicates (e.g. LAVOIE et al. 2005, BESSE-LOTOTSKAYA et al. 2006) and replicate counts on one slide (PRYGIEL et al. 2002). Still, it is necessary to have an idea about the laboratory variation to assess the variability of e.g. spatial scales.

The present study analyzes and compares the size of small-scale variation of diatom assemblages in a mesotrophic stream on different substrates. We calculate if spatial variation is smaller on a restricted area than across an entire stream section and assess if eventual differences are reflected in diatom bioindices with consequences for monitoring.

Our hypothesis are 1) small-scale variation of diatom assemblage is smaller than medium-scale variation, 2) spatial variation of diatom assemblages is similar on stones and plants (macrophytes and mosses) if sampled at the same scale, and 3) diatom indices pooled

according to the EU standard can be different between stones and plants if plants are sampled on a restricted spot and stones at a larger spatial scale.

Materials and methods

Sampling

Benthic diatom samples were collected at one sampling site in Broströmmen, a mesotrophic stream in Middle-Sweden (598280,7+0187355,3/ ISO 6709, average water chemistry: 44 μg total phosphorus L^{-1} , pH 7.1), August 2010. Sampling followed EU standard (SIS 2003). Samples were taken with a syringe brush sampler (opening diameter of 1 cm) at random in a nested design (Figs. 1a–b) to cover the variance at small (cm) and medium

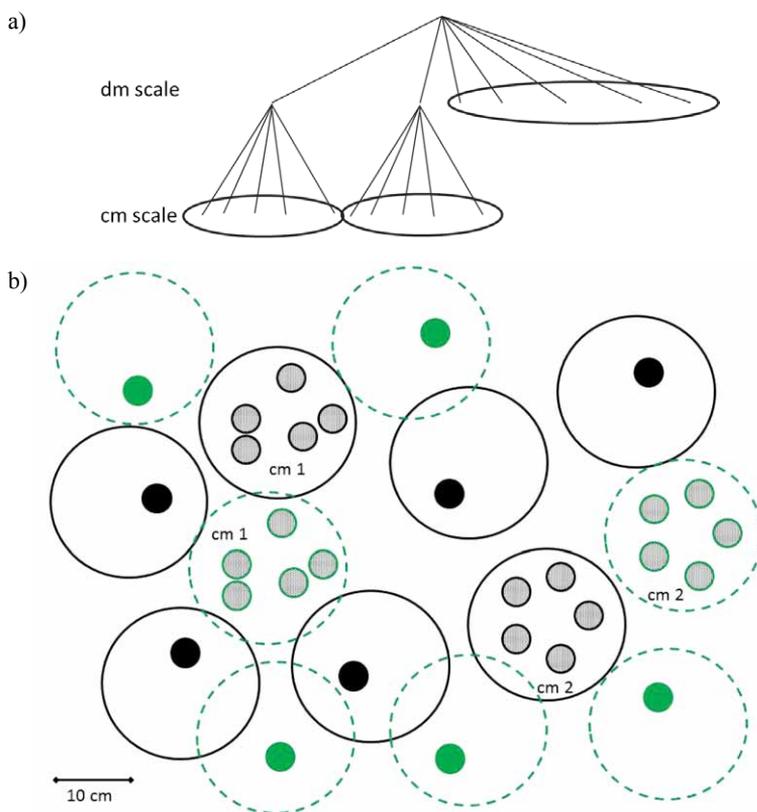


Fig. 1. a) Nested sampling design used in studied stream. Five subsamples were taken with a distance of some centimeters (cm-scale). This cm-scale sampling was performed in two different randomly chosen areas. Additionally, five single samples were taken from one substrate with a distance of 20–100 cm (dm-scale); b) Nested sampling design for both substrates, schematic. Larger circles with solid line represent sub-area for epilithon samples, while those with dashed line represent sub-area for epiphyton samples. The five subsamples in cm-scale are shown as shaded grey small circles, the two sub-areas are marked with cm 1 and 2 for each substrate. The five subsamples in dm-scale are shown as filled small circles for each substrate. Note that samples were taken at random, so this schematic picture is not reflecting a map.

scale (dm). Two different diatom assemblages were sampled in the same area using the same design: epilithon from cobble stones and epiphyton from a mixture of submerged macrophytes and mosses. Five subsamples were taken with a distance of some centimeters (cm-scale). This cm-scale sampling was performed in two different randomly chosen areas. Additionally, five single samples were taken from one substrate with a distance of 20–100 cm (dm-scale). All samples were analyzed following standard procedures (Sis 2005), identification followed Swedish standards (DYNNTAXA 2013). To get an assessment of the variation at laboratory scale, two stone samples were counted in four laboratory replicates.

To meet the demands of the EU standard for diatom sampling, we also pooled a subsample of the replicates taken at the two restricted areas, and of the replicates taken at medium scale. In that way it was possible to compare the effect of sampling on the pooled samples directly.

Diatom species composition

For a comparison of diatom species composition non-metric multidimensional scaling (NMDS) (KRUSKAL 1964) was used to display dissimilarities (metric: relative Sorensen) between samples and between substrates. Unidentified taxa, taxa identified to genus, and species which were only observed once were removed from the analysis. Diatom relative abundance values were arcsine square root transformed prior to the analyses. Non-parametric multivariate analysis of variance (NPMANOVA) (ANDERSON 2001) was used pooling all samples to test if species composition differed in general significantly between substrates. SIMPER analysis (similarity percentage, (CLARKE 1993), Bray-Curtis metric as default for dissimilarity) was used to identify the species which were typical for either substrate. NMDS and NPMANOVA were performed with the software PCOrd 6.15 (MCCUNE and MEFFORD 2011), SIMPER with PAST 2.17 (HAMMER et al. 2001). Additionally, we calculated the number of diatom taxa and diversity (SHANNON 1948) for each level as general metrics for species composition.

Diatom indices

To analyze the impact of community differences on diatom indices, we calculated the indices used for Swedish monitoring, i.e. indice de polluo-sensibilité spécifique (IPS) (CE-MAGREF 1982), trophic diatom index (TDI) and proportion of pollution tolerant (% PT) valves (KELLY and WHITTON 1995, KELLY 1998). To test if the indices varied depending on substrate, we calculated t-tests with the five dm-scale samples as replicates.

Spatial scale variation

The impact of the fixed factor substrate and the random factors dm- and cm- scale on the variation of the diatom indices was assessed by calculating coefficients of variance (CV%) for each sampled scale (cm-scale $n = 5$, dm-scale $n = 5$) for the diatom indices and for the number of taxa and the diversity of both substrates. The variation of the spatial scales was compared with the variation between substrates, for which the pooled standard samples for each substrate were averaged ($n = 2$). For stones, also the CV% of the laboratory scale ($n = 4$) was assessed. Boxplots were created to visualize the results.

Results

Overall, we found that the variation was similar at the smallest sampled scale of centimeters and at the medium-scale of decimeters in the studied stream. The variation was about the same for both studied substrates and contributed only negligibly to the large differences between substrates.

Diatom species composition

161 diatom taxa were found in Broströmmen, 105 of them were used for the analyses of differences in species composition. The five most common taxa were *Cyclotella meneghiniana* Kützing (average relative abundance 16%), *Amphora pediculus* (Kützing) Grunow (12%), *Cocconeis placentula* incl. varieties Ehrenberg (9%), *Planothidium frequentissimum* Lange-Bertalot (7%) and *Navicula capitatoradiata* Germain (6%). Small-scale variation of the diatom assemblage was on the plant substrate smaller than in dm-scale. However, for stones, one of the small areas had a lower and the other one a higher variation than the larger area, which is illustrated by the NMDS analyses (Figs. 2–3). After the removal of singletons, 70 taxa were included in the NMDS analysis for stones and 68 taxa for plants. Three major gradients captured most of the variance in the epilithon communities, with a final stress of 9.8. For epiphyton, two major gradients captured most of the variance, giving a final stress of 10. So, small-scale variation of the diatom assemblage was not always smaller than medium-scale variation, thereby falsifying our first hypothesis. Spatial variation of assemblage species composition was about the same for stones and plants, thereby verifying our second hypothesis. This similar size of variation is illustrated by the NMDS-analysis (Fig. 4) where two major gradients captured most of the variance when analyzing both communities together, giving a final stress of 10.1. The diatom flora was significantly different between stones and plants (Bray-Curtis dissimilarity 57.7%, NPMANOVA, $p <$

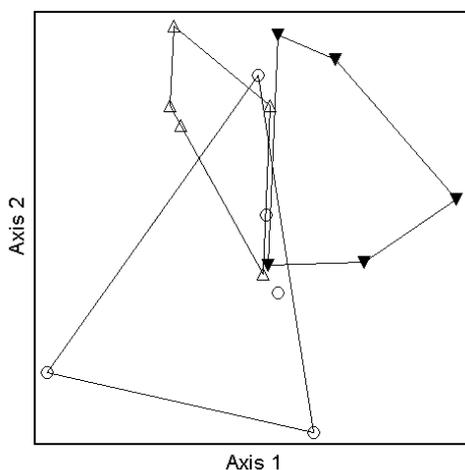


Fig. 2. Differences in diatom assemblages scraped from stones with replicates at different spatial scales. Δ , \circ – replicates in cm-scale (2 different areas), \blacktriangledown – replicates on dm-scale. NMDS – non-metric multidimensional scaling (KRUSKAL 1964) on 70 diatom taxa, final stress for 3-dimensional solution = 9.8.

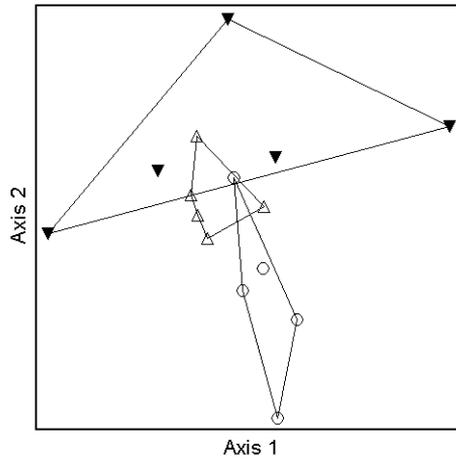


Fig. 3. Differences in diatom assemblages scraped from plants (submerged macrophytes and mosses) with replicates at different spatial scales. Δ , \circ – replicates in cm-scale (2 different areas), \blacktriangledown – replicates on dm-scale. NMS – non-metric multidimensional scaling (KRUSKAL 1964) on 68 diatom taxa, final stress for 2-dimensional solution = 10.

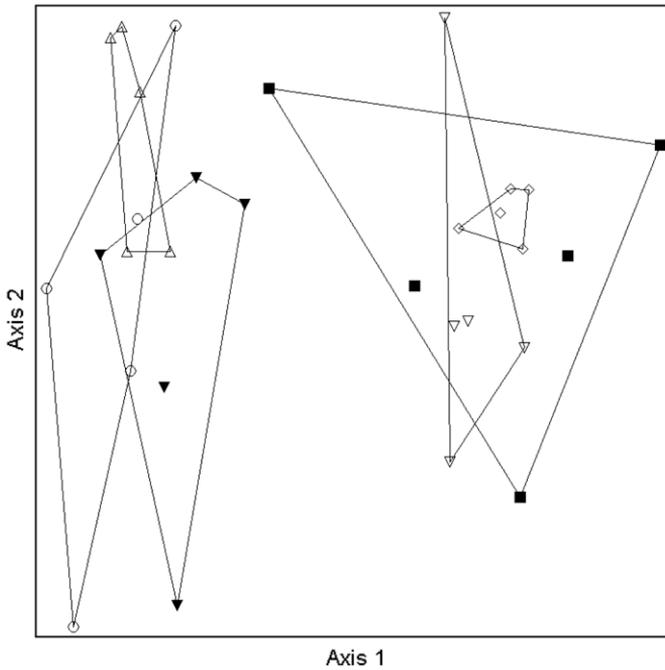


Fig. 4. Differences in diatom assemblages scraped from stones (left) and plants (right, submerged macrophytes and mosses) with replicates at different spatial scales. Stones: Δ , \circ – replicates in cm-scale (2 different areas), \blacktriangledown – replicates on dm-scale. Plants: \diamond , ∇ – cm-scale, \blacksquare – dm-scale. NMS – non-metric multidimensional scaling (KRUSKAL 1964) on 100 diatom taxa, final stress for 2-dimensional solution = 10.1.

0.001). Differences in the relative abundance of only 16 taxa explained 50% of the difference in taxon assemblages between stones and plants (SIMPER Similarity Percentage analysis, Tab. 1). The number of taxa and the diversity was higher on stones than on plants (Tab. 2).

Tab. 1. Taxa contributing most to the significant difference of diatom assemblages on stones and plants, respectively, in Broströmmen (SIMPER analysis, NPMANOVA, $p < 0.001$. Bray-Curtis dissimilarity 57.7). For each taxon, the sensitivity values for the diatom indices IPS -indice de polluo-sensibilité spécifique (CEMAGREF 1982) and TDI – trophic diatom index (KELLY and WHITTON 1995, KELLY 1998) are given.

Stone substrate				Plant substrate			
Code	Taxon	IPSs	TDIs	Code	Taxon	IPSs	TDIs
APED	<i>Amphora pediculus</i> (Kützing) Grunow	4	5	CMEN	<i>Cyclotella meneghiniana</i> Kützing	2	0
PLFR	<i>Planothidium</i> <i>frequentissimum</i> Lange-Bertalot	3.4	5	NCPR	<i>Navicula capitatoradiata</i> Germain	3	3
KALA	<i>Karayevia laterostrata</i> (Hustedt) Bukhtiyarova	4.5	4	CPLA	<i>Cocconeis placentula</i> incl. varieties Ehrenberg	4	3
KASU	<i>Karayevia suchlandtii</i> (Hustedt) Bukhtiyarova	4.5	4	SCON	<i>Staurosira construens</i> var. <i>Construens</i> Ehrenberg	4	4
CBAC	<i>Caloneis bacillum</i> (Grunow) Cleve	4	3	SSVE	<i>Staurosira venter</i> (Ehrenberg) Cleve & Moeller	4	4
PRST	<i>Planothidium rostratum</i> Lange-Bertalot	4.4	5	NZSU	<i>Nitzschia supralitorea</i> Lange-Bertalot	1.5	4
PTLA	<i>Planothidium lanceolatum</i> Lange-Bertalot	4.6	5				
NSEM	<i>Navicula seminum</i> Grunow	1.5	5				
EOMI	<i>Eolimna minima</i> (Grunow) Lange-Bertalot	2.2	5				
PTCO	<i>Platessa conspicua</i> Lange-Bertalot	4	5				

Consequences for diatom indices

The main index IPS was significant higher on stones than on plants (t-test, $p < 0.01$, Tab. 2, Fig. 5). The results of the comparison of the samples pooled according to the EU standard confirmed that IPS still was very different between substrates, independently of sampling scale (Tab. 2, Fig. 5). The same results were found for the supporting index TDI (t-test, $p < 0.01$, Tab. 2, Fig. 6). Following this, our third hypothesis was rejected, at least in the investigated stream. The variation of IPS and TDI at the spatial scales reflected the variation of the diatom assemblages, with somewhat lower variation at cm-scale than dm-scale for the plant substrate, and about similar variation at both scales for the stone sub-

Tab. 2. Means and coefficients of variance (CV%) for IPS20 – indice de polluo-sensibilité spécifique (CEMAGREF 1982), TDI – trophic diatom index, % PT – proportion of pollution tolerant valves (KELLY and WHITTON 1995, KELLY 1998) and number of taxa (nr taxa), and diversity (Shannon) at different scales and substrates in Broströmmen.

Substrate	Scale	n	IPS20	CV%	TDI100	CV%	% PT	CV%	Nr taxa	CV%	Diversity
Both	stream reach	2	11.7	18.1	70.9	17.1	6.9	20.5	43.0	52.6	4.0
Stone	dm	5	13.8	6.9	79.6	4.0	9.6	50.0	39.0	11.2	4.6
Stone	cm 1	5	13.4	3.1	86.9	4.0	14.0	27.1	37.4	13.4	4.0
Stone	cm 2	5	13.8	6.5	83.5	2.8	10.7	35.1	39.2	14.8	4.3
Stone	laboratory 1	4	14.3	1.8	83.3	1.2	5.5	66.8	36.5	3.5	4.0
Stone	laboratory 2	4	13.6	2.7	83.5	2.7	10.6	20.4	38.5	4.5	4.1
Plant	dm	5	10.8	10.8	62.7	13.7	12.5	35.8	31.4	10.0	3.7
Plant	cm 1	5	10.9	2.8	56.1	4.2	8.8	25.1	37.2	11.0	3.7
Plant	cm 2	5	11.3	5.6	62.5	5.3	8.8	20.8	37.6	7.7	3.8

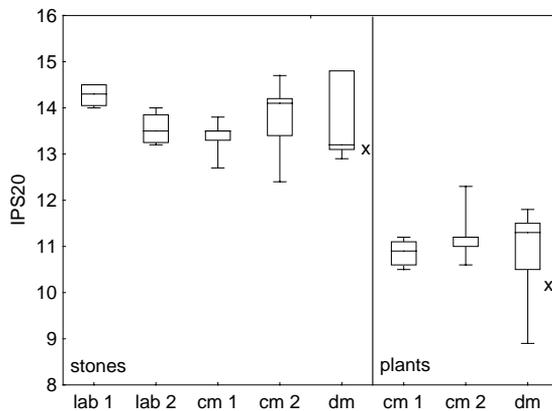


Fig. 5. Variation of diatom index IPS – indice de polluo-sensibilité spécifique (CEMAGREF 1982) at different scales and substrates (stones and plants) in Broströmmen. Median, interquartile range and total range are shown in the boxplots for scales at laboratory (n = 4), samples taken with cm distance (n = 5) and dm distance (n = 5). × represents the pooled sample taken according to EU standard procedures (Sis 2014).

strate (Tab. 2, Figs. 5–6). The laboratory replicates of IPS and TDI varied less than the replicates taken in the field (Tab. 2, Figs. 5–6). % PT did not differ between substrates (t-test, $p > 0.05$, Tab. 2, Fig. 7).

Discussion

We found that already at very small spatial scales, with a distance of a few centimeters, diatom species composition can vary substantially, similar to the differences at medium distance of several decimeters. As expected, laboratory variation contributes only a minor

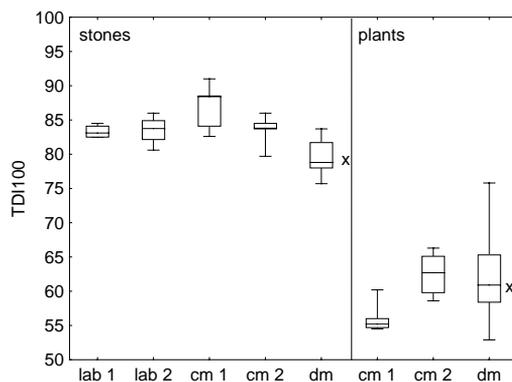


Fig. 6. Variation of diatom index TDI – trophic diatom index (KELLY and WHITTON 1995, KELLY 1998) at different scales and substrates (stones and plants) in Broströmmen. See Fig. 5 for more information.

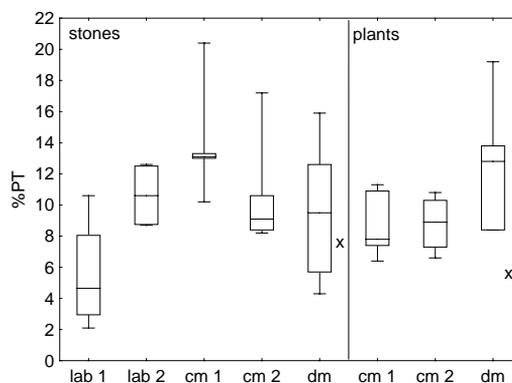


Fig. 7. Variation of diatom index %PT – proportion of pollution tolerant valves (KELLY and WHITTON 1995, KELLY 1998) at different scales and substrates (stones and plants) in Broströmmen. See Fig. 5 for more information.

part to that variation. The spatial variation was similar on stones and on plants. However, the spatial variation was not contributing significantly to the much larger variation of the diatom assemblages between stones and plants. This substrate variation in the studied mesotrophic stream was the same even if stones from a large area were compared with plants from a small area, a sampling strategy that might be applied when plants only are available from a restricted area.

Factors impacting at spatial scale

Earlier studies on the diatom flora of intertidal sandflats indicate that patchiness at small scales is random up to a scale of several decimeters (SABUROVA et al. 1995). Similar results were found for algae on hard substrate in marine and freshwater environments (RINDI and CINELLI 2000, SOININEN 2003). These large scale patterns have been attributed to environ-

mental factors such as water chemistry and tidal effects in the example of sandflats. The patterns at smaller scales have been attributed to biotic species interactions and as SABUROVA et al. (1995) point it out »a complex of abiotic conditions in the sediments«. Other studies highlight the impact of dispersal. Still, both MACHOVA-CERNA and NEUSTUPA (2009) and our study indicate that variation at the smallest scale with a distance of centimeters can be substantial. MACHOVA-CERNA and NEUSTUPA (2009) discussed dispersal limitation and niche adaptation as explanations. According to our study, we can assume that similar factors impact the spatial distribution of diatom species on the two substrates because spatial variation is similar on both studied scales when the substrate is sampled from the same spot in the stream. Certainly spatial variation depends on the sampled environment. We suggest that in a medium-order stream that it is mainly velocity that is shaping the diatom community at the decimeter scale, as shown by PASSY (2001). At smaller scales, grazing could have an important impact on algal communities (GOTHE et al. 2013, O'DRISCOLL et al. 2014), probably together with historical events following dispersal (MÜLLER-HAECKEL 1976) and also the named »complex interaction of abiotic conditions« (e.g. KEMP and DODDS 2001). The small scale variation can be of similar size as the medium scale variation, which shows that factors acting on very small scales can cause similar large variations in diatom diversity as shown for velocity (PASSY 2001). However, to analyze the importance of these factors, it is necessary to include them directly in an analysis along with the temporal aspect as earlier events are shaping the community of later stages.

Bioindices

Regarding the impact of diatom flora variation on the bioindices used for monitoring, we found the difference on substrates to be most important. The diatom index IPS on stones indicated a moderate ecological status class with a tendency to good ecological status according to the Swedish classification (NATURVÅRDSVERKET 2007). On plants, IPS also indicated a moderate ecological status class which shows the robustness of the method to assess ecological status of a water body. However, IPS had a clear tendency to poor ecological status class on plants. Contrary to this result, the TDI was higher on stones than on plants, indicating a higher nutrient level on stones (KELLY and WHITTON 1995). Diatom taxa on stones had on average higher sensitivity values for both IPS and TDI than the taxa on plants, explaining the somewhat unexpected differences of IPS and TDI. IPS was constructed to reflect a general pollution, integrating both aspects of eutrophication and organic pollution (CEMAGREF 1982), whereas TDI was constructed to solely reflect nutrient conditions (KELLY and WHITTON 1995).

A streams' diatom flora is expected to reflect mainly a streams' integrated water chemistry, the factor diatom indices were developed and are meant to use for. However, the species composition certainly always reflects other aspects of the environment than just water chemistry. In our study, the taxa on plants being mainly responsible for the difference to the stone diatom flora can be divided into three subgroups, probably representing three types of diatom groups. First, *Cyclotella meneghiniana* Kützing, *Staurosira construens* var. *construens* Ehrenberg and *S. venter* (Ehrenberg) Cleve & Moeller are tychoplanktonic taxa. They are probably entangled in the plant substrate and would be swept away on smooth stones. Second, *Cocconeis placentula* Ehrenberg is known as epiphyte, adapted to a life on plant, moss or macroalgal substrate. Third, *Nitzschia supralitorea* Lange-Bertalot belongs to the genus *Nitzschia* where many, maybe all, species have the ability to grow heterotrophic on

organic substrates in complete darkness (TUCHMAN 1996). Probably not only tychoplanktonic diatoms, but also detritus gets entangled within the plant substrate, offering the opportunity for the diatoms to use included organic substances. The combination of the factors restricted current, availability of organic substances and adaptation to plant substrate then leads to a diatom assemblage which has a on average low IPS value. At the same time, the assemblage on plants has a relatively low average TDI value. A possible explanation could be that the above mentioned factors are more important on the plant substrate than the nutrient level from the overlying water. *C. meneghiniana*, comprising on average 30% of the plant assemblage, has no appointed TDI value as the TDI excludes planktonic taxa. Similar results for the difference in IPS and TDI have been shown in a large study covering streams across Europe (BESSE-LOTOTSKAYA et al. 2006), which indicates that the discussed mechanisms could be valid for other streams than only our study site.

These differences between substrates should be kept in mind when sampling from plant substrate. Better information about the effect of substrate type on diatom assemblages is yet missing, and the existing studies are contradicting. Differences are obviously not consistent but varying at least with time, with diatom assemblages getting more dissimilar between substrates during succession (WINTER and DUTHIE 2000, MACHOVA-CERNA and NEUSTUPA 2009). So depending on time, diatom samples could be quite similar even on different substrates. There is not a simple typical plant or stone diatom flora, different taxa have been reported to dominate different substrates in different studies (REAVIE and SMOL 1997, WINTER and DUTHIE 2000). Still, BESSE-LOTOTSKAYA et al. (2006) large European study showed that differences in diatom indices was larger between streams with a different impact than between substrates sampled within each stream, so indices are robust enough to reflect an anthropogenic impact, a result confirmed by other studies showing that between stream variation is larger than within stream variation on different substrates (JUTTNER et al. 1996, ROTHFRITZ et al. 1997). We want to point out that our study only reflects the conditions of a single stream and a single occasion. Still, not much has been done in freshwater to unravel the impact of very small scales on diatom species composition, and other conditions might give different results especially with respect to diatom bioindices.

In conclusion, our results show that spatial variation was similar on both small and medium scales, and on both studied substrates, in the studied stream. This implies that it does not matter if a small or a larger area is sampled for biomonitoring as long as no major environmental factor impacts certain sites systematically. This high patchiness at both studied scales implies that by chance, one can get a quite homogenous pooled sample by taking five subsamples, but also high variation in other cases. Only frequent sampling can minimize the risk of getting outliers into the analysis. In comparison to the studied environmental factor substrate, spatial variation was relatively small and variation on a slide was unimportant, results that confirm earlier findings that small-scale spatial variation is not a problem when using diatoms to detect deteriorate impacts to a stream or lake (LAVOIE et al. 2005, MACHOVA-CERNA and NEUSTUPA 2009).

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