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A practical and sensitive approach to large river periphyton monitoring: comparative performance of methods and taxonomic levels

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The reliability and sensitivity of two periphyton monitoring methods were assessed in the large boreal Kymi River. First, results from 11 years of monitoring of periphyton biomass on artificial substrates, expressed as chlorophyll *a*, were analysed. Second, in a one-year survey, the sensitivity of periphyton biomass was compared with the analysis of epilithic diatom community structure. Finally, diatom community analysis using three practical approaches based on different taxonomic resolutions was tested. The long-term monitoring results of periphyton biomass revealed significant differences among the sites and years. Although long-term results showed significant correlation in the annual nutrient concentrations and periphyton biomass, the diatom indices demonstrated a higher sensitivity to changes in water quality. Analysis of concordance showed that diatom community analysis based on generic level alone, or specifically combined with the identification of only the abundant species, could offer a practical and reliable biomonitoring tool for large rivers.

Introduction

Surface water monitoring in Finland started during the 1960s. Monitoring was first based on water chemistry alone, but soon more emphasis was placed on studying the biomass and community structure of freshwater biota. Research efforts were primarily focused on lakes and coastal areas, and studies of lotic ecosystems have therefore remained relatively scarce. Until very recently, biomonitoring methods designed for lentic environments (e.g. grab and corer samplers for benthic macroinvertebrates) have also been applied, mainly without criticism, to lotic environments.

During the early phase of national surface water monitoring, numerous Finnish rivers were drastically polluted and even relatively robust methods were adequate for detecting wastewater impacts. Since then, however, water quality of even the most polluted rivers has substantially improved (e.g. Niemi *et al.* 1997), while routine monitoring practices have experienced little advance. However, with increasing water quality more sensitive methods may be required to reliably assess human impacts. The EU Water Framework Directive (EC Parliament and Council 2000) states that biomonitoring methods should be accurate and efficient. It is therefore likely that at least some of the common monitoring methods may not meet these challenges.

Traditionally, periphyton responses to anthropogenic impacts in lotic environments have been monitored and assessed by measuring the algal standing crop or community composition using various artificial substrata (e.g. Lowe and Gale 1980, Chessman 1985). In Finland, periphyton monitoring of large rivers and lakes is usually conducted by incubating plastic sheets or glass-fibre filters in the water for 2–3 weeks, after which chlorophyll *a* (mg m^{-2}) is determined (Heinonen and Herve 1984, Mäkelä *et al*. 1992). One advantage of this method is that periphyton biomass growth per unit of time is obtained. Besides the composition of the local species pool, the biomass of periphytic algae is, however, largely dependent on the physical growing conditions (e.g. current velocity, shading, water depth) prevailing at a site (Biggs 1996, Chételat *et al*. 1999). Thereby, local physical conditions at a study site may have notable, yet largely unknown, effects on the results obtained, and thus on the subsequent management efforts in the river channel and its drainage basin. Moreover, there is considerable inconsistency in the literature concerning the importance of nutrient control to lotic algal communities, and some studies have even found no association between nutrient concentrations and algal standing crops in streams (e.g. Kjeldsen 1994), while others have indicated a strong relationship between algal biomass and nutrient concentrations (Biggs and Close 1989, Dodds *et al*. 2002, Carr *et al*. 2005).

Investigating diatom community composition in lotic systems has been found to be a highly applicable tool for river classification and monitoring in several European countries (Whitton *et al*. 1991, Prygiel and Coste 1993, Whitton and Rott 1996, Prygiel *et al*. 1999). The applicability of benthic diatoms has also been tested in Finland (e.g. Eloranta 1995, 1999, Eloranta and Andersson 1998, Eloranta and Soininen 2002). However, diatom species identification requires considerable taxonomic expertise, being a major disadvantage to their widespread use in biomonitoring. As one possible solution, lowering the taxonomic resolution from the species to the genus level might alleviate some of the problems related to diatom community analysis. Although possibly losing part of the information obtained by congeneric species, genus level identification has been demonstrated to offer a relatively promising tool for river biomonitoring (Kelly *et al*. 1995, Wu and Kow 2002). Tests of the reliability of genus level identification have, however, typically been based on comparative studies of different diatom indices (e.g. Kelly *et al*. 1995). A reliable comparison of the taxonomic levels would require that the indices respond to the same environmental variable or variables, which may not always be the case (*see* Prygiel and Coste 1993). A more objective and rigorous comparison between the two taxonomic levels of algal identification is thus required. An alternative practical approach is to restrict identification to the most abundant species (Round 1991) or to identify rare taxa to a higher taxonomic level, but this approach has received little attention thus far.

Although numerous biomonitoring methods have been developed, comparative studies of the methods are still scarce. Before any method is recommended, the efficiency and applicability of the potential approaches in different environments should be examined. With regard to benthic algae, current monitoring methods may be insensitive to anthropogenic impacts, or they may require high taxonomic expertise, which is not always available. Our purpose in this study was to (i) evaluate the usefulness of the artificial substrate method as a periphyton biomonitoring tool for large boreal rivers, (ii) compare the reliability and sensitivity of the artificial substrate method and the method based on diatom community structure, and (iii) test the reliability of diatom community analysis using three approaches based on different taxonomic resolution.

Material and methods

This study was conducted in the Kymi River, one of the largest rivers in Finland (drainage area 37 107 km^2), located in the southeastern part of the country (Fig. 1). The discharge of the river varies considerably within and between years, but in 2003 the average discharge was 189 m^3 s^{-1} (measured at the city of Kuusankoski near site 2). The river is impacted by treated wastewaters

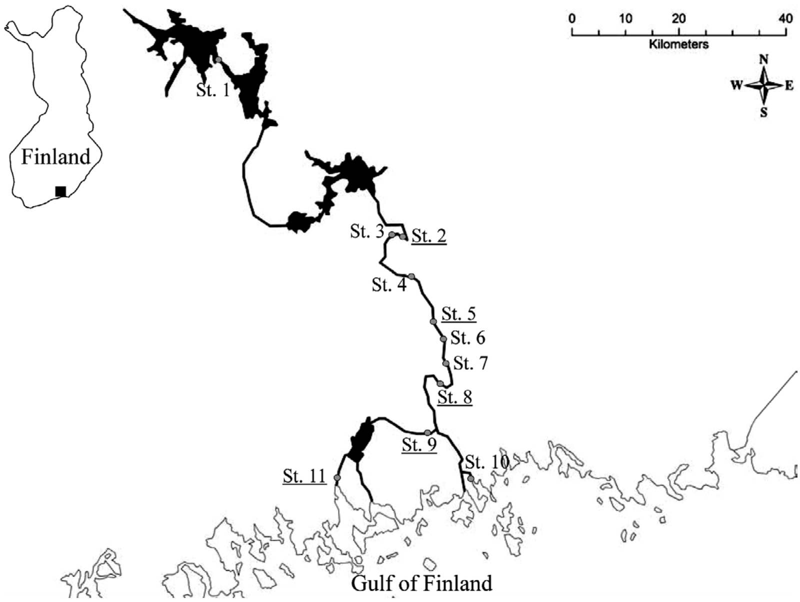

Fig. 1. Location of the sampling sites in the Kymi River, southern Finland. The five long-term monitoring sites are underlined.

from paper and pulp mills and from domestic wastewater purification plants. The major impact sites are located above sampling sites 3, 6 and 7.

The water quality of the river has been monitored since the 1960s, and periphyton monitoring with plastic sheets as artificial substrates has been included in the annual monitoring program since 1993 (Mäkelä *et al.* 1992). During the study period (1993–2003), periphyton monitoring was conducted by incubating three plastic sheets (10 × 15 cm) for two weeks at five sites in the Kymi River (sites 2, 5, 8, 9 and 11, Fig. 1). Incubation was replicated three times during a summer. The total sample size for each site was thus nine measurements per year. The three sheets were attached to an anchored cultivation frame, mounted at a depth of 0.5 m. The exact places for the incubation of the sheets were chosen according to Mäkelä *et al.* (1992), avoiding turbulent and shaded river beds and areas of fast current velocity (> 1 m s^{-1}). After the two-week incubation period, the sheets were closed in plastic bags and preserved in a freezer. The sheets and plastic bags were cleaned in the laboratory with de-ionised water and the chlorophyll *a* concentration of the water was determined.

Two-way analysis of variance with Dunnett's test for pair-wise comparisons was used for testing differences in average chlorophyll *a* content among the years and sites (at sites 2, 5, 8, 9 and 11). The unimpacted site 2 was set as a reference when testing for differences between the sites and the data from 1993 were the control in among-year comparisons. Long-term results in chlorophyll *a* and nutrient concentrations (total phosphorus and total nitrogen, TP and TN) were further examined using multiple linear regression analysis. The analysis was based on monitoring data from 1993 to 2003. Only sites 2, 8 and 11 were examined because similar water quality data was unavailable from the sites 5 and 9. Yearly averages of the variables were used in the analyses.

Second, we compared the reliability and sensitivity of the artificial substrate method and the method based on benthic diatom community structure. Samples were collected from 11 sites along the Kymi River during summer 2003 (Fig. 1), one diatom sample and one set (three sheets) of periphyton chlorophyll *a* samples from each site. Periphyton biomass was measured as in previous years, and diatoms were sampled concurrently from the natural stony substrata using a toothbrush. Sampling was performed according to the diatom-based river monitoring standard (SFS-EN 2003). At least five pebble-to-cobble (5–15 cm) sized stones were brushed and the suspension was placed in small glass vials. Samples were cleaned of organic material in the laboratory using wet combustion with acid (HNO_3:

H_2SO_4, 2:1) and then mounted in Naphrax. At least 250 diatom frustules per sample were identified and counted using phase contrast light microscopy (magnification 1000×). Species identification was performed according to Krammer and Lange-Bertalot (1986–1991). The relationship between the diatom indices as well as the chlorophyll-*a* content of the sheets and local water quality parameters (TP, TN, NH_4-N, NO_2+NO_3, COD_{mn}, conductivity and turbidity) was studied with multiple linear regression, using stepwise (forward) data entry. Prior to the analysis all parameters were log-transformed.

Third, the effect of taxonomic resolution on the reliability of diatom indices and community analysis was tested. Three diatom indices (GDI: Generic Diatom Index, Coste and Ayphassorho 1991; TDI: Trophic Diatom Index, Kelly and Whitton 1995; and IPS: Pollution Sensitivity Index, Coste in CEMAGREF 1982) were used as they all have been considered highly appropriate for Finnish freshwaters (e.g. Eloranta and Andersson 1998). The indices were calculated using Omnidia ver. 4 software. All three indices were used in the sensitivity comparison of the methods described above. The effect of exclusion of rare species (abundance < 2% of the counted cells) on the two species level indices (TDI and IPS) was analysed with the paired samples *t*-test. The effects of taxonomic resolution and exclusion of rare species on diatom community analysis were examined with four types of data set: (i) species level identification, (ii) generic level identification, (iii) abundant (> 2% of total abundance) species only and (iv) a combination of the abundant species and generic level identification (i.e. rare taxa with abundance < 2% were analysed on the generic level).

Nonmetric Multidimensional Scaling (NMDS) was first applied to ordinate the samples, using PC-ORD version 4.25 software (McCune and Mefford 1999). NMDS is non-parametric ordination method well suited to data that are nonnormal or are on arbitrary, discontinuous or otherwise questionable scales. The main advantages of the NMDS are: (i) it avoids the assumption of linear relationship among variables, (ii) it allows the use of any distance measure, and (iii) its use of ranked distances tends to linearize the relationship between distances in environmental space (relieving the "zero-truncation problem"). For these reasons, NMDS has been considered as the most effective ordination method for ecological data (McCune & Grace 2002). We used the Sørensen/Bray-Curtis coefficient as a distance measure and autopilot-mode in the analysis, allowing the program to choose the best solution (i.e. lowest stress) from 40 separate runs of real data (McCune and Grace 2002). Prior the analysis, diatom data sets were arcsine-squareroot-transformed, since most data sets benefit from one or more transformations that, for instance, make the distance measure work better and emphasise informative species at the expense of uninformative species (McCune and Grace 2002).

Concordance between species level ordination and ordinations obtained using approaches ii–iv (*see* above) was then tested. For a tight match, the dimensionality of the original data matrices was first reduced to two dimensions (i.e. using ordination scores of the first two axes). The congruence between the two-dimensional ordinations was then analysed using Procrustean analysis. The analysis works by scaling, rotating and dilating one ordination solution and then superimposing it on a second ordination, maximising the fit between corresponding observations of the two ordination configurations (Jackson 1995, Peres-Neto and Jackson 2001). The squared residuals between the two configurations were then used as a measure of association or concordance with low values indicating strong concordance. PROTEST extends Procrustean analysis by providing a permutation procedure to assess the statistical significance of the Procrustean fit (Jackson 1995). The statistical power of the method has been shown to be equal to or even superior to that of the Mantel test (Peres-Neto and Jackson 2001).

Results

Long-term monitoring data

A significant interaction term (site × year: $F_{39,401} = 3.7$, $p < 0.0001$) of the two-way ANOVA indicated that the change in chlorophyll *a* content through time was not similar at the study sites. The long-term trend in the chlorophyll-*a* content

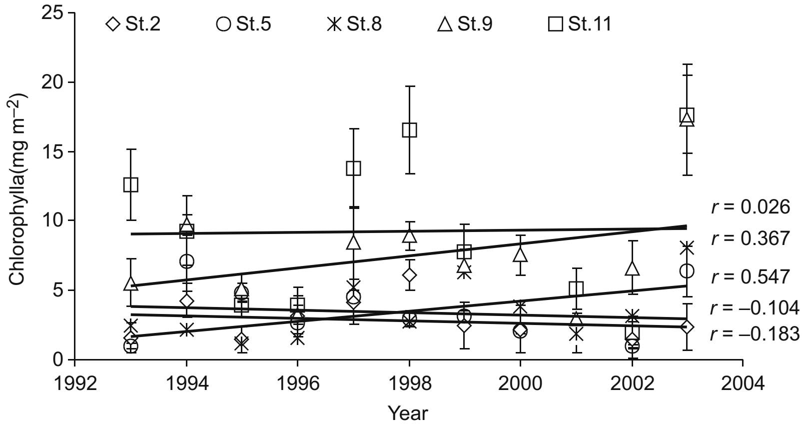

Fig. 2. Long-term trend (from 1993 to 2003) of periphyton biomass (chlorophyll *a* average ± 1 SE; mg m^{-2}) at five monitoring sites in the Kymi River. The *r* values are also shown.

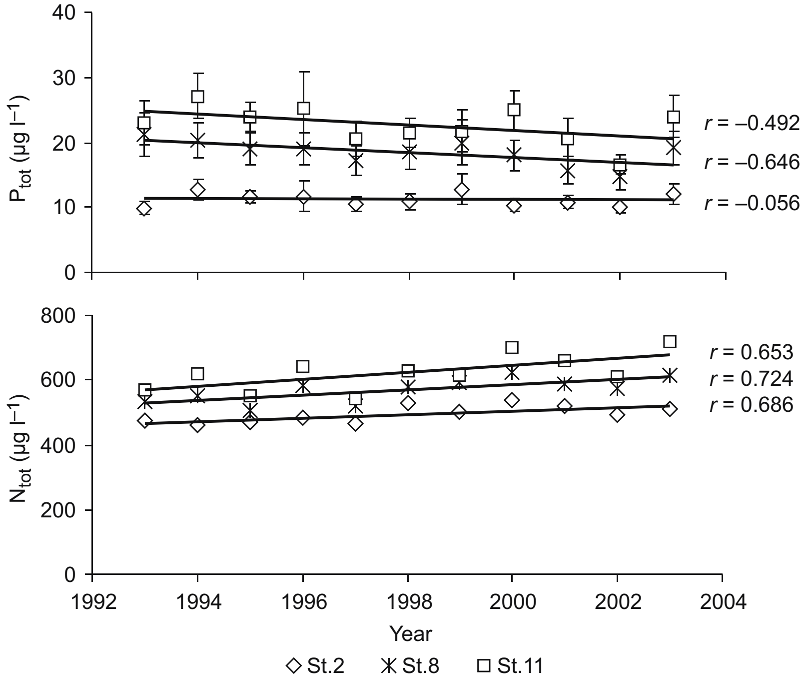

Fig. 3. Long-term trends (from 1993 to 2003) of total phosphorus (average ± 1 SE; µg l^{-1}) and total nitrogen (µg l^{-1}) concentrations at three monitoring sites in the Kymi River. The *r* values are also shown.

showed a decline at sites 2 and 5 but an increase at sites 8, 9 and 11 (Fig. 2). At site 11, within- and between-year variation was especially high and the increasing temporal trend was weak. The variance in chlorophyll-*a* values also increased significantly (Levene's test: $p < 0.0001$) in the downstream direction. Between-site comparisons (Dunnett's test) revealed that only two impacted sites in the lower reaches of the river (sites 9 and 11) had a significantly higher chlorophyll-*a* content as compared with that at the reference site 2. Between-year comparisons showed that the data from 1998 and 2001–2003 differed significantly ($p < 0.05$ in all comparisons) from the first (1993) monitoring data set. Multiple linear regression analysis indicated a significant association between annual nutrient (TP and TN) concentrations and chlorophyll *a* ($F_{2,28} = 7.5$, $p < 0.01$, $R^2 = 0.34$, sites 2, 8 and 11). Interestingly, a long-term declining trend for TP was recorded at all three sites, whereas TN concentrations seemed to increase during the study period (Fig. 3).

Comparison of the methods

In the one-year data from 11 sampling sites, the highest algal biomasses were detected at sites

9 and 10 (on average 25.4 mg m^{-2} and 19.4 mg m^{-2} of chlorophyll *a*; *see* Table 1). However, between-site variation in chlorophyll *a* was high even in the lower courses of the river, since the average amount of algae at site 11 was only 5.3 mg m^{-2}. According to the artificial substrate method, the most oligotrophic sites during the study period were sites 3 and 4 (on average 1.3 mg m^{-2} and 0.8 mg m^{-2} chlorophyll *a*, respectively). Stepwise regression analysis indicated a statistically non-significant correlation between periphyton chlorophyll *a* and conductivity ($F_{1,9}$ = 3.6, $p = 0.09$, $R^2 = 0.28$) among the 11 sites.

By contrast, diatom indices indicated that the water quality was poorest at sites 7, 9 and 11, i.e. near the estuary and below the wastewater discharges, and highest at the unimpacted reference sites 1 and 2 (Table 1). When comparing the index values to the proposed limit values of five water quality classes (*see* Eloranta and Soininen 2002), water quality at the sites ranged from moderate to poor (GDI index), from high to moderate (IPS) and from oligo- to oligo-mesotrophy (TDI). Stepwise regression analysis indicated that the GDI index correlated most strongly with TN ($F_{1,9} = 6.8$, $p < 0.05$, $R^2 = 0.42$), TDI with TP ($F_{1,9} = 7.9$, $p < 0.05$, $R^2 = 0.47$) and IPS with TN ($F_{1,9} = 9.5$, $p < 0.05$, $R^2 = 0.53$). In contrast to chlorophyll *a*, all index-water quality correlations were statistically significant. Thus, the diatom indices had a closer association (in terms of variation explained) with water quality than did chlorophyll *a* on the plastic sheets.

Comparison of taxonomic levels

In total, 161 diatom taxa were identified from the 11 samples. The number of diatom genera was 30, the same as the number of abundant (> 2% of counted cells) species. Relative proportions of the abundant species varied from 58% (site 4) to 80% (site 1). The four NMDS ordinations resulted in either 2-dimensional (data sets i and ii, *see* Material and methods) or 3-dimensional solutions (data sets iii and iv). The concordance of the species and generic level NMDS scores were highly significant (Table 2). The whole diatom community at the species level and the abundant species alone or combined with rare taxa at the genus level also showed statistically significant concordance. However, the index values based on the whole community and those obtained using only abundant species differed significantly (paired *t*-test: $p < 0.01$; $p < 0.0001$ for the IPS and TDI indices, respectively).

Discussion

The long-term monitoring results of chlorophyll *a* measured from artificial substrata showed that only the two impacted sites at the lower reaches of the river differed significantly from the unimpacted reference site. Between-year comparisons showed that the periphyton biomass data sets from 1998 and 2001–2003 differed significantly from the first data set (1993), and the significant interaction term (sites × years) indicated that the change in periphyton biomass through time was

Table 1. Average, minimum and maximum values of periphyton biomass (chlorophyll *a*), diatom indices (GDI, TDI and IPS, as calculated with Omnidia software) and nutrient (TP and TN) concentrations in the Kymi River in 2003.

Measure	Avg.	Min.	Max.
Chl. *a* (mg m^{-2})	7.1	0.8 (site 4)	25.4 (site 9)
GDI index	13.6	11.7 (site 11)	14.8 (site 2)
TDI index	33.1	22.2 (site 1)	42.9 (site 7)
IPS index	16.5	13.9 (site 11)	17.9 (site 2)
P_{tot} (µg l^{-1})	21.5	6.0 (site 1)	28.0 (site 11)
N_{tot} (µg l^{-1})	453.6	410.0 (site 2)	530.0 (site 11)

Table 2. Level of concordance and statistical significances obtained using the Procrustean Rotation Test (PROTEST) on the diatom data matrices. Site scores were obtained from Nonmetric Multidimensional Scaling (NMDS) ordination.

	Sum of squared residuals	*p*
Species		
vs. genera	0.2670	0.0002
Species		
vs. common species (> 2%)	0.5937	0.0434
Species		
vs. common species + genera	0.3975	0.0163

not similar at all sites. Within- and among-year variation in the monitoring results was especially high in the lower courses of the river, where the drainage area is heavily impacted by agricultural activities. Some of the variation was probably related to higher within-year variability in water quality near the river estuary. However, variance was shown to also increase with increasing mean values (Morin and Cattaneo 1992). Despite the opposite trends of nutrient concentrations (TP decreasing and TN increasing), multiple linear regression analysis indicated a significant association for annual nutrient concentrations and periphyton biomass. The results thus indicate that at least major changes in river water quality can be monitored with the artificial substrate method.

Comparison of the methods revealed that the chlorophyll-*a* content on plastic sheets increased towards the estuary, which was in accordance with the general trophic gradient of the river. The method was, however, unable to reliably discriminate subtle differences between the unimpacted sites and slightly impacted sites, because sites 3 and 4 (i.e. sites that receive wastewaters) supported the lowest algal biomass. Conductivity was the only variable incorporated into the regression model, explaining 28% of variation in chlorophyll *a*. Obviously, periphyton growth on plastic sheets was determined to a large degree by some unmeasured environmental factors rather than, for example, nutrient concentrations (*see* also Kjeldsen 1994).

Biomass production of periphytic algae is typically determined not only by nutrient concentrations, but also by local physical factors such as current velocity, depth, light intensity, and also by the spectrum of algal species present in the regional species pool (e.g. Biggs 1996, Chételat *et al*. 1999). Often, the main part of the algal biomass can be accounted for by filamentous macroalgae such as *Cladophora* (Dodds 1991), and their absence, for whatever reason, is strongly reflected in the algal biomass. Thus, periphyton biomass exhibits large variability at multiple spatial and temporal scales, as was also shown by the present results.

A further major drawback of the use of artificial substrata is that algal communities on, for instance, plastic substrates may differ considerably from communities on natural substrates, largely reflecting among-species variability in the ability to attach to the substratum (Schagerl and Donabaum 1998). Therefore, this may seriously impair the validity of monitoring results based on the plastic sheet method. Although chlorophyll-*a* measurement is commonly considered as a "quick and dirty" method for estimating periphyton abundance, the sampling effort becomes relatively high if more than one incubation period within a summer is performed, as is often the case with the artificial substrate method. Therefore, with several incubation periods the method can be considered as a relatively cost-intensive and laborious tool for monitoring the water quality of large rivers. Moreover, lowering the sampling effort to, for example, one incubation period would not alleviate this problem entirely, since this would result in low statistical power and increase the probability of committing a type II error. *In situ* measurement of chlorophyll *a* from stones (*see* e.g. Uehlinger 1991) would be more cost-effective than the artificial substrate approach. However, as stone size and periphyton biomass are typically correlated (Uehlinger 1991), and as the algal standing crop largely depends on the sampling occasion with regard to community development through the growing season, the standardization of the growing and sampling period and sampled area in the *in situ* measurement of chlorophyll *a* from stones is difficult.

As compared with analyses of the periphyton biomass on artificial substrates, the diatom community analysis seemed to be a more sensitive and reliable tool to assess water quality. When using diatom indices, the unimpacted reference sites (1 and 2) were evaluated as sites with good water quality, whereas sites downstream from wastewater discharges and at the lower reaches of the river received lower index values, thus indicating reduced water quality. Regression analysis also showed that water quality parameters explained more of the variation in the diatom indices than in chlorophyll *a*. Interestingly, the GDI index, which in Finnish rivers is considered to share the same limit values of water quality as IPS (Eloranta and Soininen 2002), showed lower values, which in this study lowered the ecological water quality inferred from diatoms at many sampling sites by up to one water quality class (e.g. from moderate to poor quality). This implies that the diatom index used may have an important role in determining the ecological quality of the sampling sites. Therefore, limit values proposed for GDI or IPS indices may require some modification, or at the very least, further examination. Nevertheless, sampling diatoms from the natural stony substrata and preparing permanent slides are relatively easy to conduct. Thus, the community-based method seems more sensitive and cost-effective than the traditional biomass-based monitoring method.

Analysis of concordance indicated that NMDS ordination scores derived from the generic and species level data were strongly concordant, while ordinations based solely on abundant species or combined with rare taxa at generic level showed weaker, although significant, congruence with the species data. Algal data from other Finnish streams (J. Soininen unpubl. data) also support the relatively strong congruence among the generic and species level data, suggesting that benthic algal communities might assemble from clusters of congeneric species having rather similar and narrow ecological tolerances. Thus, generic level identification, or this combined with the locally abundant species, could offer a practical and reliable biomonitoring tool for large rivers, at least within watersheds with relatively limited regional diatom species pools. Rare species are certainly important in biodiversity inventories (*see* Cao *et al*. 1998), but our results imply that, at least in large boreal rivers, excluding rare diatom species or identifying data to the genus level may have negligible effects on the results of ordination analyses.

In our data, abundant species constituted only 30 out of 161 taxa, but 58%–80% of the counted cells. However, diatom index values for data using merely abundant species were significantly different from those obtained using the complete data set. The differences were due to the tendency of the abundant species data to underestimate the water quality at the sampling sites. Thus, locally rare species (abundance < 2%) may be important in detecting subtle differences among the study sites. This implies that if merely locally abundant or core species are included in diatom indices, interpretations of the results have to be made very carefully. Moreover, for each watershed or ecoregion, an inventory for regionally abundant and frequently occurring species must first be conducted. Nevertheless, on the grounds of cost-effectiveness, reliability and sensitivity, the diatom-based method is to be recommended over the traditional method for biomonitoring large boreal rivers, regardless of the identification level.

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