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Primary Research Paper

Diatoms and biomonitoring: should cell size be accounted for?

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Abstract

Despite the fact that biovolume calculation is a common procedure in most phytoplankton and periphyton studies, diatom community analyses are usually based on relative abundance data. In a biomonitoring context, a community metric that accounts for cell size could be of interest due to the potential differences that might exist in nutrient uptake between large and small-sized species. This paper addresses the question of whether diatom community analysis should be based on relative abundance, biovolume or cell surface. The results show that although community structure expressed as relative proportion of taxa varied according to the metric used, the ordinations conducted with each metric were similar. The explained percentage of species variance was slightly higher with the relative abundance metric compared to the metrics based on relative biovolume or cell surface area. Partial CCAs showed that each water chemistry variable generally explained a higher portion of species variance when the relative abundance was used. The analyses conducted with two size groups (small and large taxa) expressed as relative abundance and relative biovolume showed similar results. Moreover, our data showed that there is no significant relationship between diatom size and total phosphorus. According to these results, it seems that relative abundance would be the most appropriate metric to use for biomonitoring purposes. The biovolume and cell surface area calculations added substantially to the total analysis time due to the numerous measurements required, but did not improve the variance explained in community structure, and site ordinations were not significantly different.

Introduction

Studies dealing with the relationship between nutrient enrichment and algal communities are often based on biomass measurements. Phytoplankton biomass has proven to be strongly related to nutrient supply in lakes (e.g. Schindler, 1974; Nicholls & Dillon, 1978; Maberly et al., 2002). However, the relationship between periphyton biomass and stream nutrient levels is not straightforward. Algal biomass reflects the response of the pioneer species pool to the interaction of light, temperature, nutrient availability, hydrology, and grazing by invertebrates, among others. The mixed influence of these factors makes total periphyton biomass a restricted indicator of nutrient enrichment in streams (Biggs, 2000; Stelzer & Lamberti, 2001; Dodds et al., 2002; Bernhardt & Likens, 2004). This fact was also pointed out in nutrient addition experiments (Borchardt, 1996; Francoeur, 2001). Weak relationships between periphyton biomass and nutrient concentrations in streams have been attributed mainly to frequent disturbance due to floods and drying events, and significant habitat heterogeneity in lotic systems (Biggs, 2000; Dodds et al., 2002; Lavoie et al., 2004). In small forested streams, periphyton biomass may be more limited by light

or grazers than by nutrients (Rosemond, 1993; Rosemond et al., 1993; Hill, 1996; Wellnitz et al., 1996), and the relative impact of these factors may shift seasonally (Rosemond et al., 2000).

On the other hand, significant relationships have been observed between stream nutrient levels and periphyton community structure (e.g. Harding et al., 1999; Stelzer & Lamberti, 2001). The contribution of each species to the overall periphyton community may be assessed by quantitative methods that allow an estimation of the absolute or relative development of each taxon. This can be achieved by expressing community structure as absolute or relative abundance, biovolume or biomass of each species. The biomass of each taxon cannot be measured directly due to the diversity of periphytic communities. To overcome this limitation, the biomass of each taxon can be estimated from biovolume using biometric formulas. Standard formulas have been developed to calculate the biovolume of co-occurring algae varying in shape and size (e.g. Hillebrand et al., 1999; Sun & Liu, 2003) and to convert microalgal biovolume to carbon content (Rocha & Duncan, 1985).

Studies of total periphytic or phytoplanktonic communities are generally based on cell volume (e.g. McCormick & Stevenson, 1991; Mulholland et al., 1995; Ghosh & Gaur, 1998; Vavilova & Lewis, 1999; Lavoie et al., 2004) because this allows an evaluation of the contribution of each algal group or each taxa to primary production (and hence C store). Moreover, algal communities are often composed of filamentous taxa or aggregates of small cell cyanobacteria that are difficult to enumerate as individuals. Despite the fact that biovolume calculation is a common procedure in phytoplankton and periphyton studies, diatom community analysis is usually based on counts of a fixed number of diatom valves since individual cells are clearly distinct. The community structure is then expressed as the relative abundance of each taxon irrespective of cell size (e.g. Kelly et al., 1995; Pan et al., 2000; Winter & Duthie, 2000a, b, c; Jüttner et al., 2003; Potapova & Charles, 2003; Gosselain et al., 2005). In addition, most diatom-based indices were develop using relative abundance data (e.g. Kelly & Whitton, 1995; Kelly, 1998; Prygiel et al., 1999), as for paleoenvironmental reconstruction models (e.g. Smol & Cumming, 2000; Fallu et al., 2002).

Relative abundance may inadequately represent diatom community structure because it does not account for volume differences among taxa. There are differences in size range among diatom species and among individuals from the same species. The influence of abundant small species may therefore be overestimated relative to large taxa. Cell size influences the species contribution to the community biomass and division rate; smaller cells often have faster turnover rates than larger ones (Cox, 1991). On the other hand, pressure by grazing is likely to be higher for small species; an important population of small species could therefore represent ''successful'' growth in that environment, but low relative abundance of a large species should not always be interpreted as indicative of poor growth (Cox, 1991). Except for the studies conducted in the Baltic and the Bothnian Sea (Busse & Snoeijs, 2002, 2003; Snoeijs et al., 2002) and in some Canadian streams (Cattaneo et al., 1997; Wunsam et al., 2002), the question of whether diatom community analysis should be based on biovolume or abundance data has rarely been addressed and the results published so far are conflicting.

In addition to relative abundance and biovolume, diatom community structure may be expressed by the cell surface area of each taxon. Although cell volume may represent an accurate estimate of species contribution to the overall community, the importance of species containing large vacuoles may be overestimated (Sicko-Goad et al., 1977). Moreover, depending on the environmental conditions, the physiological state of the cell may influence the cell size as well as the cytoplasmic constituents within the cell (Sicko-Goad et al., 1977). These two elements may add noise to the species–environment relationship. Cell surface area is an alternative metric that accounts for cell size, and might not overestimate the influence of large taxa as much as cell volume. Cell surface is a rarely used metric in biomonitoring studies, but its potential for community structure analyses has been pointed by Snoeijs et al. (2002). Valve area (2-D measurements) could also be a metric of interest because it accounts for cell size, and is affected to a lesser extend by errors associated with differences in vacuole size. To our knowledge, no study has evaluated diatom community structure using valve area so far.

In a biomonitoring context, a community metric that would account for cell size could be of interest due to the potential differences that might exist in nutrient uptake between large and small-sized species. The aim of this study was to evaluate if increasing the weight of larger species by accounting for cell size would provide a different community response to the environment compared to the more traditional data set based on relative abundance.

Materials and methods

Study area and sampling locations

A total of 410 diatom samples were collected and analysed. These samples were collected at 126 sampling locations distributed along 32 streams and creeks in the St. Lawrence River basin, Québec, Canada (Fig. 1). The sampling locations were chosen from sites included in the water quality monitoring network of the Ministry of the Environment (Québec Government). They were selected according to the availability of physico-chemical data and on the basis of land use information with the aim of sampling across a broad gradient of ecoregions and pollution levels. In order to account for the inter-seasonal and inter-annual variability in diatom communities, sampling was conducted during the Spring (May–June) and Fall (September) of 2002 and 2003, leading to 4 diatom samples for most of our sites.

The sampling sites are distributed within three ecoregions: the Canadian Shield, the St. Lawrence Lowlands and the Appalachians (Fig. 1). The Canadian Shield catchments are mostly covered by boreal forest, but the southern part of the Shield overlaps the transition zone of the mixed and boreal coniferous forests. The streams sampled are low in nutrients, conductivity and suspended solids (SS), and exhibit circumneutral pH (Table 1). These catchments are considered less impacted. The St. Lawrence Lowlands are characterized by intensive farmlands, large industrial centres, and are the location of most of Québec's population. The streams sampled are high in nutrients, conductivity and SS, and have higher pH (Table 1).

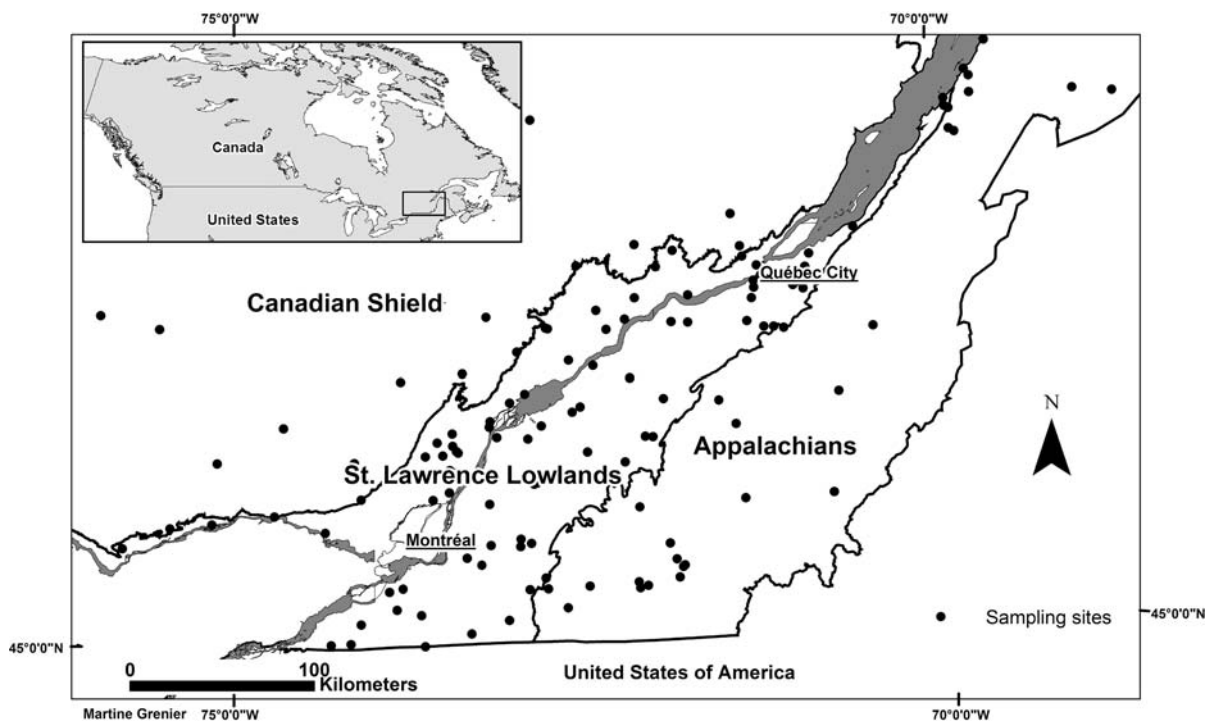

Figure 1. Ecoregions and sampling locations in the St. Lawrence Basin (Québec, Canada).

Table 1. Median values for water chemistry variables arranged according to ecoregions of the St. Lawrence basin (Québec, Canada)

Description	Tranf.	Canadian Shield		St. Lawrence Lowlands		Appalachians	
		Spring	Fall	Spring	Fall	Spring	Fall
TP (μg l^{-1})	Log	18.6	17.3	44.0	50.6	25.8	24.0
Soluble phosphorus (μg l^{-1} P)	Log	10.0	10.0	14.2	22.5	10.0	10.4
Total diss. nitrogen (μg l^{-1} N)	Log	297.5	210.0	950.0	633.3	433.3	410.3
Nitrates-nitrites (μg l^{-1} N)	Log	118.5	51.7	606.7	315.0	193.3	160.8
Ammonia (μg l^{-1} N)	Log	22.5	20.0	45.0	36.0	26.7	25.0
Chlorophyll *a* (mg m^{-3})	Log	2.3	2.5	7.1	7.3	4.6	4.2
pH	–	7.0	7.3	7.9	8.1	7.8	8.0
Conductivity (μS cm^{-1})	SQR2	29.2	37.6	181.0	273.4	145.1	163.3
Water temperature (°C)	–	12.2	20.2	14.0	21.5	12.0	21.2
Dissolved oxygen (mg l^{-1})	–	10.8	9.1	10.3	9.1	11.0	9.2
Turbidity (UNT)	Log	1.2	1.3	5.9	5.6	3.0	2.7
SS (mg l^{-1})	Log	2.8	2.4	10.7	7.3	6.3	3.3
Coliforms (UFC)	Log	10.7	43.8	251.8	277.0	126.7	122.6
Diss. organic carbon (mg l^{-1})	Log	4.9	4.6	6.4	6.0	5.8	5.7

These catchments exhibit a gradient from slightly impacted to very impacted streams, most of the latter being located in the Upper St. Lawrence plain. Located in south-eastern Canada, the catchments located in the Appalachians are also impacted by farming, but to a lesser extent. The streams sampled have intermediate levels of nutrients, conductivity and SS. Many streams have their source in the Canadian Shield or the Appalachians and flow through the St. Lawrence Lowlands. As a result, the water chemistry of some streams flowing thought the lowlands reflects the characteristics of the ecoregion upstream.

Water analyses

Water analyses were performed by the Ministry of the Environment (Québec Government) as part of a water quality monitoring programme started in the 1970's. Water samples were collected every 4 weeks. The following parameters were considered in this study: total phosphorus (TP), soluble reactive phosphorus (SRP), total dissolved nitrogen (TN), total nitrate (nitrate + nitrite) (NO_3-N), ammonia-nitrogen (NH_3-N), chlorophyll *a* (CHLA), pH, conductivity (CON), temperature (TEMP), dissolved oxygen (O_2), turbidity (TUR), SS, coliforms (COLI) and dissolved organic carbon (DOC). Some water chemistry data were transformed in order to improve normality (Table 1). Because diatoms are known to integrate stream water chemistry through time, seasonal averages were used in the analyses instead of one-time measurements. Spring averages were calculated from the six measurements taken in May and June, and fall averages were calculated from the six measurements taken in August and September.

Diatom data

Benthic diatoms were scraped from the top surface of 5 rocks (composite sample) collected within a $\sim$5 m^2 area. Sampling depth varied from 20 to 50 cm, depending on turbidity and water level. The algae were collected from riffles and unshaded areas where possible. The samples were preserved with Lugol's iodine and stored until the samples were processed. The samples were digested in hydrogen peroxide and mounted onto microscope slides using Naphrax. As recommended by Prygiel & Coste (1993), a minimum of 400 valves per slide were counted and identified to the most precise possible taxonomic level. Taxonomic identifications followed mostly Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Reavie & Smol (1998), Fallu et al. (2000), Krammer (2000, 2002, 2003)

and Lange-Bertalot (2001). Diatoms were identified and counted at 1250 × under a Zeiss Axioskop II microscope with differential interference contrast imaging (DIC). Pictures were taken for each species with a Zeiss Axiocam digital camera (1.3 Mega pixels). Diatom length and width measurements were obtained from our picture database. Diatom depths were obtained from our pictures when a girdle view of the species was available. We referred to the taxonomic books listed above to obtained a girdle view when it was not available from our picture data base. The depth measurement for some taxa was evaluated by an estimate of the distance from the bottom to the top focus of the frustule when we could not find any girdle view picture for a taxa. Cell depth was expressed as a fraction of cell width. In the case of taxa with a wide range of sizes (e.g., the length of *Surirella amphioxys* W. Smith ranged from 20 to 51 μm), the biometric measurements were calculated based on at least five individuals (up to 36). The biometric measurements of taxa having a more constant size were calculated based on an average of three individuals. Calculation of cell volume, cell surface and valve area were obtained according to geometric forms and formulas presented in Hillebrand et al. (1999). The valve area formula for the cymbelloid form was not available from Hillebrand's work and was calculated by Alain Chalifour (pers. comm.) from the Department of Mathematics at the Université du Québec à Trois-Rivières. We are aware that vacuole size (or cell wall) may influence cell "active" biovolume (Sicko-Goad et al., 1977). However, we did not consider vacuole size in our biovolume calculations since the variability associated with environmental conditions makes this estimate impractical for routine uses.

Data analysis

Diatom counts and biometric measurements were processed in a *MS ACCESS* database. A taxa was not included in the analyses if less than four valves were counted in the total set of 410 samples. Of the 460 taxa identified, 319 met the above criterion. The abundance, biovolume, cell surface area and valve area were calculated as the relative contribution (%) of each taxa to the total community. All four metrics are thus composed of percentage data. Diatom data were square root transformed and rare species were downweigthed. Detrended correspondance analyses (DCAs) with detrending by linear segments (26) and nonlinear rescaling of axes, and canonical correspondance analyses (CCAs) were conducted using CANOCO version 4.5 (Ter Braak & Smilauer, 2002). The gradient lengths along DCA axes 1 (from 3.64 to 4.09 standard deviation units, depending on matrices) justified the subsequent use of unimodal ordination methods (Ter Braak & Prentice, 1988).

DCA total inertia, eigenvalues and coordinate shifts on axes 1 and 2 were analysed to detect any differences between relative abundance data and biovolume or cell surface data. Procrustean randomization tests (PROTEST, Jackson, 1995) were conducted on the first 2 DCA axes to compare the matrices concordance. The relative abundance metric was compare with the relative biovolume, the relative cell surface and the relative valve area metrics. Procrustes analysis rotates and scales a matrix to maximum similarity with a target matrix minimizing sum of squared differences. The m^2-value (goodness-of-fit statistic) is a measure of the degree of concordance between two matrices and its significance is evaluated with a randomization test (9999 permutations) (Jackson, 1995). Canonical Correspondence Analyses (CCAs) constrained to water chemistry variables were conducted in order to compare the eigenvalues and the percentage of variance in species data explained by environmental variables using the different metrics. SRP, NO_3-N and SS had a variance inflation factor (VIF) exceeding 10 (Pan et al., 1996) and were not included in the CCAs since they were highly correlated with other variables. All remaining variables were significant ($p \leq 0.05$) as tested with Monte Carlo permutation tests (with 499 unrestricted permutations). PROTESTs were conducted on the first 2 CCA axes to compare the matrices concordance. Finally, a series of CCAs constrained to one variable (partial CCAs) were run to evaluate the percentage variance of species data explained by each variable for each community metric.

A second set of analyses was conducted on two datasets based on diatom size. The diatom data set was separated into two size classes based on biovolume (small species <500 μm^3, large species ≥ 500 μm^3). This classification was used because

cell biovolume increased markedly after 500 μm^3. CCAs and partial CCAs were conducted using the same environmental variables and the diatom data expressed as relative abundance and relative biovolume for the small and large taxa. A total of 216 taxa were classified as small cells and 103 as large cells. PROTESTs were conducted on the first 2 CCA axes to compare the concordance between the ordinations based on the small-cell and large-cell metrics expressed as relative abundance and relative biovolume.

Results

Biometric comparison

The smallest taxon was *Fragilaria* cf. *microstriata* Marciniak with cell biovolume, cell surface area and valve area of 12 μm^3, 22 μm^2 and 10 μm^2 respectively. The largest taxon was *Surirella splendida* (Ehrenberg) Kützing with cell biovolume, cell surface area and valve area of 56,409 μm^3, 9578 μm^2 and 2115 μm^2 respectively. The size differences between the smallest and the largest taxa had a factor of approximately 4700 × for biovolume, 435× for surface area and 200 × for valve area. Common small diatoms such as *Navicula minima* Grunow, *Nitzschia inconspicua* Grunow and *Achnanthidium minutissimum* (Kützing) Czarnecki ranged from 28 to 74 μm^3 for biovolume, 65–120 μm^2 for cell surface and 20–27 μm^2 for valve area. Relatively common large-sized diatoms such as *Cymbella tumida* (Brebisson) Van Heurck, *Navicula peregrina* (Ehrenberg) Kützing and *Surirella amphioxys* W. Smith ranged from 4851 to 5538 μm^3 for biovolume, 1455–1898 μm^2 for cell surface area and 399–638 μm^2 for valve area. Most of the identified taxa (90%) ranged from 36 to 5267 μm^3 for biovolume, 77 to 1936 μm^2 for cell surface and 22 to 580 μm^2 for valve area.

Depending on the metric used, the relative importance of a species in a sample could be quite variable. For example, sample 1148 composed of 15% *Navicula lanceolata* (Agardh) Ehrenberg and 71% *Navicula gregaria* Donkin based on relative abundance showed a very different structure when the metric was expressed as relative biovolume, where *N. lanceolata* increased to 41% and *N. gregaria* dropped to 37% (Table 2). Major

Table 2. Examples of diatom community structure based on relative abundances (a) and relative biovolumes (b)

Taxa name: see Electronic Supplementary Material for authorities	Vol μm^3	Samples with no major community shift										Samples with major community shift							
		1078		1098		1123		2125		4178		1107		1148		2147		4152	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Small species																			
Navicula minima	28															18	**1**	16	**2**
Achnanthidium minutissimum	74	79	**53**	77	**49**							65	**12**						
Intermediate species																			
Nitzschia palea	230							81	**88**							11	**2**		
Fragilaria capucina var. *vaucheriae*	254	7	**16**	4	**10**														
Navicula gregaria	312					8	**2**							71	**37**				
Rhoicosphenia abbreviata	465																	44	**70**
Large species																			
Surirella brebissonii	1664													3	**9**				
Navicula lanceolata	1665					85	**94**							15	**41**				
Tabellaria flocculosa	2358											8	**45**						
Melosira varians	2973									81	**91**								
Nitzschia littoralis	21,923															6	**79**		

Data are expressed as the relative contribution (%) of each taxa to the total community.

shifts in community structure were observed when the diatom assemblages were composed of a large number of small species (e.g. *A. minutissimum* and small *Navicula* and *Nitzschia* species) and a small number of large species (Table 2). For example, sample 1107 had a high relative abundance of *A. minutissimum* and a low abundance of *Tabellaria flocculosa* (Roth) Kützing resulting in an important shift in community structure when relative biovolume was used. On the other hand, minor changes were observed when large species were absent or, conversely, when the community was mainly composed of large species (Table 2). For example, sample 2125 in Table 2 was dominated by intermediate sized species, *Nitzschia palea* (Kützing) W. Smith, and had undergone no major community shift due to the absence of large species. Similarly, sample 1123 was dominated by a relatively large species (*N. lanceolata*) and showed no major community shift due to low abundance of small or intermediate sized species.

The mean shift in percentage data between the relative abundance and relative biovolume of each taxa was less than 3% for 75% of the taxa sampled. The difference in community structure was lower when the surface area or valve area metrics were used instead of biovolume. Biometric values for each taxon are listed in supplementary material[1].

Community analyses

Detrended correspondance analyses

Total variance was higher when the species data were expressed as relative biovolume (4.39), relative cell surface (4.05) and relative valve area (4.11) compared with relative abundance (3.75) (Table 3a). Eigenvalues and length of gradient were slightly higher for the relative biovolume metric than for the other metrics tested. The eigenvalue is an importance measure of the ordination axes while the length of gradient is a measure of how unimodal the species responses are along an ordination axis (Ter Braak & Smilauer, 2002). The cumulative percentage variances of species data were similar for each metric (Table 3a). Superimposed DCA ordinations (axes 1 and 2) showed that the overall site score shifts were negligible when the community structure was expressed as relative biovolume or cell surface area or valve area compared with relative abundance site scores (Fig. 2). The root mean square (RMS) shifts in DCA site scores (Fig. 2a, b, c) were 3.7, 2.5 and 2.6% on axis 1 and 5.0, 4.4 and 4.9% on axis 2 respectively. The maximum shifts were 12.8, 7.9 and 6.9% on axis 1 and 16.2, 13.5 and 14.3% on axis 2 respectively. Larger differences in community ordination (site scores) between the metrics were generally associated with sites dominated by the small-sized *A. minutissima* while the smaller differences were associated with community dominated by intermediate size species. The fit of the DCA ordinations evaluated with PROTEST was greater than expected due to random chance (relative abundance vs. relative biovolume: $m^2 = 0.031$; $p \leq 0.0001$; relative abundance vs. relative surface area: $m^2 = 0.016$; $p \leq 0.0001$ and relative abundance vs. relative valve area: $m^2 = 0.016$; $p \leq 0.0001$) indicating that there were no significant differences between site scores derived from the four community metrics.

Canonical correspondance analyses

CCA analyses were run using species data expressed as relative abundance, relative biovolume, relative cell surface and relative valve area. CCA ordinations for the four different species metrics were almost identical (only the ordination for relative abundance is shown, Fig. 3). TP, TN, NH_3-N, CON, pH, TUR, COLI, and CHLA were associated with the first axis, which reflects a "pollution gradient". The second axis was associated with TEMP and O_2, which reflects seasonality. In these CCAs, each environmental variable explained a significant ($p < 0.05$) and independent (VIF < 10) direction of variance in the diatom data. Axis eigenvalues are shown in Table 3b. The ratios of CCA and DCA eigenvalues on axis 1 were high, indicating that a large amount of variance in species data was explained by the water chemistry variables. The first two axes accounted for 68.7% of the species–environment relationships for the relative biovolume metric and 70.1% for the relative abundance metric (Table 3b), indicating that the variables used in the analyses accounted for the major gradients in the diatom community structure. The cumulative (first two axes) percentage of explained variance in species

[1] Electronic Supplementary Material is available for this article at http:// www.dx.doi.org/10.1007/s10750-006-0223-z

Table 3. Results of the DCAs (a), CCAs (b) and partial CCAs (c) conducted on the four species metrics (319 taxa and 410 samples)

	Relative abundance		Relative biovolume		Relative surface area		Relative valve area	
(a) DCAs								
Axes	1	2	1	2	1	2	1	2
Eigenvalues	0.36	0.21	0.40	0.24	0.38	0.23	0.38	0.23
Length of gradient	3.75	2.44	3.90	2.69	3.81	2.56	3.82	2.57
Cumulative percentage variance of species data	9.60	15.3	9.10	14.6	9.40	15.0	9.20	14.7
Total inertia	3.75		4.39		4.05		4.11	
(b) CCAs								
Axes	1	2	1	2	1	2	1	2
Eigenvalues	0.29	0.15	0.31	0.17	0.31	0.16	0.28	0.16
Cumulative percentage variance of species data	7.70	11.7	7.10	11.0	7.70	11.7	6.70	10.6
Cumulative percentage variance of species–environment relation	46.1	70.1	44.3	68.7	45.2	69.7	43.4	68.4

	Relative abundance variance explained (%)	Relative biovolume variance explained (%)	Relative surface area variance explained (%)	Relative valve area variance explained (%)
(c) Partial CCAs				
TP	4.2	3.7	3.9	3.9
TN	5.5	4.8	5.1	5.1
NH_3-N	2.3	2.1	2.2	2.2
CON	6.8	6.2	6.5	6.5
pH	5.1	5.1	5.2	5.2
TUR	3.9	4.4	3.8	3.8
DOC	1.5	1.5	1.5	1.5
COLI	2.8	2.6	2.7	2.7
O_2	1.0	0.9	1.0	1.0
TEMP	4.2	4.1	4.2	4.2
CHLA	4.2	3.7	3.9	3.9

All water chemistry variables were significant ($p \leq 0.05$).

distribution ranged from 11.0% for the relative biovolume metric to 11.7% for the relative abundance metric. These values are comparable to the values found in the literature (e.g., Winter & Duthie, 2000a; Fallu et al., 2002; Ponader et al., in press). The ordination showed a clear separation of sites according to ecoregions: the less impacted sites from the Canadian Shield were distributed at the lower end of the "pollution gradient" while most of the heavily impacted sites located in the farming areas of the St. Lawrence Lowlands were distributed at the higher end of the "pollution gradient". The Appalachians sites were mostly distributed in the middle of the "pollution gradient". The ordination also showed a clear separation of samples according to sampling season, with most of the spring samples distributed on the upper part of the ordination and most of the fall samples distributed on the lower part of the ordination.

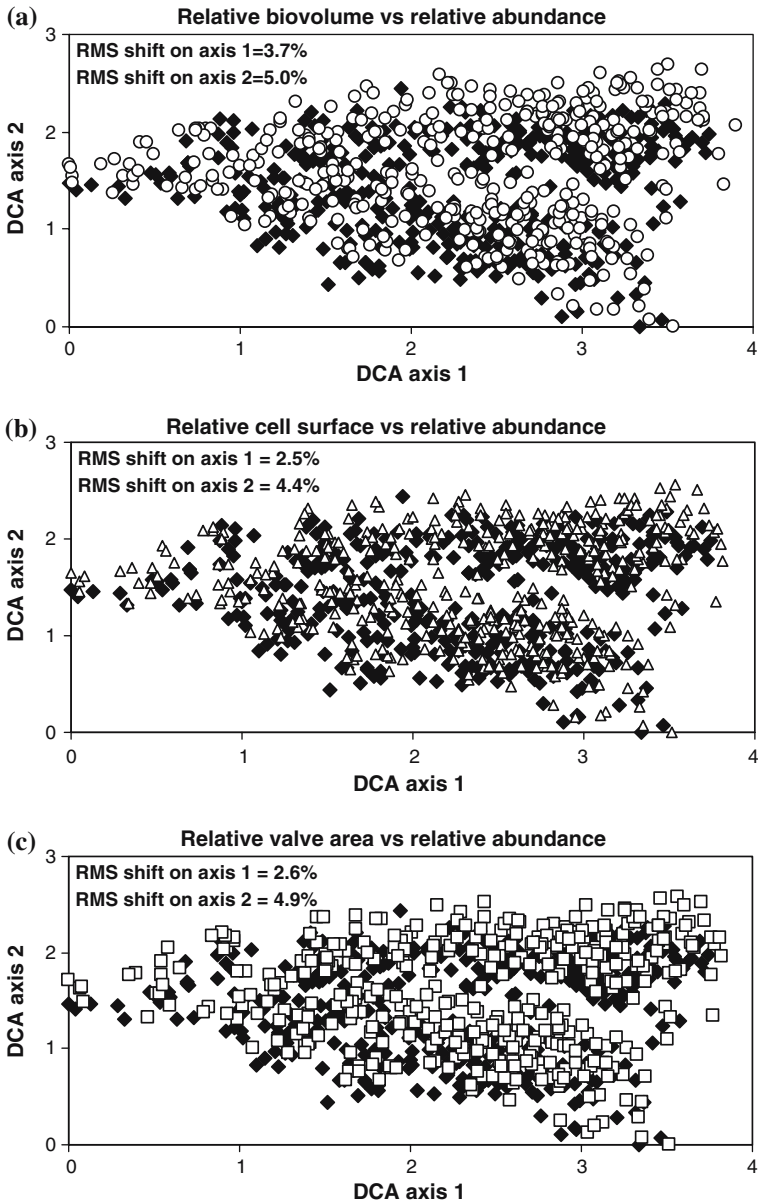

Figure 2. DCA site score differences between relative abundance (black diamonds) and (a) relative biovolume (open circles); (b) relative cell surface (open triangles); and (c) relative valve area (open squares). RMS shifts between site scores are indicated for axes 1 and 2.

Eigenvalues, cumulative percentage variance of species data, and cumulative percentage variance of species–environment relationships were similar using relative abundance, relative biovolume, relative cell surface and relative valve area (Table 3b). PROTESTs were performed to statistically evaluate the similarity between the CCA ordination for each metric. The results showed that the fit between the CCA ordinations were greater than expected due to random chance (relative abundance vs. relative biovolume: $m^2 = 0.028$; $p \leq 0.0001$; relative abundance vs. relative surface area; $m^2 = 0.014$; $p \leq 0.0001$ and relative abundance vs. relative valve area:

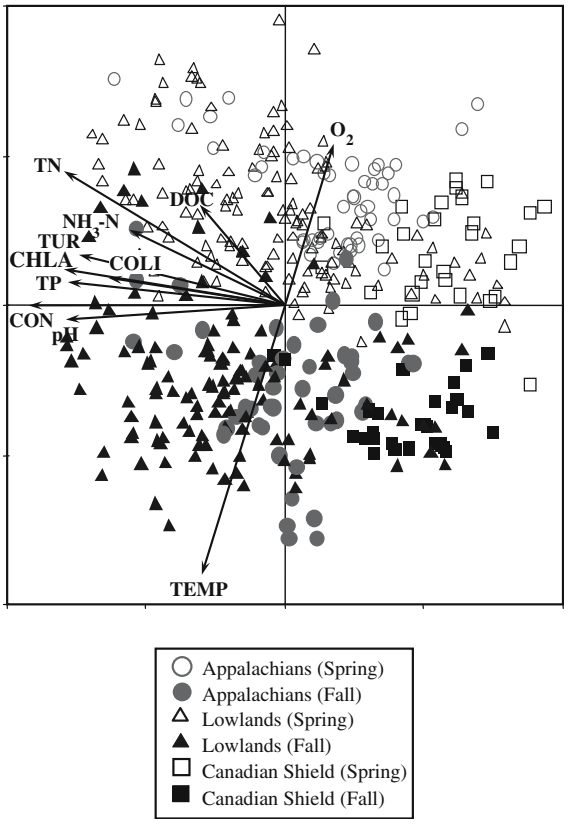

Figure 3. CCA analysis conducted on relative abundance data showing sample scores and water chemistry variables (319 taxa and 410 samples). Axis eigenvalues are shown in Table 3b. The variable codes are defined in Table 1.

$m^2 = 0.013$; $p \leq 0.0001$) indicating that there were no significant differences between site scores derived from the four community metrics.

Partial CCAs constrained to one variable at a time showed that CON, TN, pH , TP and CHLA were the water chemistry variables that individually explained the highest percentage of species variance (Table 3c). Species variance explained by each variable was generally slightly lower with the relative biovolume, relative cell surface and relative valve area metrics than with the relative abundance metric.

Small and large diatom taxa

The CCAs and the partial CCAs conducted on the two size groups expressed as relative abundance and relative biovolume showed similar results (Table 4a). The first axis eigenvalues for the relative abundance and relative biovolume CCAs were higher for the large taxa group compared with the small taxa group, as well as the percent explained variance in species data and species–environment relationship. The values were slightly higher for the data expressed as relative abundance. The partial CCAs showed that the portion of the variance in species data that can be explained by one variable is similar for the small and large taxa groups (Table 4b). TP and TEMP have slightly higher percentage values for the small taxa, while CON, pH and TUR have slightly higher percentage values for the large taxa. Except for TEMP, all variables explained a higher portion of the variance with the data expressed as relative abundance. PROTESTs showed that the ordinations for the two size groups were not significantly different when expressed as relative abundance (small vs. large: $m^2 = 0.5719$; $p \leq 0.0001$) or relative biovolume (small vs. large: $m^2 = 0.5857$; $p \leq 0.0001$). PROTESTs also showed that the ordinations for the relative abundance data and relative biovolume data were not significantly different when only the small taxa were considered (relative abundance vs. biovolume: $m^2 = 0.0123$; $p \leq 0.0001$) or the large taxa (relative abundance vs. biovolume: $m^2 = 0.0244$; $p \leq 0.0001$).

Finally, in order to test the relationship between diatom size and trophic status, we calculated the regression between average diatom size and TP (Fig. 4) and found that there is no significant relationship between diatom size and TP ($r^2 = 0.04$).

Discussion

Species metrics and environmental variables

Cell size varied significantly among the 319 identified taxa. There was a 4700-fold difference in biovolume (three orders of magnitude) between the smallest and the largest taxa. The size variations were not as important for the surface area and valve area metrics. Large differences in mean cell size were also obtained by Snoeijs et al. (2002). Our results showed variations in community structure when the species were expressed using different metrics, sometimes changing the dominant species. The most important variations were related to communities dominated with small

Table 4. Results of the CCAs (a) and partial CCAs (b) conducted on the species metrics expressed as relative abundance and relative biovolume for the small and large species

	Relative abundance				Relative biovolume			
	Small		Large		Small		Large	
(a) CCAs								
Axes	1	2	1	2	1	2	1	2
Eigenvalues	0.25	0.13	0.40	0.17	0.25	0.14	0.39	0.18
Cumulative percentage variance of species data	7.50	11.2	8.80	12.6	7.10	10.9	7.60	11.1
Cumulative percentage variance of species–environment relation	45.8	68.7	52.0	74.6	43.2	66.7	49.2	72.2

	Relative abundance variance explained (%)		Relative biovolume variance explained (%)	
	Small	Large	Small	Large
(b) Partial CCAs				
TP	4.4	3.6	4.1	3.3
TN	5.2	5.3	4.7	4.6
NH_3-N	2.1	2.2	1.9	2.1
CON	6.6	7.5	6.0	6.4
pH	5.1	6.8	4.0	5.9
TUR	3.6	4.3	3.4	3.7
DOC	1.4	1.5	1.5	1.5
COLI	2.8	2.6	2.7	2.7
O_2	0.6	0.7	0.6	0.7
TEMP	4.3	4.0	4.6	3.6
CHLA	4.2	4.1	3.8	3.7

All water chemistry variables were significant ($p \leq 0.05$).

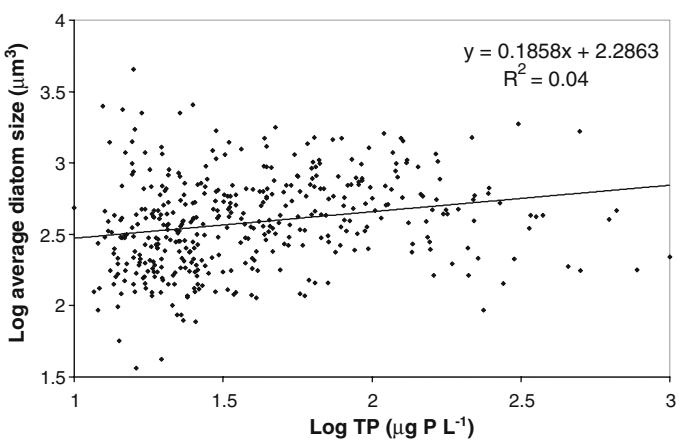

Figure 4. Regression between TP in the water column and the log average diatom size.

diatoms while the communities dominated with intermediate-sized diatoms did not fluctuate much according to the metric used.

Although community structure varied according to the metric used, DCA, CCA and partial CCA results were relatively similar. The metrics based on relative biovolume, relative surface and relative valve area generally showed a slightly lower percentage of explained species variance compared to relative abundance. This difference is

associated with the higher total inertia for these metrics. Our results showed that variations in community structure related to the metric used did not influence markedly the response to the environment. The results from PROTESTs conducted on DCA and CCA showed that site scores on the first two axes were not significantly different from one diatom metric to another. This suggests that large species provide the same information as smaller species and that a diatom assemblage (no matter if it is composed of small or large sized taxa) is an indicator of a particular environment.

Our results from the partial CCAs showed that each environmental variable generally explained a higher portion of species variance when the relative abundance metric was used. The CCAs performed with all significant and independent environmental variables showed very little variability between the metrics. According to these results, it is suggested that relative abundance would be the most appropriate metric to use for biomonitoring purposes. The biovolume and cell surface area calculations added substantially to the total analysis time due to the numerous pictures and measurements required, but did not improve the variance explained in community structure. Similarly, Snoeijs et al. (2002) concluded that the use of abundances yielded the best separation patterns in CA because larger species receive too much weight when expressed as cell volume or surface area, which created an unbalanced data set.

Small and large diatom taxa

We classified the species data set into two groups based on size in order to evaluate whether small and large species have different response to the environment. Our results showed that CCAs and partial CCAs were similar for the small and large taxa (Table 4a, b), although the water chemistry explained a slightly higher portion of the variance in species data for large taxa. Conductivity, pH and turbidity explained a larger portion of the variance in species data for large taxa. However, the higher percentage of variance explained may also be due, in part, to the lower number of species in the large taxa dataset (103) compared to the small taxa dataset (216). PROTESTs showed that the ordinations for the two size groups were not significantly different when expressed as relative abundance or relative biovolume. The results also showed that the ordinations for the relative abundance data and relative biovolume data were not significantly different when only the small taxa or the large taxa were considered.

Other studies concluded that small and large species respond differently to environmental constraints when co-occurring in the same diatom community (Busse & Snoeijs, 2002, 2003; Snoeijs et al., 2002). These studies were conducted in coastal waters with environmental gradients that greatly differ from those in the lotic freshwater ecosystems sampled in the present study. In the Bothnian Bay, which has a pronounced salinity gradient, salinity was found to be the factor with the strongest impact on large diatoms and the second largest impact on small diatoms, whereas exposure to wave action had the strongest impact on small diatoms (Busse & Snoeijs, 2002). Similar results were reported by Snoeijs et al. (2002) and Busse & Snoeijs (2003). These authors recommended that large and small species should be counted and analysed separately since important ecological information can be missed when the large species are underestimated. Our results do not clearly indicate that small and large taxa respond differently to the environment in lotic freshwater ecosystems. However, the portion of the explained variance is slightly higher for conductivity, pH and turbidity when only the larger taxa are considered, but the overall samples ordinations for small and large taxa are not significantly different. According to our results, we do not feel that counts of small and large species separately are justified for bioassessment purposes in lotic freshwater ecosystems.

Our results also showed that there is no significant relationship between diatom size and TP. This is in contradiction to the results obtained by Cattaneo et al. (1997) who found that the proportion of large diatoms was higher in nutrient-rich streams resulting in a positive relationship between average diatom size and TP. This pattern was also observed in lakes for benthic (Cattaneo, 1987) and planktonic algae (Watson & Kalff, 1981; Watson et al., 1992) and agrees with the general theory that increased resource supply leads to communities with larger individual size (Peters, 1983). However, Wunsam et al. (2002) found that

diatom size was not affected by phosphorus in either natural streams or in experimental channels that were manipulated by phosphorus additions, but was more related to a gradient of trophy that was best represented by colour. The authors cautioned that size increases cannot be unequivocally ascribed to phosphorus, which is consistent with our results.

The difference between our results and those obtained by Cattaneo et al. (1997) may in part be due to the difference in sample number and phosphorus gradient. Cattaneo et al. (1997) collected 45 samples at nine sites, while we collected 410 samples at 126 sites. Moreover, the TP gradient in their streams ranged from 4.8 to 44.8 $\mu g\ l^{-1}$, while it ranged from 10 to 1000 $\mu g\ L^{-1}$ in our study (first decile = 16 $\mu g\ l^{-1}$ and last decile = 146 $\mu g\ l^{-1}$). It seems that a relationship may exist between diatom size and TP along a short trophy gradient, but that relationships may be strongly attenuated in the overall trophy gradient in Quebec streams. Cattaneo et al. (1995) suggested exploiting a possible relationship between diatom size and trophy in order to find an alternative indicator for nutrient input. In light of our results, we do not recommend using diatom size as an indicator of trophy in Quebec streams.

Community biometrics

We realize that it is recommended in the literature to achieve about 10–25 biometric measurements for each species in each sample (e.g. McCormick & Stevenson, 1991; Mulholland et al., 1995; Ghosh & Gaur, 1998; Hillebrand et al., 1999; Snoeijs et al., 2002) in order to obtain a stable standard deviation. The number of samples used in this study (410) and the diversity of the communities (319 taxa) made this recommendation too laborious to follow. Nevertheless, biometric values for many common taxa as well as problematic taxa were calculated from numerous measurements. We compared biovolume and surface area values with the data presented in Snoeijs et al. (2002) and found that the biometrics calculated for the taxa common to both studies (~40) were similar or at least in the same order of magnitude. However, as a general trend, the biometric values recorded in Snoeijs et al. (2002) were higher than the values calculated for this study. The fact that they based their biovolume and surface area calculations on rectangularity might have overestimated the biometrics compared to our calculations based on the closest geometric forms. We do not feel that increasing the number of measurements to 25 for each taxa in each sample would improve the relationships between the metrics and the environment. We ran the same analyses with log-transformed relative biovolume in order to squeeze together the larger values and stretch out the smaller values. We obtained very similar results (not presented). This suggests that changes in biometric measurements that are in the same order of magnitude would not influence our conclusions. In most cases, it is unlikely that the biometric values would change to another order of magnitude after increasing the number of measurements for each sample.

Species ecology

In this study, we did not consider that nutrient uptake may vary according to the surface area/volume ratio or that size may influence grazing and responses to water movements (Snoeijs et al., 2002). Colony formation may also affect the surface area exposed to the environment (Snoeijs et al., 2002). In the same view, it might be relevant to consider the attachment mode, where prostrate taxa (e.g. *Achnanthes*) have less surface contact for exchanges with the environment than stalk-forming taxa (e.g. *Gomphonema*) or taxa attached at only one end (e.g. *Synedra*). Pioneer taxa forming the first layers of the biofilm might also be limited in their exchanges with the environment due to the accumulation of material in the matrix. It is also unclear if diatoms that have large biovolume as a result of multiple girdle bands (e.g. *Tabellaria*) have the same surface for exchanges with the environment as large and thin diatoms (e.g. *Surirella*). Cell size variations (seasonal, interannual, spatial, life cycle) make an average biovolume for a species throughout the year and from different sites inaccurate to use (Hillebrand et al., 1999). Moreover, cytology and morphological plasticity of algal cells is affected by environmental conditions (Sicko-Goad et al., 1977), which imply that a different vacuole correction would have to be applied for each species in every sample. It is therefore impractical to account for vacuole size in routine biovolume estimates since a single

correction factor cannot be applied. All these sources of variability are interesting questions to explore, but are beyond a realistic and user-friendly biomonitoring protocol.

Conclusion

Our results showed that increasing the weight of large species by using a metric that accounts for cell size provides the same information concerning species–environment relationships as using a relative abundance metric. The relative proportions of the taxa varied according to the metric used but did not influence the response to the environment. Analyses conducted on the two size groups separately showed that small and large taxa have similar response to the environment. Our results also showed that there is no significant relationship between diatom size and TP. The fact that the community structure expressed using the 4 metrics provided the same information, and the fact that analyses were similar when the taxa were classified in two size groups allows us to conclude that a metric that accounts for cell size is not necessary for biomonitoring purposes since no additional information is provided. Biometric measurements are time consuming and did not provide additional information on species–environment relationships in this study. We therefore recommend the use of relative abundance when studying the influence of environmental variables on diatom community structures.

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