

Diatom assemblages distribution in Catalan rivers, NE Spain, in relation to chemical and physiographical factors

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Abstract

Distribution patterns of epilithic diatom assemblages in streams in northeastern Spain and their relation to different environmental gradients are presented. Thirty-five sites were sampled covering a wide range of fluvial typologies. Gradient analysis was used to analyse the community structure and the major ecological gradients underlying variation in species composition. Two major gradients were evident; the first was a complex gradient from oligotrophic, pristine, fast-flowing highland rivers to mainly eutrophic rivers of low elevation; and the second related to altitudinal and seasonal variation of temperature. Two sets of factors were evident; one was associated with water chemistry, and the other to seasonal and physiographical variation. Variance partitioning allowed the separation of the effects of the different sets of environmental parameters. The contribution of physiography and water chemistry to diatom distribution was more unclear when the level of disturbance was intermediate. Considering the relatively high proportion of variation explained by physiographical variables alone, we suggest that when using diatoms to evaluate water quality, ecoregional characteristics of river stretches should also be considered.

1. Introduction

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Chemical analyses of water provide a good indication of the chemical quality of the aquatic systems, but do not integrate ecological factors such as altered riparian vegetation or altered flow regime and therefore, do not necessarily reflect the ecological state of the system (Karr et al. 2000). Biological assessment is a useful alternative for assessing the ecological quality of aquatic ecosystems since biological communities integrate the environmental effects of water chemistry, in addition to the physical and geomorphological characteristics of rivers and lakes (Stevenson and Pan 1999).

Diatoms are a siliceous class of algae reputed for being very sensitive to chemical conditions. They usually account for the highest number of species among the primary producers in aquatic systems (Pan et al. 1999). Consequently, they are frequently used as biological indicators of water quality and a number of methods for monitoring European rivers based on diatoms have been already proposed (Kelly et al. 1998, Prygiel et al. 2002). Diatom analysis have therefore been considered an important contribution to the European Water Framework Directive (2000/60/EC), which aims to achieve a “good status” for all waters in the EU before 2015.

Despite their potential, the use of diatoms as biological indicators of water quality of fluvial systems may be hampered because their distributional patterns respond to a multitude of different factors, ranging from biogeographical to biogeochemical and human influenced (Potapova and Charles 2002). The challenge of determining which portion of the distribution of a given community is associated with human influences and which is related to the defining characteristics of a given ecoregion needs to be resolved (e.g. Hall et al. 1999). This knowledge may be essential in the assessment of biological quality, which is based on the degree of deviation from a reference biological population. Biological integrity (Karr 1991) is measured by comparing a given site with reference ecosystems that lie in similar geomorphological and climatic settings but are not exposed to human impact. The search for reference sites is often difficult in particular in regions of extensive human activities, and this may hamper the application of this concept. Knowing in depth the autoecological characteristics of species may be extremely helpful to make an objective search for reference conditions.

Multivariate techniques (ter Braak and Verdonschot 1995) allow the elucidation of ecological factors, which explain most of the variation in diatom distribution. In particular, variance partitioning (Borcard et al. 1992) may help to separate regional and general factors influencing diatom distribution. In this paper we use multivariate techniques to elucidate the physiographical, chemical and physical factors associated with diatom community structure in NE Spain.

2. Methods

2.1. Diatom sampling, preparation and analysis

This study was carried out in a number of rivers located in Catalonia (NE Spain) (Fig. 1). These sites were selected to cover a wide range of fluvial typologies with different levels of human disturbance. Fifty-seven epilithic samples were collected during winter (February) and spring (May) 2001. Diatoms were prepared by acid oxidation with concentrated sulphuric acid and potassium dychromate. Permanent slides were mounted using Naphrax (r.i. 1.74). At least 400 valves were counted on each slide on random transects with a light microscope using phase-contrast and Nomarski differential interference contrast optics at a magnification of 1000x or 1250x. The identification mainly followed Krammer and Lange-Bertalot (1986-1991).

2.2. Site characterization

The morphological and climatic diversity of NE Spain determine an important spatial heterogeneity in the region. The Pyrenees and nearby areas have climates typical of high mountainous regions, with minimum temperatures below 0°C, annual rainfall of above 1,000 mm and heavy snowfall in the winter. Coastal areas have mild, temperate weather, with temperatures that rise and rainfall that decreases as one moves from north to south. Inland, far from the sea, the climate is typical of continental Mediterranean regions, with cold winters and very hot summers. The sites were selected to cover a large gradient ranging from pristine to polluted with different levels of human disturbance, covering the major types of geomorphological and physiographical conditions.

Chemical data (conductivity, suspended matter, dissolved oxygen, pH, BOD₅, NH₄⁺-N, Ca²⁺, PO₄³⁻-P, Mg²⁺, NO₃⁻-N, NO₂⁻-N, K⁺, Na⁺, SO₄²⁻, Cl⁻) of the sites were obtained

from the Catalan Water Agency (ACA) which monitors them routinely. Water samples were analysed following standard procedures (APHA 1989). The following physical descriptors of the sites were used for this study: water temperature, altitude, slope, stream order, catchment area, distance to source, and bifurcation ratio (i.e. the quantitative ratio determined between the number of stream segments in one order and the next). Parts of these data were derived from maps and field observations, while others were provided by the ACA.

2.3. Data analysis

Diatom data were analysed with detrended correspondence analysis (DCA) (Hill and Gauch 1980) to determine the length of the gradient for the first two axes. DCA indicated that the gradient length was greater than 2 standard deviation units (3.191); therefore, the use of unimodal ordination techniques would be appropriate (ter Braak 1987). Consequently, correspondence analysis (CA), an indirect–gradient ordination, was used to determine the major patterns of variation in species composition data without incorporating data on environmental variables. Constrained ordination (canonical correspondence analysis, CCA) was used to relate diatom assemblage structure to all predictor environmental variables and to explore the relationships among and between species and the environment (ter Braak and Verdonschot 1995). According to this preliminary CCA, we identified collinear variables and selected a subset of variables based on inspection of variance inflation factors ($VIF < 20$) (ter Braak and Šmilauer 1998). Step-wise forward selection and Monte Carlo permutation test were used to reduce further the environmental variables to those correlated significantly with the derived axes (ter Braak and Šmilauer 1998), at a cut-off point of $P=0.05$. Probabilities for multiple comparisons were corrected using the Bonferroni correction. Partial CCA was used to separate and examine the relative importance for the species data of several sets of explanatory variables on the diatom assemblages (Borcard et al. 1992). We were interested in separating the effects of the water chemistry from all the other variables and then testing whether these two different groups of variables were redundant to each other, or whether they each explained unique aspects of species composition.

Only those taxa that were observed in more than 5% of the samples were included in analyses of taxa abundances to minimize the influence of rare taxa. Taxa abundance was square root transformed in all analyses to reduce the effect of highly variable population densities on ordination scores. Environmental data (except pH and percentage slope) were logarithmically transformed before analysis to reduce skewed distributions. All ordinations were performed using CANOCO version 4.1 (ter Braak and Šmilauer 1998).

3. Results

3.1. Environmental characteristics of sites

Rivers of the study area varied widely in water quality and habitat characteristics. Conductivity increased downstream and the highest values of suspended solids were found at the lowermost sites. Nutrient concentrations also increased downstream but varied more spatially than did other major variables because the extensive application of chemical fertilizers in agriculture and diverse sewage inputs (Table 1 & 2). Some study sites were located in densely populated, heavily industrialized or intensive agricultural areas, thus receiving high inputs of organic matter and industrial sewage. Other sites were less disturbed, fast-flowing and with a stony or stony-gravel bottom, characteristic of mountainous areas. Finally, others have a lowland character, being slow-flowing.

3.2. Species composition of diatom assemblages

A total of 195 taxa were found. Only 33 taxa were observed in more than 5% of the samples. *Amphora pediculus* (Kützing) Grunow was the most abundant taxon and occurred in up to 78% of all samples. The second most abundant species were *Achnanthes minutissima* Kützing and *Navicula gregaria* Donkin. CA ordination results showed that 15.2% of diatom assemblages variance was explained on axis 1, and a further 13% on axis 2. Sites on the left side of the first CA axis (Fig. 2) lay in densely populated, industrialized or agricultural areas, receiving high inputs of organic matter and industrial discharge. Diatom taxa showing maximum abundance in samples from sites situated on the left side of the diagram included *Nitzschia capitellata* Hustedt, *Nitzschia frustulum* (Kützing) Grunow and *Navicula subminuscula* Manguin. Sites less influenced by human activities had higher values on CA axis 1 and are clustered on the right

hand side of the diagram. Diatoms abundant in these samples included *Achnanthes minutissima* Kützing, *Achnanthes biasolettiana* Grunow and *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot. The second axis is most probably reflecting seasonal differences in the diatom communities, since the winter samples had higher values, while those taken during springtime had lower values on CA axis 2.

3.3. Important environmental variables

The 21 environmental variables altogether explained 52.0% (2.067 s.d. units) of the total variation within the species data. The first two axes explained a significant portion of variance in the diatom taxa data ($P < 0.01$). The large variance inflation factors (> 20) of the exploratory CCA indicated redundancy among distance to source, sulphates, potassium, sodium, magnesium, chlorides, calcium and catchment's area, and were subsequently excluded from the environmental data set.

CCA with forward selection of species data indicated that conductivity, water temperature, BOD₅, altitude, stream order and nitrate nitrogen were the environmental variables that accounted for significant ($P < 0.05$) portions of the total variance in diatom species composition (Table 3). Conductivity explained the largest portion (7.3%) of the total unconstrained variance. CCA ordination plots (Fig. 3a, b) primarily expressed two major gradients. The first one arranged sites from oligotrophic, pristine, fast-flowing highland rivers to predominantly eutrophic rivers of low elevation. The second gradient is likely integrating a seasonal and altitudinal variation of temperature. *Cyclotella meneghiniana* Kützing, *Navicula subminuscula*, *Nitzschia frustulum* and *Nitzschia capitellata* that were mainly observed at sites with high conductivity and BOD₅, usually at lower altitudes. *Achnanthes minutissima* and *Nitzschia fonticola* Grunow were instead associated with more pristine waters and high altitude sites.

3.4. Relative importance of chemical vs. physiographical factors

Partial CCA analyses showed that 25.5% (0.526) of the total variation in the species data could be explained by both sets of variables together. Water chemistry explained a considerably high proportion (12.9%, 0.266) of the total variability in species data. The fraction of the total variance attributed to physiographical variables was 11.6% (0.239). Since the value of the

intersection (0.021, 1.0%) was much smaller than the fraction of the variation explained independently by both sets of variables, we can conclude that the two sets of variables were primarily explaining unique aspects of species composition.

The summary of the ordination gives an overall measurement of how much variation can be related to both explanatory variables. But not all species are equally well explained by the same set of environmental variables. The fit for species can be used as a measurement to find out which species are well represented and the percentage of variance fit by each set of explanatory variables. The percentage of variance explained by water chemistry and the physiographical factors with respect to the unconstrained variance differs among the different species (Table 4). *Navicula atomus* (Kützing) Grunow var. *permitis* (Hustedt) Lange-Bertalot, *Nitzschia capitellata*, *N. frustulum* and *Cyclotella meneghiniana* had their distributions mostly explained by the water chemistry. *Cocconeis pediculus* Ehrenberg and *C. placentula* Ehrenberg, *Gomphonema olivaceum* (Hornemann) Brébisson and *Nitzschia inconspicua* Grunow were amongst the most related to the physiographical group of variables. Finally, *Melosira varians* Agardh, *Navicula subminuscula*, *Cymbella minuta* Hilse ex Rabenhorst and *Nitzschia fonticola* were both explained by the physiographical and chemical variables.

4. Discussion

Approaches for the use of diatom communities as biological indicators diverge from the direct application of diatom indices, which have been developed and widely used in European waters (Descy and Coste 1991, Kelly and Whitton 1995) to multimetrics or multivariate approaches (e.g. IBI or index of biological integrity), which are preferred in the United States (Hill et al. 2000, Karr 1991). Irrespective of the method used, uncertainties still exist concerning the uniqueness or general applicability of a given diagnosis provided by the diatom communities. This is especially the case when pollution or nutrient enrichment is not driving community composition. Leland and Porter (2000) observed that incomplete prediction of phosphorus loading by diatom communities occurred systematically at lower phosphorus concentrations. Sabater (2000) showed that diatom indices successfully indicated the effect of a catastrophic heavy metal spill on a river system, but failed to reliably detect the recovery. Thus in cases

where disturbance is not extreme, ecoregional characteristics (e.g. geochemical features, temperature, water flow) and responses to environmental stressors (e.g.: nutrient enrichment, habitat alteration, toxic inputs) may combine to produce a confusing picture.

The use of multivariate analyses in the present study showed the gradient from pristine to polluted rivers as being the most important in structuring riverine diatom assemblages of river waters in NE Spain. A response of diatom communities to nutrient enrichment appeared to be evident, although the CCA showed that the effect of nutrients and conductivity on species composition was similar. Many studies have shown that diatom communities vary along gradients of ionic concentration (Sabater and Roca 1992). Most often the increase in ionic content is accompanied by nutrient enrichment (Sabater and Sabater 1988, Leland and Porter 2000). The downstream enrichment with dissolved salts, and nutrients in particular, is particularly clear in this study. The overriding effect of conductivity and nutrients on the composition of diatom communities has been recognized recently through the analysis of large data sets (Potapova and Charles 2003). In these circumstances care must be taken not to confuse the effect of ionic content with that of nutrient enrichment.

It is important to stress that diatom distribution was not only affected by chemical characteristics. CCA showed that diatom community structure could also be related to temperature, altitude and stream order. General descriptors summarizing physical habitat (e.g.: stream size and stream order) have been associated with diatom distribution (Molloy 1992). Physiographical variables, especially altitude and stream order, may provide information on how river basins are structured and, analogously, on how disturbances occurring at the basin scale (human activities, changes in land use or in vegetation) may affect diatom communities. At smaller spatial scales it has been also shown that substratum type and habitat heterogeneity are related to species composition and preferential morphological growth forms of diatom communities (Leland 1995). At the basin scale used in this study, altitudinal gradient may be partly related to the ionic enrichment of river waters. Margalef (1960) suggested that algal assemblages (including diatoms) were related to the geochemical and nutrient composition of water, as well as to the hydraulics and geomorphology of the river system. This prediction

advanced the findings based on the use of multivariate analysis, as well as the predictions of the River Continuum Concept (Vannote et al. 1980). Our analyses showed that patterns of diatom distribution could be attributed not only to water quality variation, but also to physiographical processes. The distribution of some common and abundant taxa in our data set is largely influenced by physiographical parameters. Three different groups of taxa can be identified with regard of their link to the chemical, physiographical or mixed (overlapped) influences (Table 4). Although most of these taxa have widespread distribution elsewhere, previous studies suggested that their occurrence is related to specific type of environments. For example, *Gomphonema olivaceum* has a distribution restricted to low-order streams with high alkalinity and low nutrient input (Patrick 1977), indicating strong physiographical influence. *Navicula atomus* var. *permitis* and *Nitzschia capitellata* occupied highly polluted sections of rivers, and were therefore amongst the most influenced by the water chemical characteristics of the rivers. Their occurrence was irrespective of the stream order or location (Lange-Bertalot 1979). *Melosira varians* was found mainly in downstream sections of the studied watercourses, occupying nutrient-enriched, mineralised waters (Margalef 1951). This species is therefore an expression of the overlapping influence between the chemical and the physiographical factors.

5. Conclusions

At present the influence of small-scale factors on diatom community structure is poorly understood. This makes it difficult to establish any standard methodology to assess water quality in a wide geographical area, particularly in those sites with intermediate disturbance. Setting reference standards for biological quality will require the careful development of data sets along major environmental gradients underlying species composition, in order to understand the processes that are structuring diatom assemblages. We have demonstrated that a considerable proportion of variation in the diatom assemblages can be explained by physiographical factors; it would therefore be more reliable to develop diatom-based approaches for water quality estimation that are applied at the ecoregional level or in homogeneous geographical areas, thus avoiding unwanted effects on data sets.

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References

American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. Washington DC: American Public Health Association, 1989. p 1220.

Borcard D, Legendre P and Drapeau P. Partialling out the spatial component of ecological variation. *Ecology* 1992; 73: 1045-1055.

Descy JP, and Coste M. A set of methods for assessing water quality based on diatoms. *Verh Int Verein Limnol* 1991; 24: 2112-2116.

European Commission. Directive 2000/60/EC of The European Parliament and of the Council - Establishing a framework for Community action in the field of water policy. Brussels, Belgium, 23 October 2000, 2000.

Hall RI, Leavitt PR, Quinlan R, Dixit AS and Smol JP. Effects of agriculture, urbanization, and climate on water quality in the northern Great Plains. *Limnol and Oceanogr* 1999; 44: 739-756.

Hill BH, Herlihy AT, Kaufmann PR, Stevenson RJ, McCormick FH and Johnson CB. Use of periphyton assemblage data as an index of biotic integrity. *J N Am Benthol Soc* 2000; 19: 50-67.

Hill MO and Gauch HEJ. Detrended correspondence analysis: an improved ordination technique. *Vegetatio* 1980; 42: 47-58.

Karr JR. Biological integrity: a long-neglected aspect of water resource management. *Ecological application* 1991; 1: 66-84.

Karr JR, Allen JD and Benke AC. River conservation in the United States and Canada. In: Boon PJ, Davies BR, Petts GE, editors. *Global Perspectives on River Conservation: Science, Policy, and Practice*. New York: J. Wiley, 2000. p 3-39.

- Kelly MG, Cazaubon A, Coring E, Dell' Uomo A, Ector L, Goldsmith B, Guasch H, Hurlimann J, Jarlman A, Kawecka B, Kwandrans J, Laugaste R, Lindstrøm EA, Leitao M, Marvan P, Padisak J, Pipp E, Prygiel J, Rott E, Sabater S, van Dam H and Vizinet J. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *J Appl Phycol* 1998; 10: 215-224.
- Kelly MG and Whitton BA. The Trophic Diatom Index: A new index for monitoring eutrophication in rivers. *J Appl Phycol* 1995; 7: 433-444.
- Krammer K and Lange-Bertalot H. Bacillariophyceae. In Ettl H, Gerloff J, Heynig H and Mollenhauer D, editors. Süßwasserflora von Mitteleuropa. Stuttgart, Fischer-Verlag, 1986-1991: 2. 1-4.
- Lange-Bertalot H. Pollution tolerance of diatoms as a criterion for water quality estimation. *Nova Hedwigia* 1979; 64: 285-304.
- Leland HV. Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. *Can J Fish Aquat Sci* 1995; 52: 1108-1129.
- Leland HV and Porter SD. Distribution of benthic algae in the upper Illinois River basin in relation with to geology and land use. *Freshwater Biol* 2000; 44: 279-301.
- Margalef R. Regiones limnológicas de Cataluña y ensayo de sistematización de las asociaciones de algas. *Coll Bot* 1951; 3: 43-67.
- Margalef R. Ideas for a synthetic approach to the ecology of running waters. *International Revue der Gesamten Hydrobiologie* 1960; 45: 133-153.
- Molloy JM. Diatom communities along stream longitudinal gradients. *Freshwater Biol* 1992; 28: 59-69.
- Pan YD, Stevenson RJ, Hill BH, Kaufmann PR and Herlihy AT. Spatial patterns and ecological determinants of benthic algal assemblages in Mid-Atlantic streams, USA. *J Phycol* 1999; 35: 460-468.
- Patrick R. Ecology of freshwater diatoms- diatom communities. In: Werner D, editor. *The biology of diatoms*. Oxford: Blackwell, 1977. p 284-322.

Popatova MG and Charles DF. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. *J Biogeogr* 2002; 29: 167-187.

Potapova MG and Charles DF. Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. *Freshwater Biol* 2003; 48: 1311-1328.

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

Sabater S. Diatom communities as indicators of environmental stress in the Guadiamar River, S-W Spain, following a major mine tailing spill. *J Appl Phycol* 2000; 12: 113-124.

Sabater S. and Sabater F. Diatom assemblages in the River Ter. *Arch Hydrobiol* 1988; 111: 397-408.

Sabater S. and Roca JR. Ecological and biogeographical aspects of diatom distribution in Pyrenean springs. *Br Phycol J* 1992; 27: 203-213.

Stevenson RJ and Pan Y. Assessing environmental conditions in rivers and streams using diatoms. In: Stoermer EF and Smol JP, editors. *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge: Cambridge University Press, 1999. p. 11-40.

ter Braak CJF. Ordination. In: Jongman RHG, ter Braak CJF and van Tongeren OFR, editors *Data analysis in community and landscape ecology*. Wageningen: Pudoc, 1987. p. 91-173.

ter Braak CJF and Šmilauer P. *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4)*. New York: Microcomputer Power, Ithaca, 1998. p. 352.

ter Braak CJF and Verdonschot PFM. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquat Sci* 1995; 57: 255-289.

Vannote R., Minshall GW, Cummins KW, Sedell JR and Cushing CE. The river continuum concept. *Can J Fish Aquat Sci* 1980; 37: 130-137.

