WATER QUALITY EVALUATION IN CATALONIAN MEDITERRANEAN RIVERS USING EPILITHIC DIATOMS AS BIOINDICATORS

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ABSTRACT – The main goal is to evaluate water quality in the alkaline Mediterranean rivers from the central and the south basins of Catalonia, using benthic diatoms as indicators. We sampled 72 sites spread throughout the seven studied basins: Ebre/Segre, Llobregat, Besòs, Francolí, Gaià, Foix and Riudecanyes. Most of them are from the Water Quality Surveillance Network of the Catalan Government Water Agency (ACA), with 17 new stations distributed in unpolluted watercourses from different ecoregions. The European protocol has been followed to take the epilithic samples, to treat them and to quantify species abundance. The study has also followed a full complete floristic list of the benthic diatom flora. 23 new taxa for the Iberian flora are listed. Application and optimal run of general water pollution indices IPS, CEE, IBD, EPI-D and water trophic level indices TDI and van Dam have been tested in Mediterranean basins. The comparisons between indices and chemical variables have shown IPS to be the best diatom index to be applied in Mediterranean rivers. Finally the water quality information has been synthesised in a biological water quality map.

INTRODUCTION

For the first time a broad programme to test biological methods for monitoring is being carried out in Catalan rivers by the Catalan Water Agency (ACA). Algae, macroinvertebrates, fishes and bank woodland indices have been checked in Catalanian watercourses. Diatoms have been chosen as the phytobenthos indicators organisms. The use of diatoms as indicators of biological water quality of rivers is widespread in Europe and their good performance on that issue has been demonstrated. Several indices have been created and tested with good results, mainly in central and northern European rivers (Lange-Bertalot 1979, Prygel & Coste 1996, Eloranta 1995, 1999, Coring 1999, Kwandrans et al. 1999, Eloranta & Andersson 1998, Van de Vijver & Beyens 1998, Kelly et al. 1995). Very little work has been done for Mediterranean rivers on diatoms as water quality indicators. In Italy several rivers have been biomonitored...
by diatom indices (Grandoni & Dell’Uomo 1996, Dell’Uomo & Tantucci 1997, Dell’Uomo & Grandoni 1997, Ciutti et al 2000, Capelletti et al. 2003). They always used the EPI-D index, generated by Dell’Uomo (1996) as diatom index to evaluate Italian river water quality. Only once the European indices other than EPI-D were applied in those rivers and their results compared between them, but not checked against physico-chemical variables to check which method was better (Dell’Uomo et al. 1999). Also the indices IPS and CEE have been applied in two rivers (Ziller & Montesanto 2004) and a mountain creek (Montesanto et al. 1999) in Greece, performing good results but again not checked against pollution variables.

Mediterranean rivers differ from central and northern European rivers in their hydrological pattern, which fluctuates a lot seasonally. This fluctuation has a strong effect on water pollution degree, as pollutant concentration will quickly vary with flow variation (Merino et al. 1994). On Catalan rivers few works are done on diatoms. They describe diatom communities and their relation to water quality, but no attempt of water quality evaluation has been done (Tomàs & Sabater 1985, Sabater et al. 1987, 1991, Sabater & Sabater 1988). The aim of this study is to test the usefulness of diatom indices developed and currently used in central Europe as biological variables for the evaluation of the quality of running waters in different types of rivers, in Catalonia, mainly of the Mediterranean ecotype.

MATERIAL AND METHODS

We have studied the epilithic diatom communities from 72 sites spread in rivers in Catalonia, central and south catchments (Fig. 4, Table I). 55 of them were chosen from the Water Quality Surveillance Network of the Catalan Water Agency (ACA), which are monitored monthly at chemical level. We added 17 new points placed in clean rivers or streams where very good biological quality was expected a priori. All sites were visited during summer 2002. The seven catchments sampled were Ebre/Segre, Llobregat, Besòs, Francolí, Gaia, Foix and Riudecanyes. The respective surface is 14.987 km², 5.090 km², 1.024 km², 854 km², 421 km², 310 km², 72 km² and the sampled points in each one were 16, 28, 12, 6, 4, 4 and 2 respectively. Nearly all rivers flow through the mountain belt and lowland, all of them draining calcareous substrate, except for the two larger catchments Llobregat and Ebre/Segre, which had few points in high mountain rivers which flow mainly through siliceous substrate (J032, L020, L021 and J010 at the Segre basin and J117, J078 and C715 from the Llobregat river).

Epilithic diatom samples were taken and treated following the European norm protocol (European Committee for Standardization 2002, 2003). At least five rocks from the main flowing water and well-lighted river part were broached to collect diatoms. Samples were preserved with formaldehyde at 4%. Afterwards samples were treated to obtain clean frustule suspension. Organic matter was eliminated by oxidation with hydrogen peroxide. Diluted HCl was added to remove the calcium carbonate, really abundant in these waters, in order to avoid late precipitation, which could make frustule observation and counting difficult. Finally, after distilled water cleanings, permanent slides were prepared with Naphrax®. A complete taxa list was made from each slide and at least 400 valves were counted (Prygiel & Coste 1993) to calculate species relative abundance. These data were processed with the software OMNIDIA version 3 (Lecointe et al. 1993, 1999, http://perso.club-internet.fr/clci/) which provided the resulting values for several diatom water quality indices per each list (Table I). From all of them we have chosen six indices: IPS (Specific Polluosensitivity Index, Coste in Cemagref 1982), IBD (Biological Diatom Index, Lenoir & Coste 1996, Prygiel & Coste 2000), EPI-D (Eutrophication/Po
tion Index, Dell’Uomo 1996), CEE (Descy & Coste 1990), TDI (Trophic Diatom Index, Kelly & Whitton 1995). A first group of them are considered general pollution evaluators: IPS, IBD, EPI-D and CEE (Prygiel et al. 1999). The two first have been chosen because they are widely used in France by Water Agencies. IBD is actually normalized by the French government (AFNOR 2000). IPS is usually considered as the reference index since long time (Descy & Coste 1991). EPI-D has been chosen as it is the only diatom index especially designed for Mediterranean type rivers (Dell’Uomo 1996). Most of these indices are based on the Zelinka & Marvan formula (Zelinka & Marvan 1961), which is a weighted average of species indicator values. A different number of species is taken into account by each index: IBD uses 209 taxa, EPI-D 233 taxa, and the IPS uses all known taxa. CEE index, based on a two-fold quality grid which uses 208 taxa, was already tested in Catalanian rivers of Llobregat basin by Muñoz & Prat (1994). It was also used in Andorra rivers which belong to the Ebre basin (Merino et al. 1993). The four indices range from 1 to 20, being 1 the worst quality and 20 the best.

A second group of the chosen indices are proposed to assess exclusively the trophic level (nutrients) of river waters: the TDI designed in England, uses 98 taxa, most of them at the genera level. It ranges form 0 to 100, rescaled by Kelly (1998), being 0 low nutrient concentrations and 100 very high concentrations. We have also used the van Dam diatom ecological indicator values (van Dam et al. 1994) to evaluate the trophic level by calculating an index made by Kelly (1995) using these van Dam’s trophic values and the Zelinka & Marvan equation. Its range has been modified to range from 1 (oligotrophic) to 5 (hypertrophic); a set of 948 taxa is taken into account.

Physico-chemical results have been provided by the Catalanian Water Agency, who is sampling the studied sites monthly. We have taken the values which were temporally close to our sampling date. Two variables have been chosen from all data from ACA: Total Organic Carbon (TOC) and soluble phosphorous (P) calculated as Phosphates (P2O5) (expressed as mg of phosphorous per litre). The detection limit for the analysis method was of 0.05 mg/l. They are representatives of two different pollution factors and had the more com-
complete data set from all chemical parameters. Pearson’s correlations were calculated between indices values and log transformed chemical values.

RESULTS

A total of 239 diatom taxa were identified during the study, belonging to 61 different genera. Within them, 58 taxa are new for Catalan country flora (Cambra et al. 1991), and 23 are recorded for the first time for the Iberian Peninsula (Aboal et al. 2003) (Table III). From these, Navicula antonii Lange-Bertalot, Cyclotella cyclopuncta Håkansson et Carter, Achnanthidium latecephalum Kobayasi and Navicula germanii

Table I. List of sampled sites in summer 2002, indices and chemical variables values.

<table>
<thead>
<tr>
<th>Site Code</th>
<th>Village</th>
<th>River</th>
<th>IPS</th>
<th>CEE</th>
<th>IBD</th>
<th>TDI</th>
<th>EP-D</th>
<th>van Dam</th>
<th>P values</th>
<th>TOC values</th>
</tr>
</thead>
<tbody>
<tr>
<td>A100</td>
<td>Garajonay</td>
<td>E</td>
<td>1.3</td>
<td>4.5</td>
<td>5.9</td>
<td>2.3</td>
<td>3.0</td>
<td>0.7</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>A101</td>
<td>El Seibo</td>
<td>E</td>
<td>1.3</td>
<td>4.5</td>
<td>5.9</td>
<td>2.3</td>
<td>3.0</td>
<td>0.7</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>A102</td>
<td>Pico del Teide</td>
<td>E</td>
<td>1.3</td>
<td>4.5</td>
<td>5.9</td>
<td>2.3</td>
<td>3.0</td>
<td>0.7</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>A103</td>
<td>Pico Viejo</td>
<td>E</td>
<td>1.3</td>
<td>4.5</td>
<td>5.9</td>
<td>2.3</td>
<td>3.0</td>
<td>0.7</td>
<td>1.9</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table II. Pearson’s correlation between the diatom indices. Bold numbers p < 0.001, n=73.
Wallace had relative abundances over 5% at least at one site. *Gomphonema lateripunctatum* Reichardt et Lange-Bertalot and *Navicula subalpina* Reichardt were quite widespread, but never reached abundances over 5%. A clear positive relation exists between number of species and catchment’s surface, as at the largest catchments, a greater number of taxa have been identified, while at smaller ones the species richness is less. That is because in large catchments there is a higher diversity of river ecotypes. In the Llobregat basin (second largest studied area), 169 taxa were identified from the 28 sampling points. On the other hand, in the small catchments Gaia, Foix and Riudecanyes (where 2 to 4 points were sampled), around 40 diatom taxa were found at each site. In terms of the relative species abundance, only 69 taxa reached the abundance of over 5% at least at one sampling point. These are the ones who will mainly determine the values for water quality indices.

When applying the water quality indices, the results range from the maximum to the minimum possible values (Table I and Fig. 3). Studied rivers had localities with the lowest water biological quality (minimum value found: IPS=1 at Anoia river, site J004) and the highest values (maximum value found: IBD=20 at Noguera Ribagorçana river, site J010).

Significant correlations are observed between all the indices and the van Dam trophy values except for the TDI (Table II). CEE and IPS have a strong correlation, and they also have high correlations with IBD and van Dam, whilst the correlation of each of these indices with EPI-D is less.

The indices profiles (Fig. 3) show different trends in their behaviour. IPS and CEE reach extreme values, both good and bad water quality, while IBD and EPI-D do not tend to give such extreme values. IBD do not value water quality as badly as the others do, never assessing below 3. Conversely, EPI-D tends to underestimate good quality sites, as it never goes over 13. It seems like it has a top limit around this value in Catalonian rivers. Finally, TDI results do not fit with none of the other indices results.

To test the capability of detection of the pollution degree of diatom water quality indices in these Mediterranean rivers, we compared them with two ecological variables representative of two pollution factors: phosphates (P) as eutrophication and Total Organic Carbon (TOC) as organic matter. The general pollution indices were compared with both variables, and in all cases we obtained negative correlations, and all of them were statistically sig-
significant (Fig. 1). However, better correlation values were shown by IPS and CEE for both chemical variables. Diatom indices have better correlations with TOC rather than with phosphates. The trophic indices were only checked against the nutrient variable. The van Dam values were negatively and significantly correlated with phosphates, while TDI did not correlate at all (Fig. 2).

**DISCUSSION**

Apart the study on water quality assessment by diatoms for Catalan Mediterranean rivers, a detailed benthic diatom flora check-list of these rivers was obtained. It adds information to previous works (Margalef 1951, Tomáš & Sabater 1985, Cambra *et al.* 1991, Aboal *et al.* 2003) and it helps to know better diatom communities composition and distribution in Iberian Peninsula. This is necessary for Catalan rivers, as it provides valuable information essential for the correct index application, especially for IPS, which takes into account all existing taxa in Catalan rivers. Especially interesting are the 23 new records found for the Iberian Peninsula flora. This great amount of new cited taxa can be explained by the lack of important taxonomical works on Iberian diatom flora in the last two decades. Several common taxa in European flora have not been previously cited in Iberian rivers due to a lack of studies or because some of them are taxa recently described or separated from

Table III. List of taxa identified in the studied sites. Bold: taxa which had abundance over 5% at least in one site. One asterisk: new taxa for Catalonian diatom flora (Cambra *et al.* 1991). Two asterisks: new taxa for the Iberian Peninsula (Aboal *et al.* 2003).
older species complexes, such as *Navicula antonii* Lange-Bertalot and *N. germanii* Wallace. Some species could have been confounded with others, due to their high similarity in the optical microscope. This is the case for the *Achnanthidium* species morphologically close to *A. minutissimum* (Kützing) Czarnecki: *A. catenatum* (Bílý et Marvan) Lange-Bertalot and *A. latecephalum* Kobayasi. *A. catenatum* is quite common in France (Coste & Ector 2000), but has never been inventoried for the Iberian Peninsula rivers. It has a preference for planktonic habitat rather than for benthic and was described in the Zelinka river in Bohemia (Bílý & Marvan 1959), cited recently in several lakes and rivers of Europe, for instance in France (Druart & Straub 1993, Coste & Ector 2000) and in Switzerland (Straub 2002). Thus maybe the distribution of this species is broader in Catalonian rivers but it has not been sampled at proper sites or it has been previously misidentified. Another taxa recorded for the first time for the Iberian Peninsula is *Achnanthidium latecephalum*. It was first described in low polluted Japanese rivers (Kobayasi & Ishida 1996) and has also been found in Mediterranean type rivers of France and Corsica (Rimet et al. 2002). Other interesting new taxa are *Gomphonema lateripunctatum* Reichardt & Lange-Bertalot and *Navicula subalpina* Reichardt, both characteristic for clean alkaline waters. *Gomphonema lateripunctatum* is a highly alkalophilous species of mountain and plain watercourses, common in several calcareous Mediterranean rivers. *Navicula subalpina* was first found in subalpine calcareous waters of Austria. We found it in mountain belt watercourses of the Ebre and Llobregat basins. It could have been easily confused before with *N. capitatoradiata* Germain (Reichardt 1988).

About biological water quality of the studied rivers, a general trend is the decrease of indices values as waters flow down (Fig. 4). That is due to increasing human pressure on the surrounding lands. This decrease is usually gradual along the river, except for the Besòs basin rivers and streams, where there is a sudden drop in water biological quality when rivers left the mountains and entered into the valley.

The indices for water quality assessment of general pollution (IPS, CEE, IBD and EPI-D) have shown to be good tools for detection of organic matter pollution and trophic level. Correlations with ecological variables representative of these pollution types are all negative and statistically significant. Good correlations of pollution variables with IPS, IBD and CEE have already been observed in several European rivers: Kelly et al. (1995) in England, Eloranta (1999) in Finland, Kwandrans et al. (1999) in Poland, Prygiel & Coste (1993) in France. Also EPI-D response has shown up to be sensible to river nutrient variations in west
The negative correlation obtained means that for low pollution index values are high, and conversely. This pattern fits pretty well with the TOC. However, it does not work for phosphates as shown at several localities, which had low phosphate concentration, but diatom indices indicate a bad water quality. Better relationships of these indices with organic matter parameters rather than with trophic state ones were already expressed by Prygiel & Coste (1993). Some examples of this misalliance are the sites where low phosphate concentrations were found but diatom communities were dominated by *Nitzschia palea* (Kützing) Smith, *N. frustulum* (Kützing) Grunow, *Fistulifera saprophila* (Lange-Bertalot et Bonik) Lange-Bertalot, *Eolimna subminuscule* (Manguin) Moser, Lange-Bertalot et Metzeltin and *Navicula veneta* Kützing. All of them are highly tolerant to pollution, which confers low values to indices. This misalliance is explained by three facts. First due to the
“global” nature of indices, which try to evaluate the general state of water quality and not only the trophic degree (Prygiel et al. 1999). So not a perfect adjust is expected with a single pollution variable as they are made to evaluate the global water quality, which is a combination of several variables: eutrophication, organic pollution, ionic composition, pH, salinity, etc. Also temporal integration of the variation of chemical variables to which organisms have been exposed for some time must be a factor of misalliance of these indices (Round 1991). This temporal variation is reflected by biological indices, thus a weak correlation to punctual chemical values is expected (Harris 1984). And finally the fact that these indices are based on weighted averaging of species indicator values, with the assumption that species have symmetrical unimodal distributions along a nutrient gradient, when not all the species present this distribution (Potapova et al. 2004). Anyway, the key point is that when there is eutrophication or organic matter pollution, these indices always detect so and show up a low value.

A better correlation with phosphates is expected from the indices, which focus on trophic level evaluation, but in the studied rivers they have got similar results to the other indices. That also happens in Poland and Finland rivers (Kwandrans et al. 1999, Eloranta 1999). From the two indices chosen, van Dam indicator values adaptation had significant correlation with phosphates whilst TDI did not. Actually this index was created as a specific tool for P evaluation in wastewater outflow (Kelly & Whitton 1995), but it has been tested at several European rivers with good results working well as phosphor level evaluator (Kelly et al. 1995, Coring 1999, Eloranta 1999, Kwandrans et al. 1999, Rott et al. 2003). Those good results impelled us to test it with our Mediterranean dataset, where it has not worked properly. Three reasons are supposed to be the cause: 1– It is a method mainly based on generic taxa, taken into account a reduced portion of infrageneric taxa. 2– TDI should always be valued along with the percentage of organic pollution tolerant valves. A percentage over 20 is supposed to mask the good TDI performance (Kelly 1998), and on our data set, 63% of species lists went over this percentage. And 3– A high percentage of sites got over the P concentration upper limit of effectiveness of the index, settled about the 0.3 mg/l (Kelly et al. 2001). 24 of the 54 P analyses exceed these limits.

We can conclude that from the four diatom global quality indices, EPI-D and IBD are the worse for Mediterranean rivers as weaker correlations are found for these indices with pollution variables, and their results differ most from the other two tested indices. IBD tend to overestimate water quality at middle and high polluted sites, mainly because it overvalues some small Naviculaceae such as Fistiulifera saprophila, Eolimna minima (Grunow) Lange-Bertalot, Sellaphora minisculum (Grunow) Mann, quite abundant in Catalonian polluted rivers. EPI-D underestimates water quality at clean sites (Fig. 3). The communities of these localities are dominated by Achnanthidium minutissimum, A. biasolettianum (Grunow) Round et Bukhtiyarova, which EPI-D does not consider as unpolluted waters indicator species, but weakly polluted water indicators. Furthermore, several clean alkali water species dominant at these rivers, such as Encyonopsis microcephala (Grunow) Krammer, Gomphonema lateripunctatum and Brachysira neoexilis Lange-Bertalot, are not even used by this Italian index. Even though this index has a late updated version to be checked in the future (Dell’Uomo 2004).

The fact that IPS and CEE give almost the same results in spite of their different evaluation methodology (IPS is based on the Zelinka & Marvan equation and CEE on a double entry table), confers them more credibility. The results show that IPS is the best index to apply to Catalanian Mediterranean rivers as it gives the best correlations with organic matter pollution and trophic level and because it takes into account all benthic diatom taxa identified from those rivers, while all the others do not.

Nevertheless the identification of most seriously disturbed sites is successful by any of the indices, and most sites of this study are highly polluted, as show their P and TOC concentrations, in general higher than in other European regions. A broader study focusing on less disturbed rivers is required to a better differentiation of situations in the Mediterranean ecoregion rivers.

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