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Aquatic macrophytes as biological indicators of environmental conditions of rivers in north-eastern Spain

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The aim of this work was to evaluate the potential of aquatic macrophytes as biological indicators of the environmental conditions of rivers in north-eastern Spain. To this end, twenty five river basins were studied to assess the species composition and abundance of macrophytes, and to examine their relation with different geographic, morphometric, mineralization, and trophic status parameters. Of the twenty six macrophyte species found, five appeared to be useful as biological indicators of the environmental conditions of the rivers. *L. minor* was a good indicator of high mineralization (i.e., conductivity and Cl^-) and high trophic state (especially, N-NO_2^-). *C. stagnalis* was a good indicator of high P-PO_4^{3-} concentrations. *P. crispus, P. polygonifolius,* and *R. penicillatus* seemed to be useful indicators of high N-NO_3^- concentration. We conclude that, in our study area, aquatic macrophytes can indeed be used as biological indicators of the environmental conditions of rivers.

Keywords : aquatic plant, geomorphology, high trophic state, mineralization, river, water quality.

Introduction

It is a well-known fact that the structure of aquatic vegetation in rivers can change as a result of both nutrient enrichment (Tusseau-Vuillemin 2001, Sánchez-Carrillo & Álvarez-Cobelas 2001) and the presence of pollutants (Bernez et al. 2001). Moreover, aquatic macrophytes have been reported to improve water quality (Wilcock & Nagels 2001, Thiébaut & Muller 2003) and to affect algal growth (Nakai et al. 1999) as well. Likewise, the diversity of macrophyte species can have an effect on the functioning of wetland ecosystems (Engelhardt & Ritchie 2001). Most importantly, the maintenance of this diversity is thought to enhance the numerous services that wetland ecosystems can provide to human society (Engelhardt & Ritchie 2001, Thiébaut & Muller 2003).

Aquatic macrophytes can obtain nutrients from the sediment as well as directly from the water itself (Denny 1972, Chambers et al. 1989, Levin et al. 2001, Schulz et al. 2003, Thiébaut & Muller 2003). Consequently, the availability of sediment nutrients may limit the growth and distribution of macrophytes (Spencer & Ksander 2003), thus complicating interpretation of the data concerning them (Kelly & Whitton 1998). Indeed, several studies have failed to link the distribution of macrophyte species to sediment conditions in British lowland rivers (Clarke & Wharton 2001, Clarke 2002, Mainstone & Parr 2002) and to separate, for instance, the effect of nutrient enrichment on macrophyte distribution from the effect of other environmental factors (such as conductivity), and the effect of pH from phosphate and ammonium enrichment (Dawson & Szoszkiewicz 1999, Thiébaut & Muller 1999). On the other hand, recent research has shown that local environmental conditions may be a less important factor than species colonisation processes in the distribution of macrophyte species (Demars & Harper 2005).

Despite their limitations, macrophytes are undoub-

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tedly useful indicators of the environmental conditions of rivers, such as high trophic state (Kelly 1998, Thiébaut & Muller 1999, Amoros et al. 2000, Kohler & Schneider 2003, Schneider & Melzer 2004). In fact, many authors have reported on macrophytes' role as valuable biological indicators of river water quality (Haslan 1987, Peñuelas & Sabater 1987, Romero & Onaindia 1995, Lazaridou et al. 1997, Szymanowska et al. 1999, Lehmann & Lachavanne 1999, Haslam 2000, Klumpp et al. 2002, Demirezen & Askoy 2004). In this respect, an increase in certain nutrients and the presence of pollutants are known to have an effect on the distribution of aquatic macrophytes (Bernez et al. 2001, Samecka-Cymerman & Kempers 2002). In particular, macrophytes can be used as metal accumulators (Comin et al. 1997).

Certain macrophyte species and groups of species have been described as biological indicators of morphometrical characteristics and the physical features of streams (Martínez-Taberner & Moyá 1993, Grasmück et al. 1995, Dawson et al. 2002, Ludovisi et al. 2004, Reid & Quinn 2004). Aquatic macrophytes seem to respond in a predictable way to the trophic and physical characteristics of rivers (Haury & Peltre 1993, Haury 1996, Spink & Murphy 1997, Ali et al. 1999). In fact, the correlation of aquatic plants against environmental parameters indicates the environmental preferences and ranges of each plant or plant community (Dawson & Szoszkiewick 1999). Most importantly, Harper et al. (2000) demonstrated that the development of methods to assess the ecological integrity of running waters requires the integration of both physical and chemical parameters, as well as their effects upon biological structure.

Descriptions of how river macrophytes respond to environmental factors have varied in accordance with the scale of the environmental variation observed (Jackson & Charles 1987, Onaindia et al. 1996). Also and most importantly, it is known that the range of water trophic status and mineral content over which macrophytes are found varies significantly from one region to another, with the result that aquatic macrophytes' potential as biological indicators of the environmental conditions of rivers may differ from one place to another. In consequence, the aim of the work described in this article was to determine the links between the physical and chemical variables of rivers and the distribution of aquatic macrophytes, in an attempt to evaluate the potential of these plants as biological indicators of the environmental conditions of rivers in north-eastern Spain.

Study area, materials and methods

The study was carried out in the Basque Country in north-eastern Spain. This region is topographically divided into two waterheds by the Vasco-Cantábrica mountain range (Salvada, Gorbea, Amboto, Aitzgorri and Aralar). On the northern side of this mountain range, rivers drain into the Bay of Biscay, while on the southern side they flow via the Ebro river into the Mediterranean Sea. The substrate of the studied area is limestone. Intensive agricultural crops are widespread on the southern side of the watershed (Docampo et al. 1989). Mean annual rainfall is 1,200 mm and the temperature of river waters ranges from 6-8°C in winter to 17-20°C in summer (Sánz de Galdeano & Madariaga 1992).

Thirteen main river basins and five small coastal rivers draining into the Bay of Biscay, together with seven river basins draining into the Ebro river, were studied as a representative sample of the environmental diversity within the Basque Country. Two hundred and forty six sampling stations were set up in the network of rivers.

Sampling of the vegetation was carried out over 100 m stretches of the rivers in summer, between June and August. Percentage of cover was recorded as a measure of the abundance of aquatic macrophytes, using a scale of 1 to 5: 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75% and 5=76-100% (Onaindia et al. 1996). The nomenclature in this article is in accordance with Flora Europea (Tutin et al. 1964-1980) and Flora del País Vasco (Aizpuru et al. 2000).

Geographic and morphometric parameters were also studied, together with water quality variables, as indicators of mineralization and trophic status. At each sampling station, geographic and morphometric factors, namely altitude, stream order, gradient, width of stream, mean depth, and water velocity, were measured in the field. The last three factors were used to calculate flow-rate (discharge). Water samples were taken throughout the year : winter, spring, summer and autumn. Conductivity, pH, oxygen (O_2) concentration and water temperature were also measured in the field.

Water mineralization was determined by measuring its conductivity, hardness, alkalinity, and calcium (Ca^{2+}), magnesium (Mg^{2+}), carbonate (CO_3^{2-}), sulphate (SO_4^{2-}), potassium (K^+), sodium (Na^+) and chloride (Cl^-) concentration. Trophic status was determined according to the following parameters: pH, dissolved O_2, phosphate (PO_4^{3-}), nitrite (NO_2^-), nitrate (NO_3^-), and ammonium (NH_4^+) concentration. Laboratory analysis for Ca^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_2^-, NO_3^-, and NH_4^+ was carried out following standard methods (APHA/AWNA/VPCP 1985, Golterman 1969). Hardness was calculated according to Seddon (1972). Maps (scale 1:50.000) were used to determine altitude, slope, river order, and distance from source.

Relationships between physicochemical variables and plant cover were analysed by Spearman's rank correlation coefficient. Experiment-wise error rate was adjusted (separately for each variable studied) by the use of the Bonferroni correction. Mean values of parameters were compared with ANOVA results, since extremely stenoecious species will likely not show any correlation with an environmental parameter as they grow exclusively in certain environmental conditions. Statistical analysis was performed using Stat View (Abacus Concepts 1986) and SPSS (SPSS Inc. 1999).

Results

Chemical characteristics of rivers

Mean values of some physical and chemical parameters measured in the studied rivers are given in Table 1. In this table, it can be seen that the sites where the different aquatic macrophytes were present indeed showed a wide range of physical and chemical conditions, from moderately acidic (pH= 5.1) to moderately basic (pH= 8.8), and from oligotrophic ($P^-PO_4^{3-}$ and $N\text{-}NH_4^+$ concentrations below detection limits) to meso-, eutrophic ($P\text{-}PO_4^{3-}$= 93.04 µg l^{-1} and $N\text{-}NH_4^+$= 25.00 mg l^{-1}). In these same sites, conductivity values varied between 108 and 9,560 µS cm^{-1}. These high conductivity values were most likely due, in some cases, to the influence of seashore and, in others, to pollution.

In general, rivers in the study area showed high mineral contents (data not shown), especially for Ca^{2+} (in mg l^{-1} : maximum value= 372.74, minimum value= 14.20, mean value=74.93), most likely due to the limestone substratum on which they flow.

Nutrient levels in the studied waters were relatively high. Mean values of $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, and $P\text{-}PO_4^{3-}$ at the study sites were 1.03±0.31 mg l^{-1}, 28.27±6.05 µg l^{-1}, and 5.19±1.09 µg l^{-1}, respectively.

Aquatic plant species

Twenty six different species of aquatic macrophytes were recorded at 124 of the sampling stations. Out of these twenty six species, only those present in more than 2% of the macrophyte-recorded stations (*i.e.,* stations where macrophytes were found) were considered in this study, *viz. Apium nodiflorum, Callitriche stagnalis, Groenlandia densa, Lemna minor, Myriophyllum spicatum, Nasturtium officinale, Potamogeton crispus, Potamogeton polygonifolius, Ranunculus penicillatus, Veronica beccabunga,* and *Zannichellia palustris* (i.e., a total of 11 species).

The most abundant species, present in a broad range of environmental conditions, was *A. nodiflorum* (present in 33% of the macrophyte-recorded stations). This species was not included in the ANOVA analysis as it

Table 1. Mean pooled values (with min - max in brackets) of the relevant physicochemical parameters for all macrophyte-recorded stations and for the stations where the most frequent macrophytes were found.

	Conductivity (µS cm^{-1})	Temperature (°C)	Cl^- mg l^{-1}	$N\text{-}NO_2^-$ µg l^{-1}	$N\text{-}NO_3^-$ µg l^{-1}	$P\text{-}PO_4^{3-}$ µg l^{-1}	$N\text{-}NH_4^+$ µg l^{-1}	Dissolved O_2 mg l^{-1}	pH
Macrophyte-	652	16.14	60.40	28.27	3.77	5.19	1.03	8.11	7.54
recorded stations	(108-9560)	(8.50-22)	(6.74-1910)	(0.03-631)	(0-28.61)	(0- 93.04)	(0-25)	(2.15-13.60)	(5.10-8.80)
A.nudiforum	871	16.55	101.93	48.57	2.23	10.61	1.84	7.17	7.42
	(217-9560)	(9.50-21)	(9.70-1910)	(0.35-631)	(0-22)	(0-93.04)	(0-25)	(2.15-13.60)	(6.20-8.60)
C. stagnalis	513	17.35	36.12	30.90	1.50	9.54	0.66	8.45	7.45
	(353-651)	(15.50-20)	(22.36-71)	(3-80.64)	(0.15-5.19)	(0.63-22.94)	(0-4)	(2.54-13.60)	(6.90-8.60)
G. densa	583	16.69	36.17	14.02	4.48	1.62	2.11	8.96	7.53
	(314-1524)	(12.00-22)	(10.93-87.19)	(0.23-45.01)	(0 -13.80)	(0-6.48)	(0.01-10)	(6.83-11.10)	(6.80-8.30)
L. minor	1061	17.14	125.17	70.13	3.08	5.16	0.27	7.8	7.03
	(507-2880)	(11.30-21)	(22.72-420.67)	(0.87-187.09)	(0.01-20.15)	(0.29-11.98)	(0.47-0.50)	(3.95-10.45)	(5.10-8.60)
M. spicatum	619	15.07	47.02	21.79	6.77	1.93	0.11	8.39	7.27
	(353-745)	(10.00-20)	(26.27-79.75)	(0.10-81.91)	(0.57-20.15)	(0.18-4.23)	(0.47-0.25)	(5.3-10)	(5.10-8.10)
N. officinale	421	14.99	21.38	4.04	1.96	0.48	0.07	9.26	7.89
	(217-756)	(8.50-22)	(8.58-71)	(0.70-15.26)	(0-7.20)	(0-2.48)	(0-0.30)	(7.31-11.46)	(7.10-8.80)
P. crispus	608	13.97	38.46	27.16	12.93	2.40	0.21	7.44	7.68
	(228-1008)	(11.50-16.30)	(10.98-68.85)	(0.22-124.71)	(0.68-22.54)	(0-11.14)	(0.03-0.54)	(5.12-10.40)	(7.30-8.20)
P. polygonifolius	581	13.16	50.69	0.25	9.71	0.37	0.03	6.46	7.4
	(314-777)	(11.60-14.10)	(19.78-84.78)	(0.98-0.38)	(7.2-12.03)	(0-0.86)	(0.01-0.05)	(5.2-8.70)	(7.10-7.80)
R. peniccillatus	410	13.09	26.54	0.29	11.05	0.23	0.02	8.69	7.86
	(108-837)	(10.20-16.40)	(10.93-84.72)	(0.03-0.87)	(6.25-28.61)	(0-1.63)	(0-0.09)	(5.20-11.60)	(6.60-8.50)
V. beccabunga	455	16.99	31.82	7.47	1.54	4.07	0.78	8.92	7.71
	(201-1502)	(8.60-21)	(6.74-262.7)	(0.19-34.76)	(0-7.20)	(0-29.80)	(0-10)	(2.15-13.11)	(6.70-8.60)
Z. palustris	524	19.25	29.02	49.17	1.14	2.76	2.67	8.64	7.85
	(401-723)	(19.00-20)	(12.78-48.99)	(4.68-81.91)	(0-2.70)	(0.39-5.82)	(0.10-10)	(6.94-10.51)	(7.50-8.30)

introduced high variability. By contrast, the least frequent species was *P. polygonifolius,* present in just 2% of the macrophyte-recorded stations.

Geographic and morphometric parameters

Plant species distribution was significantly affected only by water temperature ($F_{9,70}$= 2.58, $p < 0.02$) (Fig. 1a). In this respect, *Z. palustris* and *R. penicillatus* were found at the highest and lowest values of mean water temperature, respectively (Fig. 1a).

Mineralization parameters

Regarding plant distribution and mineralization parameters, significant differences were found only for conductivity ($F_{9,71}$= 2.25, p = 0.028) (Fig. 1b) and Cl^- concentration ($F_{9,71}$= 2.09, p = 0.041) (Fig. 1c). *L. minor* was found at the highest conductivity (1061.00±329.41 µS cm^{-1}) and Cl^- concentration values (125.17±58.57 mg l^{-1}), with no significant differences being observed in the distribution of the other macrophyte species (Fig. 1a,b). Mean conductivity and Cl^- concentration values for *A. nodiflorum* were 871.12 ± 237.19 µS cm^{-1} and 101.93±48.39 mg l^{-1}, respectively.

Trophic level

The distribution of macrophyte species was significantly affected by $N\text{-}NO_2^-$ ($F_{9,72}$= 4.94, p < 0.0001), $N\text{-}NO_3^-$ ($F_{9,72}$= 5.93, p < 0.0001), and $P\text{-}PO_4^{3-}$ ($F_{9,68}$= 2.24, p = 0.03) concentration (Fig. 2). *L. minor, Z. palustris,* and *C. stagnalis* were found at the highest $N\text{-}NO_2^-$ concentrations (70.13±22.14, 49.17±16.63, and 30.90±15.52 µg l^{-1}, respectively), while *P. polygonifolius, R. penicillatus, N. officinale,* and *V. beccabunga* were observed at the lowest concentrations of this same anion (0.25±0.08, 0.29±0.08, 4.04±1.44, and 7.47±2.08 µg l^{-1}, respectively) (Fig. 2a). *A. nodiflorum* also appeared at high levels of N-NO2- (48.57±16.58 µg l-1). Finally, A. nodiflorum and L. minor were positively correlated with $N\text{-}NO_2^-$ concentration (r = 0.54, p = 0.001 and r = 0.54, p = 0.0003, respectively).

On the other hand, *P. crispus, R. penicillatus,* and *P. polygonifolius* were found at the highest $N\text{-}NO_3^-$ concentrations (12.93±3.99, 11.04±2.13, and 9.71±1.39 µg l^{-1}, respectively), while *Z. palustris, C. stagnalis,* and *V. beccabunga* were found at the lowest concentrations of this same anion (1.14±0.58, 1.50±0.76, and 1.54±0.62 µg l^{-1}, respectively) (Fig. 2b). No correlation was found between the distribution of macrophyte species and river $N\text{-}NO_3^-$ concentration.

Regarding $P\text{-}PO_4^{3-}$ concentration and macrophyte species distribution (Fig. 2c), *C. stagnalis* and *L. minor* were found at the highest $P\text{-}PO_4^{3-}$ concentration values (9.54±4.25 and 5.16±1.77 µg l^{-1}, respectively). *A. nodiflorum* also appeared at high $P\text{-}PO_4^{3-}$ values (*i.e.,* 10.60±3.10 µg l^{-1}). Finally, *R. penicillatus, P. polygonifolius,* and *N. officinale* were found at the lowest P-

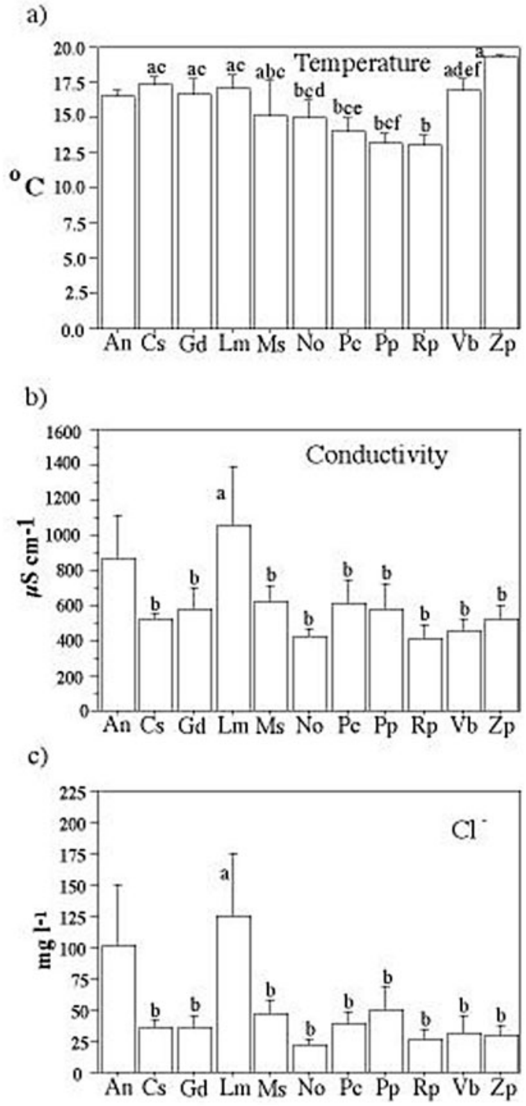

Fig. 1. Macrophyte species distribution (mean ± SE) in relation to temperature (a), conductivity (b) and chloride concentration (c). Within graphs, means with the same lower case letter are not significant at p<0.05. *Apium nudiflorum* was not included in the analysis as explained in the text. An= *Apium nodiflorum,* Cs= *Callitriche stagnalis,* Gd= *Groenlandia densa,* Lm= *Lemna minor,* Ms= *Myriophyllum spicatum,* No= *Nasturtium officinale,* Pc= *Potamogeton crispus,* Pp= *Potamogeton polygonifolius,* Ra= *Ranunculus penicillatus,* Ve= *Veronica beccabunga,* and Za= *Zannichellia palustris.*

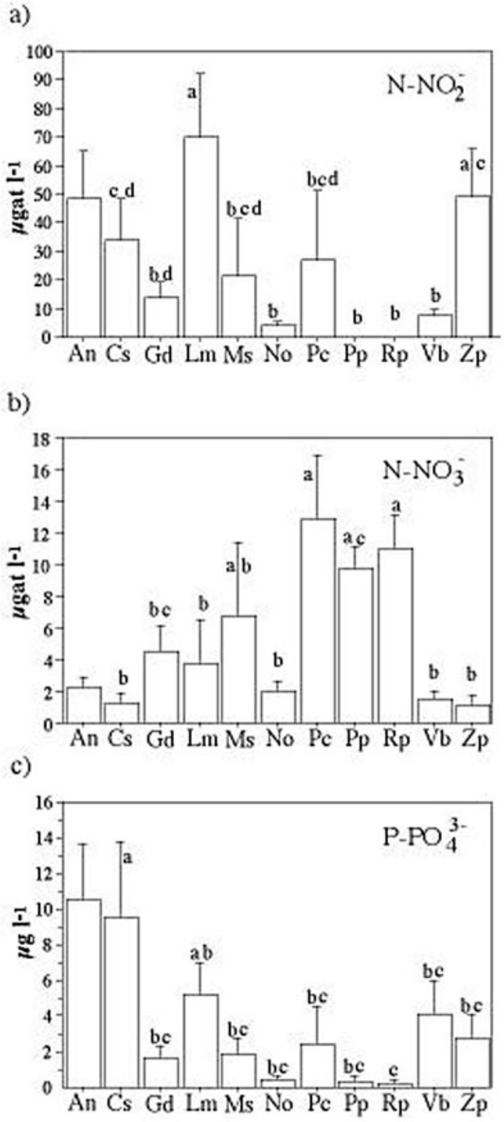

Fig. 2. Macrophyte species distribution (mean ± SE) in relation to nitrite (a), nitrate (b), and phosphate (c) concentration. Within graphs, means with the same lower case letter are not significant at p<0.05. *Apium nudiflorum* was not included in the analysis as explained in the text. An= *Apium nodiflorum,* Cs= *Callitriche stagnalis,* Gd= *Groenlandia densa,* Lm= *Lemna minor,* Ms= *Myriophyllum spicatum,* No= *Nasturtium officinale,* Pc= *Potamogeton crispus,* Pp= *Potamogeton polygonifolius,* Ra= *Ranunculus penicillatus,* Ve= *Veronica beccabunga,* and Za= *Zannichellia palustris.*

PO_4^{3-} concentrations (0.23±0.16, 0.37±0.25, and 0.48±0.19 μg l^{-1}, respectively). Again, no correlation was found between macrophyte species distribution and river P-PO_4^{3-} concentration.

In relation to N-NH_4^+ and macrophyte species distribution (Table 1), although no significant ($p > 0.05$) differences were found, *A. nodiflorum* was indeed correlated with N-NH_4^+ values (r= 0.49, $p < 0.0001$). *Z. palustris* and *R. penicillatus* were found at the highest (2.67±1.44 mg l^{-1}) and lowest (0.02±0.00 mg l^{-1}) N-NH_4^+ concentrations, respectively.

Finally, dissolved O_2 and pH did not show any correlation with the studied species, except for *A. nodiflorum* that was negatively correlated with pH (r = -0.34, p = 0.0002) and showed a mean distribution value of 7.42±0.09 (Table 1).

Discussion

Distribution of macrophyte species in the studied rivers was certainly discontinuous. Although twenty six different species of aquatic macrophytes were recorded at 124 sites, only eleven of those were present in more than 2% of the macrophyte-recorded stations (*i.e.,* where macrophytes were found). Most importantly, only *A. nodiflorum* and *L. minor* showed correlations with the measured variables (*i.e., A. nodiflorum* was positively correlated with N-NO_2^- and N-NH_4^+ concentration and negatively correlated with pH; *L. minor,* in turn, was positively correlated with N-NO_2^- concentration). Thus, in our study area, it might be concluded that the potential of aquatic macrophytes as biological indicators of the environmental conditions of rivers was somewhat restricted by the discontinuity of their distribution. This discontinuity in macrophyte distribution might be due to the specific dispersal and regeneration abilities of the macrophyte species present, as indicated by Demars & Harper (2005).

In any case, regarding their effect on macrophyte distribution, physicochemical parameters clearly dominated over geographic and morphometric variables, probably due to the geological homogeneity of the substrate and/or the relatively high values of nutrient content and mineralization found in these waters, which would mask other effects. In this respect, dominance of geochemical parameters over chemical ones has been previously related to substrate (Grasmück et al. 1995) and morphological heterogeneity (Haury 1996) in other European rivers.

In this study, *A. nodiflorum* was the most abundant species, being distributed in all the studied rivers and within a broad range of environmental conditions. Similar results were described by Demars & Harper (1998) in British rivers. Besides, as abovementioned, *A. nodiflorum* showed a positive correlation with N-

NO_2^- and $N\text{-}NH_4^+$, as well as a negative one with pH. This species has the ability to oxygenate its substrate (Chorianopoulou et al. 2001) and has been reported to be a good competitor in eutrophic waters (Szakowski & Kolsowski 2001). Its abundance at the study area might be due to its good adaptation to the high values of nutrient content and mineralization found in these river waters. Thus, in our study area, *A. nodiflorum,* despite having been reported as a valuable indicator of high mineral content in other areas of Europe (Martínez-Taberner & Moyá 1993), cannot be considered a useful biological indicator of the environmental conditions of rivers. After all, biological indicators must, by definition, appear only within a narrow range of a certain parameter.

Some mineralization parameters, namely, conductivity and Cl^- concentration, differentiated *L. minor* distribution from that of the other macrophytes here studied, and can thus be considered a useful indicator of those two parameters. *L. minor* has also been reported to be a good indicator of mesotrophic waters (Dawson et al. 1999). *Z. palustris* has previously been reported as a good indicator of high conductivity and eutrophic sites in British rivers (Whitton et al. 1998) but in this study the distribution of this macrophyte species did not show correlation with any of the mineralization parameters here determined.

In our study area, water nutrient enrichment was the most significant factor for the differentiation of macrophyte species distribution, as already reported for other European rivers (Thiébaut & Muller 1999, Harper et al. 2000, Lafont 2001, Thiebaut et al. 2002, Gomarasca et al. 2004). In this work, most importantly, the distribution of aquatic macrophytes depended on the specific nutrient under study. In fact, *C. stagnalis* was distributed in sites showing high $P\text{-}PO_4^{3-}$ concentrations, moderate levels of $N\text{-}NO_2^-$, and low values of $N\text{-}NO_3^-$ concentration. This species has previously been found in base-rich or acidic and impoverished rivers (Palmer et al. 1992, Rodwell et al. 1995). Other authors have found it mainly in eutrophic waters (Palmer et al. 1992, Swedish EPA 2002, Thiebaut et al. 2002) and positively affected by the presence of SO_4^{2-} (Gomarasca et al. 2004).

Here *L. minor* was mainly located at sites with high concentrations of $N\text{-}NO_2^-$, as described for Italian rivers (Gomarasca et al. 2004), and moderate levels of $P\text{-}PO_4^{3-}$. This species has been reported as a useful indicator of a moderate degree of high trophic state (Grime et al. 1988, Palmer et al. 1992, Papastergiadou & Babalonas 1993, Rodwell et al. 1995, Thiebaut et al. 2002, Swedish EPA 2002).

In the study sites, *P. crispus* and *P. polygonifolius* appeared in stations having high $N\text{-}NO_3^-$ concentrations and low levels of $P\text{-}PO_4^{3-}$. *P. crispus* has previously been linked to meso-, eutrophic sites in British rivers (Palmer et al. 1992, Rodwell et al. 1995, Dawson et al. 1999, James et al. 2002, Swedish EPA 2002). On the contrary, in French rivers, *P. polygonifolius* has been found to be an indicator of oligotrophic waters (Thiébaut & Muller 1999, Thiébaut et al. 2002, Swedish EPA 2002). Other species of this genus, such as *P. perfoliatus* and *P. pectinatus,* have been associated to high trophic state in rivers of France, Great Britain, Poland and Germany (Grasmük et al. 1995, Rodwell et al. 1995, Dawson et al. 1999, Dawson & Szoszkiewicz 1999, Clarke & Wharton 2001, Szoszkiewicz et al. 2002, Shneider & Melzer 2004, Gomarasca et al. 2004).

N. officinale, a species previously described for other Basque rivers as a good indicator of low mineralization (Onaindia et al. 1996), has also been found in the study area under low values of nutrient content and mineralization, but the statistical analyses have not shown its potentiality.

R. pennicillatus was mainly found at low $P\text{-}PO_4^{3-}$ concentrations and high levels of $N\text{-}NO_3^-$. This species has been described in British rivers as typical of mesotrophic environments (Rodwell et al. 1995, Demars & Harper 1998).

In conclusion, in our study area, *L. minor, C. stagnalis, P. crispus, P. polygonifolius,* and *R. pennicillatus* can be used as biological indicators of nutrient enrichment. *L. minor* is a good indicator of high mineralization (*i.e.,* conductivity and Cl^-) and high trophic state (especially, $N\text{-}NO_2^-$). *C. stagnalis* appears to be a good indicator of high $P\text{-}PO_4^{3-}$ concentrations. *P. crispus, P. polygonifolius,* and *R. penicillatus* can be considered useful indicators of high $N\text{-}NO_3^-$ concentration.

Finally, it must be emphasized that the morphometric parameters, the range of trophic state conditions, the mineral contents, etc. over which the macrophytes here studied were found, differ from those of other regional and/or European rivers. Consequently, the results here obtained regarding the potentiality of different aquatic macrophytes as biological indicators of the environmental conditions of rivers should not be assumed to be appropriate for extrapolation to other areas.

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