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# EPIPHYTIC DIATOMS AS WATER QUALITY INDICATORS IN SPANISH SHALLOW LAKES

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EPIPHYTIC DIATOMS
BACILLARIOPHYCEAE
SHALLOW LAKES
WATER QUALITY
BIOMONITORING
NUTRIENTS
TROPHIC STATE
DIATOM INDICES
WATER FRAMEWORK DIRECTIVE
MEDITERRANEAN REGION

ABSTRACT – The Water Framework Directive (WFD) requires that the European Union countries determine the biological state of their waters with respect to sites of high quality. Despite bioindicators have been widely applied in running waters and deep lakes throughout all Europe, little is known about their applicability in shallow lakes. In this context, the objective of this work is to check the effectiveness of epiphytic diatoms as indicators of the trophic state in six shallow lakes of León Province (NW Spain). Epiphyton was sampled from helophyte stems in six shallow lakes covering different ecological conditions. A significant correlation was found between the total nitrogen concentration and the diatom indices SPI (Specific Polluosensitivity Index) and BDI (Biological Diatom Index), this correlation being lower with respect to the total phosphorus concentration. The classification of the lakes according to the trophic and saprobic levels based on diatoms corresponds to that obtained from the analysis of the limnological and chemical parameters of the studied systems. Despite the use of these indices has been traditionally limited to rivers and channels, our results show the effectiveness of epiphytic diatoms as biological indicators of the quality of water in Mediterranean shallow lakes and the applicability of common diatom indices for biomonitoring purposes in these aquatic ecosystems.

DIATOMÉES ÉPIPHYTES
BACILLARIOPHYCEAE
LACS PEU PROFONDS
QUALITÉ DE L'EAU
BIOMONITORING
NUTRIMENTS
ÉTAT TROPHIQUE
INDICES DIATOMIQUES
DIRECTIVE CADRE SUR L'EAU
RÉGION MÉDITERRANÉENNE

RÉSUMÉ – La Directive Cadre sur l'Eau (WFD) exige que les pays membres de l'Union Européenne déterminent l'état biologique des eaux continentales en référence à des sites de haute qualité. Même si les bioindicateurs ont été largement appliqués dans les eaux courantes et les lacs profonds en Europe, ils ont peu été utilisés dans les lacs peu profonds. Dans ce contexte, l'objectif de ce travail a été de vérifier l'efficacité des Diatomées épiphytes comme indicateurs de l'état trophique dans six lacs peu profonds de la Province de León (NO de l'Espagne). L'épiphyton a été échantillonné sur les tiges des hélophytes dans six lacs peu profonds qui couvrent un vaste spectre de conditions écologiques. Une corrélation significative a été trouvée entre la concentration en azote total et les indices diatomiques SPI et BDI. La corrélation est moindre avec le phosphore total. La classification des lacs selon les indices trophique et saprobique basée sur les Diatomées correspond à celle obtenue par l'analyse des paramètres limnologiques des systèmes étudiés. Bien qu'habituellement l'utilisation de ces indices soit limitée aux faciès lotiques, nos résultats démontrent l'efficacité des Diatomées comme indicateurs de la qualité de l'eau dans les lacs peu profonds méditerranéens et l'applicabilité des indices diatomiques traditionnels pour le biomonitoring de ces écosystèmes aquatiques lacustres.

## INTRODUCTION

In order to follow the requirements of European Union Water Framework Directive (European Parliament & The Council of the European Union 2000), state members are developing several methods to assess the biological quality of their waters using bioindicators. The final goal is to determine the quality state with respect to high-quality sites. During the last 30 years, diatoms have been successfully used as biological indicators in streams and deep lakes (Descy 1976, Lange-Bertalot 1979, Round 1991, Whitton *et al.* 1991, Hoffman 1994, Whitton & Rott 1996, Dell'Uomo & Grandoni 1997, Dell'Uomo *et al.* 1999, Prygiel *et al.* 1999b, Eloranta *et al.* 2002). Some studies have demonstrated the efficacy of planktonic and/or periphytic diatoms as indicators of trophic status in shallow lakes and wetlands (Hawes & Smith 1993, McCormick & O'Dell 1996, McCormick *et al.* 1996, Mayer & Galatowitsch 1999, King *et al.* 2000, Pan *et al.* 2000, Kitner & Poulícková 2003). This corresponds to the experimental evidence that relates the concentration of nutrients (mainly N and P) and the composition and abundance of the diatom community in these ecosystems (Fairchild & Everett 1988, Blanco *et al.* 2003). This work presents results concerning six lakes in León Province (NW Spain), covering a wide range of nutrient concentration. The study is part of the pan-European project ECOFRAME focused to establish water quality typologies in shallow lakes following Water Framework Directive requirements (Moss *et al.* 2004, Nõges *et al.* 2004). Our aim is to assess the efficacy of epiphytic diatoms as indicators of the trophic and saprobic status in shallow lakes by means of the employment of common diatom indices.

## MATERIAL AND METHODS

Studied lakes (Fig. 1) surface ranged from 1.0 to 9.4 ha and their total phosphorus concentrations from 30 to 446 $\mu g \cdot L^{-1}$ (Table I). All were permanent systems at 800 m a.s.l., fully described in Fernández-Aláez *et al.* (1999a, 1999b, 2002) and Moss *et al.* (2004). Morphometric and physicochemical variables (mean depth, area, specific conductance, pH, chlorophyll *a* concentration [Chl *a*], total phosphorus and nitrogen concentrations [$P_{tot}$], [$N_{tot}$], Secchi depth and hydrophyte cover) were measured two times in July and August 2000 following the procedures described in Moss *et al.* 2003 and Nõges *et al.* 2004. Submersed helophyte stems (*Scirpus lacustris* L. and *Typha latifolia* L.) were sampled in July 2000 in southern exposure mats by triplicate in each lake using a Kornijów sampler (Kornijów & Kairesalo 1994). Stems were cut 2-5 cm under water surface to avoid aerial exposition and the influence of waves. Samples consisted in 4-6 fragments of stems of 10 cm length, and were preserved in 10% formaldehyde until processing. Epiphyton was removed from all the fragments using a toothbrush and diatoms were cleaned and mounted on permanent slides according to standard European and French protocols (Kelly *et al.* 1998, AFNOR 2000, CEN 2002). At least, 400 valves were counted and identified on each slide. Diatom taxonomy followed Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot (1993), Round *et al.* (1990) and other taxonomical reference works. Biological Diatom Index (BDI, Lenoir & Coste 1996, AFNOR 2000), Specific Polluosensitivity Index (SPI, Coste in CEMAGREF 1982, version 2003 actualised in OMNIDIA v.3) and Van Dam's trophic and saprobic values (Van Dam *et al.* 1994) were calculated using OMNIDIA software (Lecointe *et al.* 1993, 1999).

## RESULTS

Table I, middle and Fig. 2 show the main diatom taxa observed in the six shallow lakes. Typical periphyton-growing families, such as Achnanthidiaceae and Gomphonemataceae were dominant in many lakes (Fig. 3). Centric diatoms were found exceptionally. In Lake La Baña, *Eunotia naegelii* Migula accounted for more than 80% in abundance of all taxa. This species is rarely reported in the Iberian freshwater phycoflora (Varela 1983, Catalán *et al.* 1993, Almeida 1998, Aboal *et al.* 2003). *Achnanthidium minutissimum* (Kützing) Czarnecki was the most abundant species in three of the lakes (Villaverde, Redos and Laguna Grande), while the morphologically similar *Gomphonema* aff. *angustatum* (Kützing) Rabenhorst, *G. parvulum* f. *saprophila* Lange-Bertalot & Reichardt and *G.* aff. *gracile* Ehrenberg dominated in Lake Villadangos. The highest species richness appeared in Lake Zotes, where *G. parvulum* and Nitzschiaceae (*Nitzschia fonticola* Grunow and *N. amphibia* Grunow) dominated (Fig. 3). *Nitzschia radicula* Hustedt, in Lake Laguna Grande, is the first record of this species in the Iberian Peninsula (Aboal *et al.* 2003). Diatom indices and corresponding water quality status categories are shown in Table I, bottom. Lakes La Baña and Villaverde are considered oligotrophic, while Zotes and Villadangos are eutrophic, the rest fitting in different degrees of mesotrophy. Only Lake La Baña is oligosaprobic, the others being $\beta$-mesosaprobic or reaching the $\alpha$-meso-

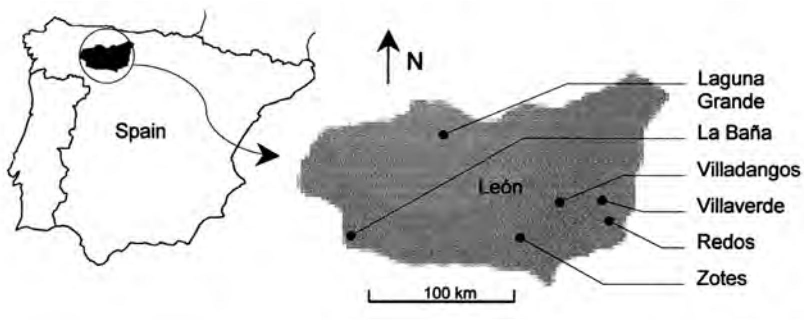



Fig. 1. Geographic location of the studied shallow lakes in Spain and León Province

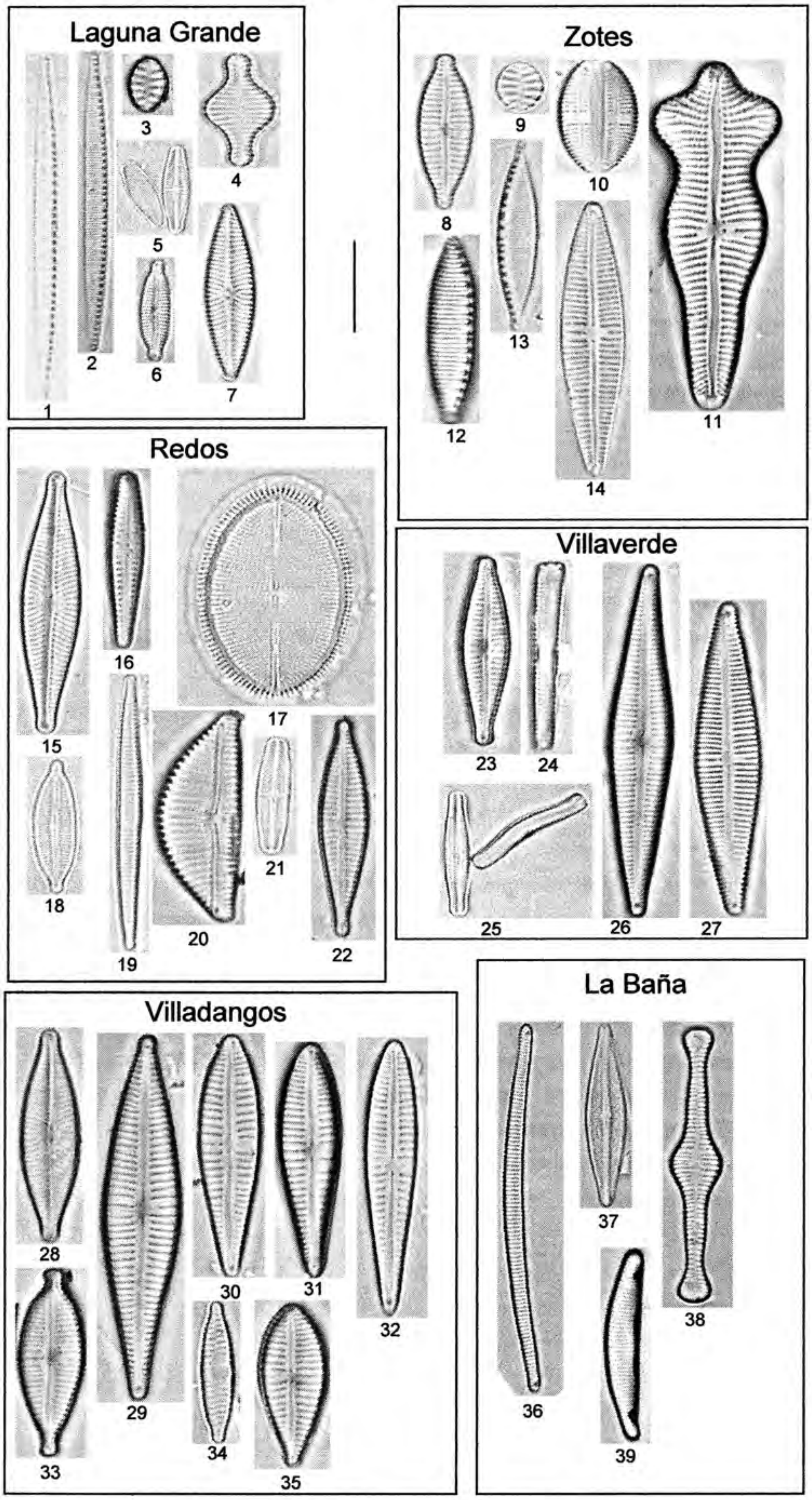
Table I. – Top, Limnological and chemical characteristics of the studied lakes. Average summer values. Data from Moss *et al.* (2004). Middle, Main diatom taxa (⩾ 1 % abundance) found in the six shallow lakes of León Province (NW Spain). Bottom, Diatom indices values SPI (Coste in CEMAGREF 1982) and BDI (Lenoir & Coste 1996, AFNOR 2000) and corresponding biological quality status categories, trophic and saprobic status (Van Dam *et al.* 1994) for the studied lakes. See Material and methods for details.

| Lake | Mean depth (m) | Area (ha) | Conductivity ($\mu S \cdot cm^{-1}$) | pH | [$P_{tot}$] ($\mu g \cdot L^{-1}$) | [$N_{tot}$] ($\mu g \cdot L^{-1}$) | [Chl *a*] ($\mu g \cdot L^{-1}$) | Secchi depth (m) | Hydrophyte cover |
|---|---|---|---|---|---|---|---|---|---|
| La Bañia | 1.1 | 6.5 | 9 | 5.9 | 30 | 380 | 2 | > 1.1 | 25-50 % |
| Villaverde | 0.6 | 1.7 | 128 | 7.5 | 77 | 860 | 6 | > 0.6 | < 25 % |
| Redos | 0.6 | 1.0 | 86 | 9.6 | 54 | 300 | 21 | > 0.6 | > 50 % |
| Laguna Grande | 1.3 | 2.5 | 111 | 9.6 | 55 | 480 | 34 | 1.0 | < 25 % |
| Villadangos | 0.4 | 9.4 | 234 | 7.3 | 446 | 2250 | 81 | 0.35 | 0 % |
| Zotes | 0.9 | 1.0 | 339 | 7.9 | 102 | 3750 | 30 | 0.8 | 0 % |

| Taxon | Code |
|---|---|
| *Achnanthidium* sp. | ACHS |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | ADMI |
| *Achnanthidium saprophilum* (Kobayasi & Mayama) Round & Bukhtiyarova | ADSA |
| *Amphora pediculus* (Kützing) Grunow | APED |
| *Brachysira neoexilis* Lange-Bertalot | BNEO |
| *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck | CPLI |
| *Craticula submolesta* (Hustedt) Lange-Bertalot | CSBM |
| *Encyonema silesiacum* (Bleisch) D.G. Mann | ESLE |
| *Encyonopsis microcephala* (Grunow) Krammer | ENCM |
| *Eunotia incisa* Gregory | EINC |
| *Eunotia naegelii* Migula | ENAE |
| *Fragilaria capucina* Desmazières var. *capucina* | FCAP |
| *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot | FCVA |
| *Gomphonema acuminatum* Ehrenberg | GACU |
| *Gomphonema* aff. *angustatum* (Kützing) Rabenhorst | GANG |
| *Gomphonema clavatum* Ehrenberg | GCLA |
| *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt | GEXL |
| *Gomphonema gracile* Ehrenberg | GGRA |
| *Gomphonema lagenula* Kützing | GLAG |
| *Gomphonema parvulum* Kützing var. *parvulum* f. *parvulum* | GPAR |
| *Gomphonema parvulum* f. *saprophila* Lange-Bertalot & Reichardt | GPAS |
| *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot | GPUM |
| *Navicula cryptocephala* Kützing | NCRY |
| *Navicula cryptotenella* Lange-Bertalot | NCTE |
| *Nitzschia amphibia* Grunow | NAMP |
| *Nitzschia fonticola* Grunow | NFON |
| *Nitzschia lacuum* Lange-Bertalot | NILA |
| *Nitzschia palea* (Kützing) W. Smith | NPAL |
| *Nitzschia radicula* Hustedt | NZRA |
| *Staurosira construens* (Ehrenberg) Williams & Round | SCON |
| *Staurosirella pinnata* (Ehrenberg) Williams & Round | SPIN |
| *Tabellaria flocculosa* (Roth) Kützing | TFLO |
| *Ulnaria ulna* (Nitzsch) Compère | UULN |

| Lake | SPI / Ecological quality status | BDI / Ecological quality status | Trophic status (Van Dam *et al.* 1994) | Saprobic status (Van Dam *et al.* 1994) |
|---|---|---|---|---|
| La Baña | 20.0 / High | 19.9 / High | Oligotrophic | Oligosaprobic |
| Villaverde | 18.9 / High | 18.0 / High | Oligotrophic | β-Mesosaprobic |
| Redos | 19.2 / High | 17.7 / High | Oligo-mesotrophic | β-Mesosaprobic |
| Laguna Grande | 17.5 / High | 16.7 / Good | Meso-eutrophic | β-Mesosaprobic |
| Villadangos | 10.9 / Moderate | 12.0 / Moderate | Eutrophic | α-Meso-polysaprobic |
| Zotes | 11.1 / Moderate | 11.4 / Moderate | Eutrophic | β-Mesosaprobic |

Fig. 2. Main diatom taxa observed. Scale bar: 10 µm. 1-2: *Nitzschia radicula*. 3: *Staurosirella pinnata*. 4. *Staurosira construens*. 5: *Achnanthidium minutissimum*. 6: *Encyonopsis microcephala*. 7: *Navicula cryptotenella*. 8: *Gomphonema parvulum*. 9: *Staurosirella pinnata*. 10: *Amphora pediculus*. 11: *Gomphonema acuminatum*. 12: *Nitzschia amphibia*. 13: *Nitzschia fonticola*. 14: *Gomphonema gracile*. 15: *Navicula cryptocephala*. 16: *Gomphonema pumilum*. 17: *Cocconeis placentula* var. *lineata*. 18: *Encyonopsis* aff. *microcephala*. 19: *Fragilaria capucina*. 20: *Encyonema silesiacum*. 21: *Achnanthidium minutissimum*. 22-24: *Gomphonema exilissimum*. 25: *Achnanthidium minutissimum*. 26-27: *Gomphonema gracile*. 28: *Navicula cryptocephala*. 29: *Gomphonema* aff. *gracile*. 30-32: *Gomphonema* aff. *angustatum*. 33: *Gomphonema* aff. *lagenula*. 34: *Fragilaria capucina* var. *vaucheriae*. 35: *Gomphonema parvulum* var. *parvulum* f. *saprophila*. 36: *Eunotia naegelii*. 37: *Brachysira neoexilis*. 38: *Tabellaria flocculosa*. 39: *Eunotia incisa*.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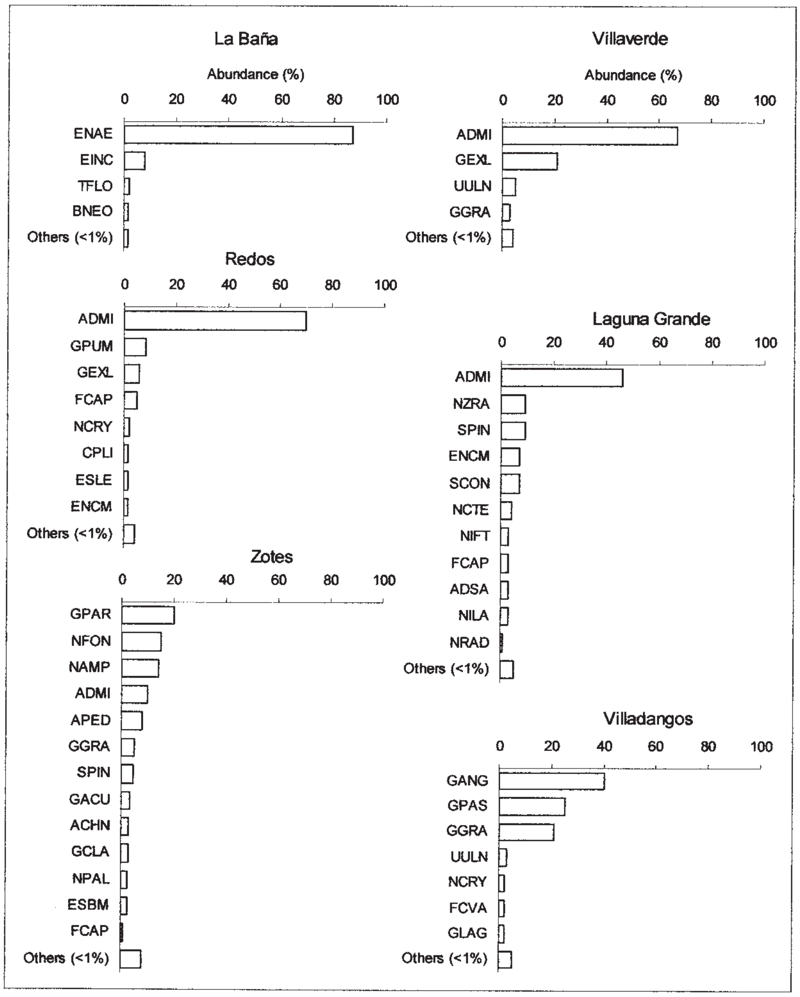


Fig. 3. Relative abundance (%) of diatom taxa in the epiphytic samples of the six shallow lakes. See taxa codes in Table I.

polysaprobicity in Lake Villadangos (Table I, bottom). BDI and SPI output did not differ significantly (t-test: 0.22, n.s.). Log [$N_{tot}$] was significantly ($p < 0.05$) correlated to BDI and SPI (Fig. 4). Regression coefficient values were lower in the case of the total phosphorus concentrations. SPI and BDI are not significantly correlated to log [$P_{tot}$].

## DISCUSSION

It has been stated that the structure and dynamics of shallow lakes differ significantly from that of the deep ones (Jeppesen 1998, Scheffer 1998). Since the concentration of nutrients is higher in shallow lakes (see e.g. Quirós 2001), the traditional classification of lake trophic status according to several physicochemical variables (Vollenweider & Kerekes 1980, Wetzel 1983, Novotny & Olem 1994) cannot be strictly applied to shallow lakes, especially in the Mediterranean region. As consequence of natural eutrophication and of much higher nutrient variations in shallow than deep lakes, different TP and chlorophyll *a* concentrations have to be chosen as explaining ranges for shallow lake functioning (Jeppesen *et al.* 2000). In our study, lakes such as La Baña or Villaverde, will fit within the oligotrophic or the eutrophic categories depending on the variable chosen for the classification (summer [Chl *a*] and [$P_{tot}$], respectively). Thus, the use of more integrative indicators such as diatoms would probably achieve a more realistic approach for water quality assessment in these ecosystems. In this work, diatom abundances were distributed accordingly to their autoecological valences (Coste in CEMAGREF 1982, Van Dam *et al.* 1994, Lenoir & Coste 1996) within the different trophic status in the lakes, suggesting that these parameters, calculated from running rivers but also low channels and ditches, are also applicable to lenthic facies in shallow lakes. However, our preliminary dataset do not let us to state whether there are typical lacustrine species with poorly known ecological ranks. In any case, three major problems can be inferred from our results about the applica-

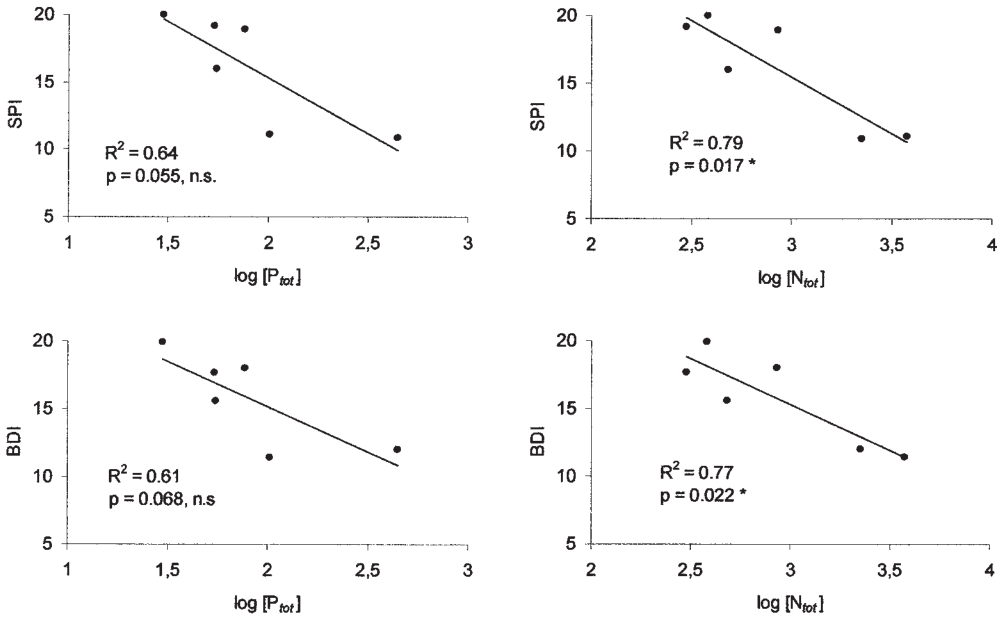


Fig. 4. Correlation between diatom indices (SPI, BDI) and total phosphorus or total nitrogen concentrations (log units).

bility of these diatom indices: a) the dominance of euryoic species such as *Achnanthidium minutissimum* sensu lato in many of the studied lakes, b) the dominance of taxa such as *Eunotia naegelii* that are not taken into account for the calculation of some indices (e.g. BDI), c) the presence of species complexes that are difficult to identify using standard taxonomic techniques (e.g. *Gomphonema* species in Lake Villadangos).

These facts could explain to a certain extent the discrepancy between the SPI and BDI values in some lakes and, thus, the presence of outlier points in the log [$P_{tot}$] regression lines (Fig. 4). In order to avoid these and other effects, constant efforts are being done in France and other European countries for the improvement of indices calculation by intercalibration tests (Prygiel *et al.* 1999a, 2002). A new BDI version will be available in OMNIDIA version 4 at the beginning of 2004 including the new investigations about the diatom ecology of hundreds new sampling sites of all France (J Prygiel pers comm) and other countries of Europe. New methods could also be tested or adapted in south of Europe, for instance the trophic indication methods (P and N) elaborated in Austrian rivers by Rott *et al.* (1999, 2003).

Cazaubon (1996) summarizes methodological problems of the use of epiphyton for biomonitoring in rivers. The relationship between epiphyton and water chemistry has been demonstrated (Lakatos 1989, Ács *et al.* 1991, 1994, Cambra 1992, 1993) and submerged macrophytes have been recommended over emergent ones (helophytes) for routine monitoring (Kelly *et al.* 1998). Nevertheless hydrophytes are absent in lakes when planktonic chlorophyll *a* is high (Scheffer 1998), and differences in the epiphytic communities among macrophyte species or plant parts have proved to be significant (Cattaneo & Kalff 1980, Eminson & Moss 1980, Blindow 1987, Cattaneo *et al.* 1998, Romo & Galanti 1998). Such differences can be reduced increasing the number of replications (Iserentant & Versailles 1989) or using the same macrophyte species, which strongly decrease the applicability of these substrates. In comparison with rivers, where stones or sediments are always present as common substrates for attached algae, only the submerged part of helophytes and sediments can be considered as universal substrates in shallow lakes. *Typha* spp., *Scirpus* spp., *Phragmites* spp. and other emergent species are commonly found in any lenthic systems, and studies have proved the homogeneity of their periphytic communities, as long as their composition is more related to chemical characteristics of the lake water than to the type of plant substrates (Cattaneo *et al.* 1998), especially in eutrophic systems (Eminson & Moss 1980). Moreover, differences in the epiphytic community between dead and alive stems have proved to be no significant (Caput & Plenkovic-Moraj 2000), which valuate helophytes as useful substrata for sampling in any season. Helophytes present additional advantages: in order to avoid shading from phytoplankton (Hansson 1992) and the deposition of suspended solids and dead algae (frustules or valves), vertical surfaces have been recommended for sampling in slow-flowing waters (Kelly *et al.* 1998). Moreover, they present comparatively regular architectures and easily measurable surfaces allowing also quantitative estimations of periphytic productivity, al-most impracticable in the case of several hydrophytes.

A few comparative studies have been done using diatom indices from epilithon and epiphyton samples. Hoffman (1994) found minor differences using Van Dam's values in German lakes. Similar results report Lenoir & Coste (1994) applying the SPI in French rivers.

In conclusion, our results support the hypothesis that epiphytic diatoms, especially on helophyte stems (*Scirpus lacustris* and *Typha latifolia*), are valid biological indicators for shallow lakes. The changes observed in the diatom communities responded quantitatively and qualitatively to the trophic status of the lakes, allowing the application of biological indices. As far of the lack of specific indices for lenthic waters, SPI, BDI and Van Dam's trophic values can be used for the assessment of water quality status in shallow lakes. The design of a standardized biomonitoring protocol for these ecosystems and the evaluation of substrata-periphyton interactions effect over diatom indices are future research targets.

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