

NUTRIENT MONITORING IN SPANISH WETLANDS USING EPIPHYTIC DIATOMS

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ABSTRACT. – The Water Framework Directive requires that the European Union countries determine the biological state of their waters with respect to sites of high quality. Although bio-indicators have been widely applied in running waters and deep lakes throughout Europe, little is known about their applicability in shallow lakes and wetlands. The objective of this work is to check the effectiveness of epiphytic diatoms as indicators of the trophic state in two wetlands of Álava Province (N Spain). Diatom and water chemical samples were collected during summer and autumn 2007, following standard methodology. Stems of submerged macrophyte (*Carex riparia*, *Iris pseudacorus*, *Veronica anagallis-aquatica*) were sampled in each lake. Based on the number of statistically significant correlations between diatom indices and environmental factors, the SID index was shown as the most suitable method to assess nutrients within these systems. SID was significantly correlated with TP, NTK and ammonium levels. Among three different plants, only the indices calculated from *I. pseudacorus* samples were significantly correlated with nutrients.

INTRODUCTION

The Water Framework Directive (European Union 2000) establishes the guidelines for the ecological status assessment of continental water bodies in the European Union. The use of bioindicators, including diatom communities, is considered among other methods. During the last decades many diatom metrics based on ecology and species relative abundances have been developed for lotic systems (Ector & Rimet 2005). However, studies regarding lacustrine systems are relatively scarcer. The usefulness of diatom communities as ecological indicators in lentic environments has been extensively proved. Diatoms in lake sediments have been successfully used in many countries as proxies of past and present environmental conditions. Specifically, epiphytic diatoms as indicators of nutrients and other water quality characteristics have been investigated in many studies (Hofman 1994, Linares 2003, Blanco *et al.* 2004, Denys 2004, Ács *et al.* 2005, Stenger-Kovács *et al.* 2007, Trobajo Pujadas 2007, Ács *et al.* 2008). Numerous nutrient inference models developed for lakes are also based on diatom species ecology and relative abundances.

Despite this, to our knowledge, there are no available diatom-based indices for Iberian shallow lakes. Up to date, proposed methods have been developed for temperate deep lakes (e.g., Hoffmann 1994) but their applicabil-

ity elsewhere has not been explored thoroughly. Only the TDIL has been designed specifically for shallow lakes, although the floristic spectrum considered by this index (see Stenger-Kovács *et al.* 2007) and the ecological preferences found for the dominant taxa appear barely comparable among different ecoregions. Moreover, Álvarez-Blanco (2010) demonstrated the existence of contrasting autoecological parameters in a number of common diatom species along spatial and temporal gradients, thus evidencing the need to develop specific indices based on regional surveys. However, this often implies the collection of large datasets after intensive sampling efforts in order to obtain statistically significant sample sizes and such data sets are currently unavailable in the study area covered by this work. Hence, this paper aims to provide a scientific basis for the future implementation of water quality monitoring methods (particularly eutrophication) in Spanish wetlands. Due to the absence, in many cases, of available hard substrata for diatom sampling, there is no standardized sampling protocol for lakes although some recommendations have been presented (King *et al.* 2006). Blanco & Bécares (2006) proposed a sampling method for the collection of epiphytic diatoms in shallow lakes; however the influence of the different host macrophyte species on their diatom communities needs to be addressed. This paper evaluates also the effect of the host macrophyte on the composition of epiphytic diatom

Table I. – Mean values and standard deviations of the water nutrient concentrations during the study (ppm).

	NH ₄ ⁺	NTK	TP
Arkaute	0.24 (± 0.04)	2.73 (± 0.78)	0.22 (± 0.12)
Betoño	0.15 (± 0.13)	0.90 (± 0.69)	0.01 (± 0.01)

community in lentic habitat, and, as a result, on the water quality assessment by means of diatom indices developed for lotic systems.

MATERIAL AND METHODS

The Arkaute and Betoño lakes studied are located in the Salburúa wetland (42°51' N, 2°38' W; 216.38 ha; min. and max. altitude: 509-514 m a.s.l.; Lobo Urrutia & Sesma Ausejo 2006). This is one of the most valuable continental wetlands in the Basque Country and is a Ramsar Wetland of International Importance. It is affected by agriculture and most of its water bodies have subsaline (bicarbonate-dominated) and meso/eutrophic water. Depth of Arkaute lake in the sampled sites is approximately 20 cm (Bolue Ingurumen Ikerketak 2008). In Betoño lake mean annual depth varies between 50 and 80 cm (Ekos 2006). The mean nutrient concentrations during the study are shown in Table I.

Diatom samples were taken fortnightly five times during the summer and autumn of 2007. Simultaneously, the concentrations of total phosphorus (TP), total nitrogen (NTK) and ammonium (NH₄⁺) were measured following APHA (1998). Sampling method follows the protocol for the collection of epiphytic diatoms in shallow lakes described by Blanco & Bécars (2006). First of all, a visual survey of the lakes was made to identify the most abundant and widely distributed macrophyte species present in the system: *Carex riparia* Curtis, *Iris pseudacorus* L., *Veronica anagallis-aquatica* L. Submersed macrophyte stems were sampled in triplicate for each lake. Epiphyton was removed from all the fragments by gently shaking the samples for 2 min according to Zimba & Hopson (1997). The method followed ensures the collection of more than 90 % of the diatoms attached to the stems. Resulting suspensions were cleaned by oxidation with hot hydrogen peroxide 30 % v/v and then rinsed three times with distilled water. Air-dried aliquots were mounted on permanent glass slides using the refractive resin, Naphrax®, according to standard European protocols (CEN 2003). On each slide, at least 400 diatom valves were counted and identified to the lowest possible taxonomic level (species, sub-species, variety or form) based on the taxonomy and nomenclature proposed in Krammer & Lange-Bertalot (1986-1991), Lange-Bertalot (1995-2009) and Lange-Bertalot (2000-2009). Based on the floristic inventories obtained, 18 common diatom indices were calculated using OMNIDIA version 4.1 software (Lecointe *et al.* 1993, 1999). The results of these indices are numeric values standardized from 1 to 20, representing respectively the theoretical minimal and maximal water quality status.

Table II. – Correlation matrix between calculated diatom indices and nutrient concentrations. Spearman's coefficients (ρ_s^2) shown

	NH ₄ ⁺	NTK	TP
SLA	0.25	0.24	0.41
DESCY	0.01	0.00	0.02
LMA	0.24	0.27	0.42
SHE	0.11	0.04	0.19
WAT	0.30	0.33	0.41
TDI	0.00	0.02	0.00
% PT	0.19	0.22	0.33
GENRE	0.38	0.14	0.24
CEE	0.02	0.09	0.16
IPS	0.37	0.27	0.41
IBD	0.15	0.35	0.40
IDAP	0.44	0.23	0.40
EPI-D	0.33	0.07	0.22
DI_CH	0.15	0.24	0.13
IDP	0.16	0.12	0.02
SID	0.20	0.50	0.44
TID	0.52	0.23	0.38

Since some of the measured variables did not follow normal distributions (Kolmogorov-Smirnov test, $p < 0.05$), non-parametric statistical analyses were used. Spearman's correlation coefficients between diatom indices and chemical variables were calculated and the Kruskal-Wallis one-way analysis of variance was carried out to identify significant differences among index scores for the different plant species and lakes. Additionally, a nonparametric similarity analysis (ANOSIM permutation test, Clarke 1993) was carried out using (a) diatom relative abundances and (b) presence-absence data to assess the influence of contrasting plant substrata on the composition of epiphytic diatom communities. Spearman's correlation and Kruskal-Wallis tests were calculated using STATISTICA 7.0 software (StatSoft 2001) and ANOSIM test with Community Analysis Package version 3.11 (Pisces Conservation Ltd 2004).

RESULTS

169 diatom taxa have been identified including species, sub-species, varieties and forms. The species with the highest occurrence in both lakes and also the most abundant was *Achnantheidium minutissimum* (Kütz.) Czarn. *Navicula trivialis* Lange-Bert., *Gomphonema italicum* Kütz. and *G. gracile* Ehrenb. dominated the assemblages

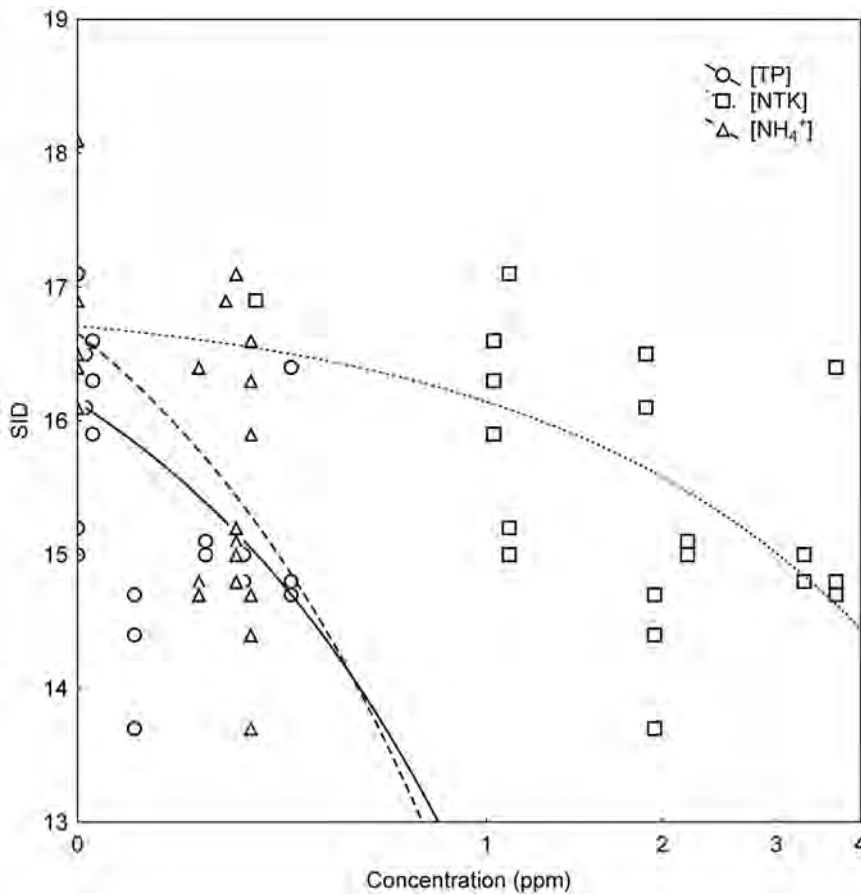


Fig. 1. – Plot of the SID index scores versus nutrient concentrations in the studied lakes. Data fitted to linear regression lines.

found at Lake Arkaute, while the most abundant taxa at Lake Betoño were *Epithemia adnata* (Kütz.) Bréb., *Gomphonema acidoclinatum* Lange-Bert. & E. Reichardt and *Achnanthydium pyrenaicum* (Hust.) H. Kobayasi.

Considering the average correlation indices between diatom indices and environmental factors, the SID index (Rott *et al.* 1997) was found to be the most suitable method to diagnose the chemical quality of the water within these systems (mean $\rho_s^2 = 0.38$, Table II). When data for all substrata were pooled together SID was negatively and significantly correlated with TP ($p < 0.001$), NTK ($p < 0.001$) and ammonium ($p < 0.05$) levels (Fig. 1). In correlation analysis by type substratum, SID was not correlated with any chemical variable when the sampled macrophyte was *Carex riparia* and *Veronica anagallis-aquatica*. In the case of *Iris pseudacorus*, SID was significantly correlated with TP ($p < 0.01$) and NTK ($p < 0.01$), but not with ammonium concentration ($p = 0.36$) (Fig. 2).

Kruskal-Wallis test results showed that SID index values were significantly higher in Lake Betoño than in Arkaute ($H = 15.92$, $p < 0.001$). No significant differences were found in SID index values among different plant substrata ($H = 0.85$, $p = 0.65$) (Fig. 3).

Results of ANOSIM test on the relative abundance of diatom species showed significant differences between the diatom communities of the two different lakes

($R = 0.002$; $p < 0.05$). No significant differences were found in the relative abundance of diatom species on the three macrophytes whether data for each lake were analysed independently or pulled together. ANOSIM test with presence-absence data also showed significant differences between specific composition of the diatom communities in the two lakes ($R = 0.001$; $p < 0.05$). No significant differences were found for the specific composition of the diatom communities developed on the three macrophytes whether data for each lake were analysed independently or pulled together.

In Arkaute lake, water quality decreased towards the end of the study as the TP concentration increased. Water quality in Betoño lake, evaluated by the SID index, remained higher and was more stable, corresponding to lower nutrient concentrations (Fig. 3).

DISCUSSION

Given the variety of diatom indices (Ector & Rimet 2005) it is necessary to select the most suitable index for each system. In the present study SID index (Rott *et al.* 1997) was found to be the most appropriate index based on the correlations with the chemical variables, despite being a saprobic index developed for Austrian streams.

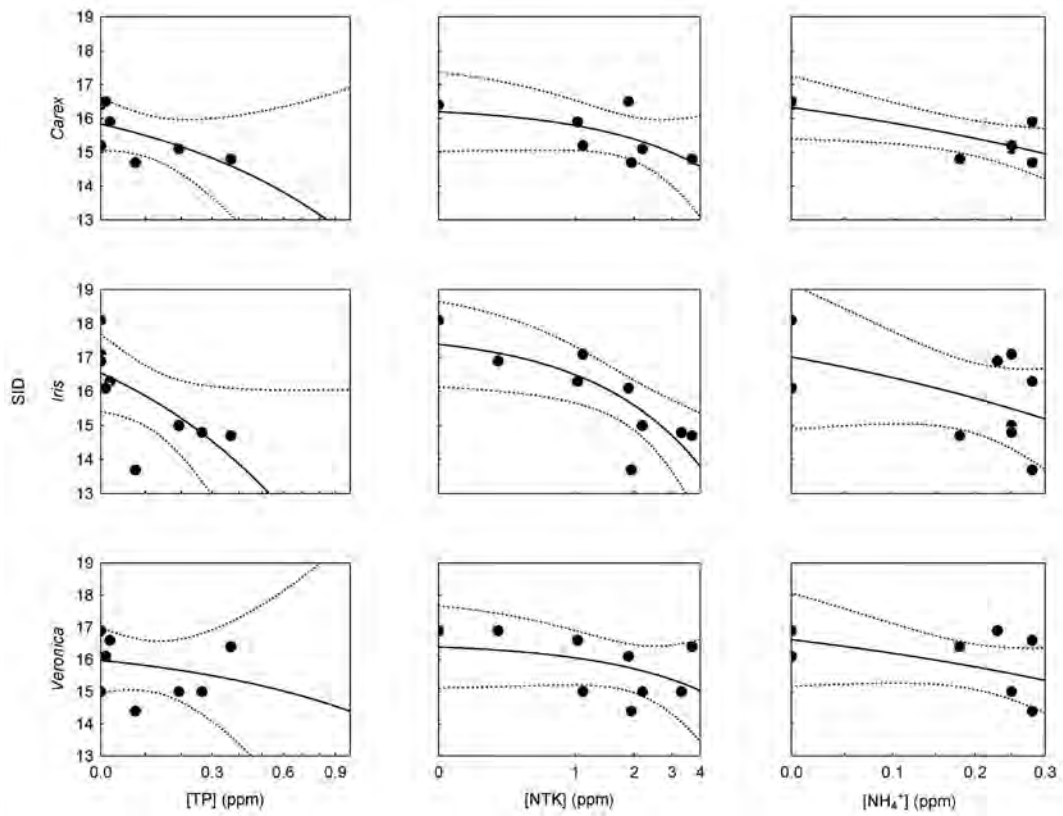


Fig. 2. – Regressions of the SID index scores calculated for the diatom assemblages growing on different plant substrata on nutrient correlations. Regression bands (95 % confidence limits) shown (dashed lines). Data fitted to linear regression lines.

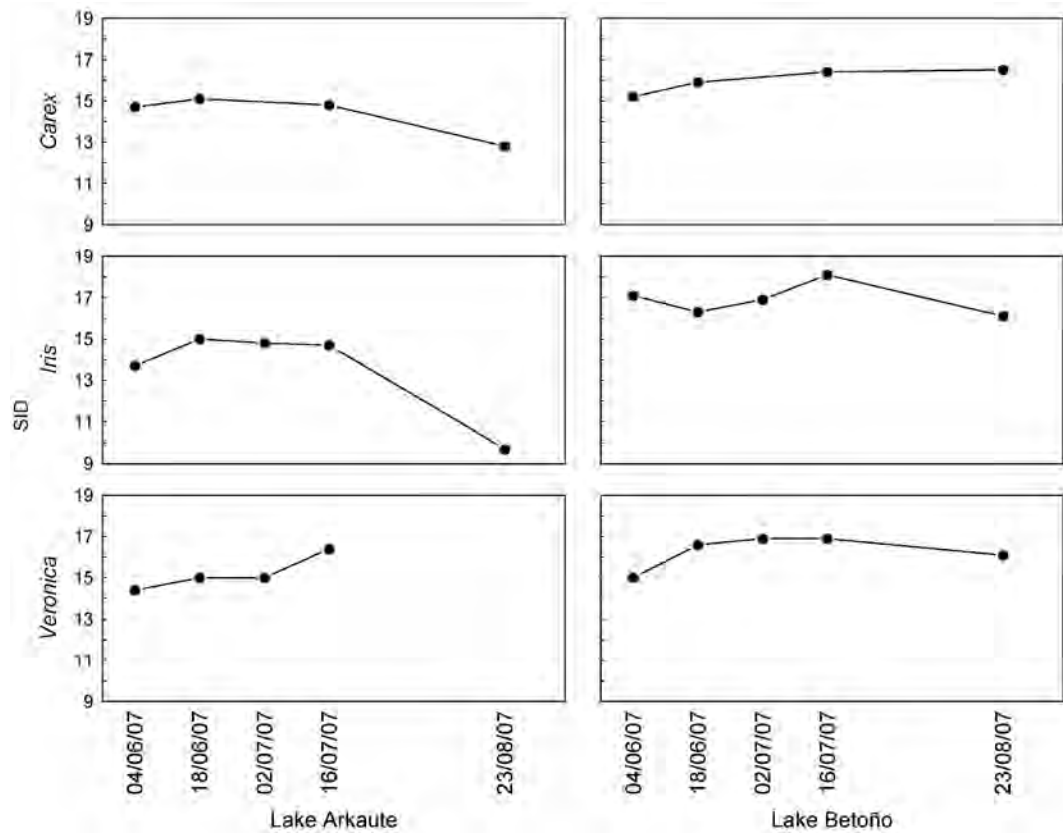


Fig. 3. – Temporal changes in the SID index scores calculated for the diatom assemblages growing on different plant substrata.

On the other hand, the applicability of the indices can vary depending on the type of substratum (macrophytes, stones or sediments) sampled (Besse-Lototskaya *et al.* 2006). This work showed that SID was significantly correlated with chemical variables only when the sampled macrophyte was *Iris pseudacorus*, although Kruskal-Wallis test results demonstrated no significant differences in index values among different plant substrata.

Similarity test showed no significant differences in composition of diatom assemblages developed on the three sampled macrophytes. Many studies have focused on the effect of substrata on the diatom communities growing on them (e.g., Cattaneo & Amireault 1992) with contrasting results. Poulícková *et al.* (2004), comparing diatom communities from different substrata (stones, mud and young reed stalks), found different specific compositions among different substrata and trophic statuses. Potapova & Charles (2005) obtained different results regarding species richness, diversity and diatom biovolume in assemblages from hard (rocks and wood) and soft (sand and silt) substrata but, in this case, the relationship with environmental factors was similar for both types. Kíter & Poulícková (2003) also found significant differences in species richness on diatom communities growing on different substrata (rocks, plants and sediment). This could lead to a differential response of epiphytic assemblages to limnological variables, as evidenced in our study. Nevertheless, Winter & Duthie (2000) did not observe solid structural differences comparing diatom assemblages from different substrata (epilithic, epipellic and epiphytic) but they did find that some taxa were more abundant in certain habitats.

Different results have been obtained for epiphytic communities as well. Eminson & Moss (1980) suggested that epiphytic algal communities are more influenced by water physico-chemical parameters than by host macrophytes except in oligotrophic systems. Cattaneo *et al.* (1998) found similar taxonomic composition of epiphytic algae on other plants but different diversities and biomass when comparing plants with different morphology. We found significant relationships between epiphyton and abiotic factors only for certain host macrophytes, although the reliability of diatom-based biotic indices inferred from different plants has not been explored elsewhere. Some other authors have reported affinities of diatom species for a particular habitat. For instance, Blindow (1987) found larger differences, in epiphyton composition, between *Chara globularis* Thuill. and *C. tormentosa* L. than between the former and *Nitellopsis obtusa* (Desv.) J. Groves and Messyas & Kuczyńska-Kippen (2006) found that *Eunotia lunaris* (Ehrenb.) Bréb. ex Rabenh. preferred *Typha angustifolia* L. stands to *Chara tormentosa* L. stands. Differences in epiphytic diatoms growing on various plants may be caused by several factors such as plant architecture (Cattaneo *et al.* 1998) or allelopathic effects, although phytoplankton is more sensitive to

allelopathic substances than epiphyton (Hilt 2006). These floristic differences could explain to a certain extent the contrasting performances of computed ecological indices in our study.

In summary, the significant correlation among SID index and chemical variables proves the effectiveness of epiphytic diatom communities as potential ecological indicators in lentic systems according to the guidelines established by the Water Framework Directive. Particularly, we propose SID index for routine biomonitoring of these two Spanish lakes. In order to standardize the methodological protocol and based on our results, we recommend sampling only on *Iris pseudacorus* stems for ecological quality assessment in these lakes.

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