# MONITORIZACIÓN DE LOS ECOSISTEMAS DULCEACUÍCOLAS MEDIANTE MÉTRICAS BASADAS EN DIATOMEAS

## MONITORING FRESHWATER ECOSYSTEMS USING DIATOM BASED METRICS

**CRISTINA CEJUDO FIGUEIRAS**

# MONITORIZACIÓN DE LOS ECOSISTEMAS DULCEACUÍCOLAS MEDIANTE MÉTRICAS BASADAS EN DIATOMEAS

## *MONITORING FRESHWATER ECOSYSTEMS USING DIATOM-BASED METRICS*

CRISTINA CEJUDO FIGUEIRAS

MONITORIZACIÓN DE LOS ECOSISTEMAS DULCEACUÍCOLAS MEDIANTE MÉTRICAS BASADAS EN DIATOMEAS

*MONITORING FRESHWATER ECOSYSTEMS USING DIATOM-BASED METRICS*

Memoria de la Tesis presentada para optar al grado de Doctora en Ciencias Ambientales por la Universidad de León

Directores:

Dr. Saúl Blanco Lanza
Área de Ecología
Universidad de León

Dr. Eloy Bécares Mantecón
Área de Ecología
Universidad de León

León 2011

*"Pocos objetos hay más hermosos que los pequeños caparazones silíceos de las diatomeas: ¿fueron creadas éstas para que pudiesen ser examinadas y admiradas con los mayores aumentos del microscopio?"*

(Charles Darwin: El Origen de las Especies, cap.VI).

Autora: Cristina Cejudo Figueiras. IMARENABIO, Universidad de León, La Serna, 58 - 24007 León, España (Spain). Tel: (+34) 987293136. Fax: (+34) 987291563. E-mail: ccejf@unileon.es, ccejfig@gmail.com

# ÍNDICE

## AGRADECIMIENTOS

**Lista de artículos incluidos en la tesis.**

**I.** Cejudo-Figueiras C., Morales E.A., Wetzel C.E., Blanco S., Hoffmann L. & Ector L. 2011. Analysis of the type of *Fragilaria construens* var. *subsalina* (Bacillariophyceae) and description of two morphologically related taxa from Europe and the United States. *Phycologia*, 50 (1): 67-77.

**II.** Blanco S., Cejudo-Figueiras C., Tudesque L., Bécares E., Hoffmann L. & Ector L. Are diatom diversity indices reliable monitoring metrics? *Hydrobiologia*. Aceptado.

**III.** Cejudo-Figueiras C., Blanco S., Álvarez-Blanco I., Ector L. & Bécares E. 2010. Nutrient monitoring in Spanish wetlands using epiphytic diatoms. *Vie et milieu - life and environment*, 60 (2): 89-94.

**IV.** Cejudo-Figueiras C., Álvarez-Blanco I., Bécares E. & Blanco S. 2010. Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited. *Marine and Freshwater Research*, 61: 1457–1467.

**V.** Cejudo-Figueiras C., Álvarez-Blanco I., Bécares E. & Blanco S. 2010. Epiphytic diatoms along environmental gradients in European shallow lakes. *The Journal of the North American Benthological Society*. Enviado.

# 1. INTRODUCCIÓN

## *Introducción general*

Los ecosistemas acuáticos se encuentran sometidos a múltiples presiones tanto de origen natural como antrópico. Factores como la sequía, los trasvases de agua, la eutrofización y el aumento de la cantidad de materia orgánica por presiones agrarias y ganaderas, contaminación térmica, acidificación, turbidez o presencia de tóxicos, generan un empeoramiento del estado ecológico que afecta a las comunidades de organismos asociadas a estos ecosistemas. En las sociedades actuales, la preocupación por conservar y recuperar los ecosistemas acuáticos ha ido aumentando y alejándose del concepto tradicional de calidad química del agua, prestando más atención al equilibrio ecológico dentro del ecosistema.

Este nuevo concepto de calidad se materializó en Europa con la aprobación de la Directiva Marco del Agua (DMA, No. 2000/60/EC; European Union, 2000) cuyo objetivo es evitar el deterioro de todas las masas de agua y conseguir un buen estado ecológico de éstas en todos los países de la Unión Europea antes de 2015. La DMA establece como factores esenciales para la determinación del estado ecológico de las aguas valorar distintos aspectos referentes a la hidromorfología, los parámetros físico-químicos y las comunidades biológicas. Entre los bioindicadores propuestos (fauna piscícola, zoobentos, fitobentos y fitoplancton), las algas fitobentónicas ocupan un lugar destacado, y dentro de este grupo, las diatomeas reúnen una serie de características fisiológicas y ecológicas que las convierten en organismos idóneos para su empleo como indicadores ecológicos.

## *Características generales de las diatomeas y su papel en la bioindicación*

Las Diatomeas son organismos unicelulares eucariotas pertenecientes a la División Bacillariophyta del reino Chromista. Son organismos generalmente autótrofos, fotosintéticos que cumplen un papel esencial en la cadena trófica de los ecosistemas acuáticos. Las diatomeas son organismos cosmopolitas, existiendo miles de especies en todos los continentes, desde los trópicos hasta los polos. A su vez, son ubiquistas, por lo que podemos encontrarlas en ambientes muy distintos (marinos, salobres y dulceacuícolas). Además, la amplia distribución de algunas especies favorece su utilización como organismos indicadores.

Otras ventajas que presenta este grupo de microalgas en su función bioindicadora es su sensibilidad frente a la alteración de los parámetros físico-químicos del agua, por lo que las comunidades de diatomeas reflejan con gran precisión las condiciones ambientales del medio en que se han desarrollado. Además, se conocen las preferencias ecológicas de muchas especies con respecto a los niveles de nutrientes y de oxígeno disuelto, por lo que son indicadoras de niveles tróficos y sapróbicos. Su carácter sésil permite realizar estudios de calidad en un punto concreto y sus ciclos de vida cortos hacen que los cambios de calidad se reflejen en pocas horas. En pocos centímetros cuadrados se puede hacer un muestreo representativo de la comunidad y las muestras son fáciles de recoger y conservar. Existen especies que viven libres en la columna de agua (fitoplancton) y especies que viven asociadas a un sustrato, formando parte del perifiton. Éste se clasifica en distintos grupos en función del sustrato: epipelon (sedimentos), epifiton (plantas, musgo u otras algas), epiliton (rocas o piedras) y epipsammon (granos de arena). También hay especies epizooicas (sobre

animales).

El empleo de comunidades de diatomeas en estudios de bioindicación se ha realizado aplicando diferentes técnicas como modelos predictivos (Gevrey *et al.*, 2004; Ponader *et al.*, 2008; Feio *et al.*, 2009), métodos multimétricos (Reavie *et al.*, 2008) o análisis de similitud (Kelly, 2001), sin embargo, el método más común en el campo de la bioindicación ha sido el cálculo de índices ecológicos. Existen dos grandes grupos de índices basados en las comunidades de diatomeas:

- Los índices de diversidad se basan en el supuesto de que las presiones sobre el ecosistema (contaminación orgánica, eutrofización, etc.) provocan un declive en la diversidad, de forma que disminuye la abundancia de ciertos taxones intolerantes, mientras que los taxones tolerantes superan a los primeros (Archibald, 1972; Patrick, 1973). Sin embargo, la fertilización puede aumentar la diversidad de la comunidad favoreciendo el establecimiento y crecimiento de varias especies (Canning-Clode *et al.*, 2008). De hecho, uno de los paradigmas teóricos más fructíferos de la biología actual es la relación entre diversidad y productividad tanto en ecosistemas acuáticos como terrestres. Varios meta-análisis (Waide *et al.*, 1999; Bonn *et al.*, 2004; Gillman & Wright, 2006) muestran relaciones lineares, unimodales o no significativas entre estas variables dependiendo de la escala espacial cubierta (Foody, 2004; Drake *et al.*, 2008) y, por otra parte, de la presencia de retroalimentaciones competitivas e influencias ambientales complejas (Molis, 2002; Grace *et al.*, 2007). Esta variabilidad ha revelado que los índices de diversidad de diatomeas no resultan adecuados para evaluar las condiciones ecológicas, a pesar de la frecuencia con que se usan en estudios de bioindicación.

- Por su parte, los índices autoecológicos se basan en la sensibilidad de los diferentes taxones a la contaminación, ponderada por su valor indicador y la abundancia relativa de los mismos dentro de la comunidad, en función de la amplitud ecológica y las preferencias de hábitat de las especies o grupos taxonómicos principales (Ector & Rimet, 2005; de la Rey *et al.*, 2008). Sin embargo, tales índices diatomológicos son sensibles a diversos factores como la precisión taxonómica, la influencia del sustrato, ciertas características físico-químicas del medio o parámetros fisiográficos y geográficos. Existen más de 100.000 taxones de diatomeas (Round *et al.*, 1990) y cada año se describen alrededor de 400 nuevas especies, por lo que la velocidad a la que se actualiza el conocimiento de la taxonomía y los respectivos valores autoecológicos, es mayor que la velocidad a la que se actualizan los índices diatomológicos.

Por otra parte, la influencia del sustrato sobre la comunidad de diatomeas perifíticas aun crea controversia en la literatura científica, habiéndose realizado numerosos estudios al respecto (Eminson & Moss, 1980; Blindow, 1987; Poulíčková *et al.*, 2004; Potapova & Charles, 2005; Townsend & Gell, 2005). El efecto de otros factores como los métodos de muestreo y la preparación de muestras así como la precisión en la identificación o la consideración del tamaño celular (Cattaneo *et al.*, 1995; Kelly *et al.*, 1998; Lavoie *et al.*, 2005; Besse-Lototskaya *et al.*, 2006; King *et al.*, 2006; Lavoie *et al.*, 2009) han sido objeto de análisis en las últimas décadas.

### *Particularidades ecológicas de los lagos someros*

Una parte significativa de este estudio tiene por objetivo sentar las bases científicas para la aplicación de índices diatomológicos en lagos someros,

que son los ecosistemas lacustres numéricamente más abundantes en el mundo (Wetzel, 1990). Los lagos someros, a diferencia de los profundos, suelen estar colonizados por macrófitos y no presentan estratificación térmica en verano, ya que su escasa profundidad (< 3 m) genera una mezcla constante del agua (polimixis) (Scheffer, 2001). La acción del viento facilita la interacción entre el agua y el sedimento y los nutrientes se reciclan rápidamente, aumentando así la productividad (Bécares *et al.*, 2004). La interacción agua-sedimento depende, a su vez, de mecanismos físicos, químicos y biológicos como la morfometría, el régimen hídrico, la granulometría, la naturaleza estructural de la materia orgánica o la composición química del agua (de Vicente *et al.*, 2006).

Cuando la concentración de nutrientes es muy baja, las lagunas suelen tener poca cobertura vegetal y la riqueza de especies es mínima. Debido a la escasa producción de fitoplancton la turbidez también es baja. Con mayores niveles de nutrientes aumenta la riqueza de especies en todos los niveles y las redes tróficas son más complejas, aumentando también la cantidad de biomasa. Las aguas pueden mantener la transparencia del agua debido a mecanismos como la competencia entre macrófitos y fitoplancton por los nutrientes y la luz. La presencia de vegetación, a su vez, funciona como refugio para el zooplancton y reduce la resuspensión de sedimentos por el viento contribuyendo también a la transparencia del agua. Sin embargo, en aguas eutróficas, la vegetación desaparece drásticamente y la laguna pasa a un estado de aguas turbias. A lo largo del año una laguna puede presentar estados alternativos de aguas claras y turbias, dependiendo de los mecanismos que actúen en ella (Scheffer, 2001; Bécares *et al.*, 2004). No existe un único nivel crítico de nutrientes que determine el cambio de aguas claras a turbias, ya que pueden actuar otros factores como el clima, el tamaño de la laguna y su profundidad (Scheffer & van Nes, 2007).

Dentro de la amplia variedad de bioindicadores (Ector & Rimet, 2005), las diatomeas han destacado debido a los buenos resultados obtenidos tanto en sistemas lóticos (Prygiel *et al.*, 1999) como en sistemas leníticos tales como humedales costeros (Trobajo, 2003), lagos de montaña (Linares, 2003) o lagunas mediterráneas (Blanco *et al.*, 2004). La mayoría de los índices basados en comunidades de diatomeas se han desarrollado para ríos, sin embargo, hasta el momento sólo se ha desarrollado un índice específico (TDIL; Stenger-Kovács *et al.*, 2007) para lagunas.

Por todo esto, y considerando la heterogeneidad de las bases de datos en que se basan los índices, en muchos casos, es necesario hacer una evaluación previa para determinar qué índice es el más adecuado para cada estudio (Besse-Lototskaya *et al.*, 2006; Blanco *et al.*, 2007; Martín *et al.*, 2010, Álvarez-Blanco et al. 2010) y realizar, a mayor escala, estudios de intercalibración (Kahlert *et al.*, 2009; Kelly *et al.*, 2009).

La presente investigación está enfocada a clarificar ciertos aspectos importantes referentes a la bioindicación a partir de comunidades de diatomeas, como la influencia del sustrato y los gradientes biogeográficos en las comunidades de diatomeas y en la calidad del agua inferida a partir de éstas y la aplicabilidad de los índices de diversidad, además de esclarecer la taxonomía de especies fácilmente confundibles en microscopía óptica, presentando dos especies nuevas para la Ciencia.

## 2. OBJETIVOS

Los objetivos de la presente tesis son:

**1.** Contribuir al conocimiento taxonómico y ecológico de las diatomeas.

**2.** Investigar la validez de los índices de diversidad de diatomeas como indicadores de calidad del agua.

**3.** Identificar las variables que afectan a la composición de las comunidades de diatomeas en ecosistemas de agua dulce.

**4.** Evaluar la influencia de diferentes plantas sustrato en las comunidades de diatomeas que albergan y en la diagnosis ambiental inferida a partir de las mismas.

**5.** Analizar la influencia de las variables morfométricas y geográficas de los lagos someros sobre sus comunidades de diatomeas epífitas.

Este estudio está estructurado en cinco artículos que estudian diferentes aspectos de los temas arriba mencionados.

El **Artículo I** estudia la identidad taxonómica de la diatomea *Fragilaria construens* var. *subsalina*. Hasta este momento, la estructura valvar de este taxón no se había estudiado en detalle, mediante el análisis de fotografías de microscoía óptica (MO) y microscopía electrónica de barrido (MEB) del material lectotipo del taxón. Además, se describen dos nuevos taxones morfológicamente similares, uno de la cuenca del Duero y otro del noroeste de Estados Unidos (erróneamente identificado en Morales (2005) como *Pseudostaurosira subsalina*). El estudio se ha completado con un análisis morfométrico que confirma la separación entre especies.

En el **Artículo II** se evalúa la utilidad de los índices de diversidad de diatomeas como métricas de monitorización en hábitats lóticos, estudiando varias hipótesis: a) la existencia de una relación linear, negativa y significativa entre fósforo total (PT) y la diversidad de diatomeas, tal como señalan varios autores (e.g. Ndiritu *et al.*, 2006; Ní Chatháin & Harrington, 2008), b) patrones significativamente diferentes en las curvas de acumulación de especies, de frecuencia-abundancia, de rango-abundancia y en los diagramas de distribución de frecuencias para las comunidades de diatomeas desarrolladas bajo diferentes niveles tróficos, según la hipótesis descritas en Magurran (2004), y c) una frecuencia de ocurrencia mayor para taxones que aparecen en comunidades que se encuentran bajo condiciones mesotróficas, ya que las especies generalistas que toleran un amplio rango de condiciones ambientales tendrán distribuciones más amplias, mientras que las especies especialistas están restringidas a unos pocos lugares donde se encuentran sus requerimientos más estrictos (Heino & Soininen, 2006).

Los **Artículos III y IV** estudian las correlaciones entre las variables ambientales y las métricas basadas en comunidades de diatomeas epífitas procedentes de lagunas del humedal de Salburua y de la depresión del Duero. Además, se analiza la hipótesis del sustrato neutro (Blindow, 1987), evaluando la influencia del macrófito hospedador sobre la calidad del agua inferida a partir de los índices diatomológicos mediante análisis de la varianza, y sobre la composición de las comunidades de diatomeas desarrolladas sobre las diferentes plantas sustrato mediante análisis de similitud. Se pone a prueba, así, la aplicabilidad en sistemas leníticos de los índices de diatomeas desarrollados para sistemas lóticos.

Por último, en el **Artículo V** se estudia

el efecto de diferentes grupos de factores abióticos (químicos, morfométricos y geográficos) sobre las comunidades de diatomeas epífitas desarrolladas en 44 lagunas europeas.

## 3. MATERIAL Y MÉTODOS

### *Áreas de estudio*

Para la realización del presente trabajo se han estudiado sistemas acuáticos de agua dulce, tanto lóticos **[Artículos I, II]** como leníticos **[Artículos III, IV, V]**. Su localización y coordenadas geográficas se muestran en la Tabla 1 y la Figura 1.

Parte del trabajo realizado consiste en una investigación sobre la identidad taxonómica de un grupo de especies de identificación problemática **[Artículo I].** El material lectotípico de *Fragilaria construens* var. s*ubsalina*, fue recolectado en aguas salobres el 15 de julio de 1922 en la localidad de Schleswig-Holstein, Alemania. El material tipo de *Psedostaurosira americana*, se recogió en aguas ligeramente alcalinas con concentraciones bajas o medias de ortofosfatos y nitrógeno total y con un contenido moderado de electrolitos. Este material, así como la muestra adicional recolectada en la misma localidad provienen del río Norwalk a la altura de la localidad de South Wilton (Connecticut, EEUU) y fueron recogidas el 9 de septiembre de 1998 y el 10 de agosto de 2004, respectivamente. El material tipo de *Pseudostaurosira alvareziae* proviene de aguas oligosaprobias, meso-eutróficas y ligeramente alcalinas con bajo contenido en electrolitos. Se recolectó en el río Tormes a su paso por Salamanca (España) el 5 de agosto de 2004.

Con el fin de evaluar la aplicabilidad de los índices de diversidad dentro del campo de la bioindicación basada en comunidades de diatomeas **[Artículo II]**, se estudiaron 640 estaciones a lo largo de la cuenca del Loira- Bretaña (Francia) recogidas entre 1996 y 2000. La cuenca (44º 38' N - 48º 51' N y 4º 30' E - 4º 45' O) abarca alrededor de 156400 km² (28% de la superficie de Francia) y está formada por la cuenca del Loira y sus afluentes, las cuencas costeras de Bretaña y las cuencas costeras Vendée y Poitevin. Las precipitaciones anuales varían entre 500 y 1800 mm. La zona del Macizo armoricano y del Macizo Central se compone de rocas silíceas, metamórficas y fracturadas. Otra parte está formada por sedimento, areniscas o roca calcárea paleozoica o cenozoica.

La parte del estudio enfocada a esclarecer las cuestiones referentes a la aplicabilidad de los índices autoecológicos basados en diatomeas y la influencia del sustrato sobre la evaluación de la calidad del agua y la estructura de las comunidades de diatomeas se localizó, en el humedal de Salburua **[Artículo III]** y en las lagunas de la depresión de la cuenca del Duero **[Artículo IV]**.Se estudiaron las balsas de Arkaute (39,5 ha) y Betoño (21 ha), situadas dentro del humedal de Salburua (42º 51' N, 2º 38' O; 216,38 ha; altitud mínima y máxima: 509-514 m.s.n.m.; Lobo Urrutia & Sesma Ausejo, 2006). Se trata de un humedal Ramsar de importancia internacional localizado en un valle agrícola en las proximidades de Vitoria-Gasteiz (País Vasco) y alimentado, principalmente, por el acuífero Cuaternario de Vitoria-Gasteiz. Se encuentra entre las regiones Atlántica y Mediterránea. Los suelos de las lagunas están formados por limo, arcilla, arena y grava, depositados sobre sustrato margoso, lo que le confiere, al acuífero, un carácter semiconfinado. Las muestras se recogieron durante el verano y el otoño de 2007.

Además, se estudiaron diecinueve lagunas permanentes localizadas en la cuenca del río Duero (97290 km², 40º 17' N - 43º 1' N y 2º 56' O - 8º 41' O), situada en la Submeseta norte (700 m.s.n.m) bajo bioclima mediterráneo. La precipitación anual media de la cuenca

Tabla 1. Localización y coordenadas geográficas de los puntos de muestreo.

| Artículo | Tipo | Punto | País | Latitud | | Longitud | |
|---|---|---|---|---|---|---|---|
| IV | Laguna | Antimio | España | 42º 43' 03" | N | 05º 37' 30" | O |
| IV | Laguna | Bardal | España | 40º 51' 59" | N | 00º 07' 38" | O |
| IV | Laguna | Bercianos | España | 42º 36' 33" | N | 05º 09' 01" | O |
| IV | Laguna | Berrueces | España | 42º 10' 30" | N | 05º 06' 22" | O |
| IV | Laguna | Cabeza | España | 41º 07' 52" | N | 06º 03' 09" | O |
| IV | Laguna | Carucedo | España | 42º 42' 56" | N | 06º 46' 42" | O |
| IV | Laguna | Chozas | España | 42º 44' 35" | N | 05º 43' 13" | O |
| IV | Laguna | Diel | España | 42º 44' 49" | N | 05º 16' 24" | O |
| IV | Laguna | Muña | España | 41º 30' 31" | N | 04º 00' 09" | O |
| IV | Laguna | Redos | España | 42º 41' 48" | N | 05º 13' 29" | O |
| IV | Laguna | Ribas | España | 42º 23' 23" | N | 04º 31' 29" | O |
| IV | Laguna | Santervás | España | 42º 43' 43" | N | 04º 48' 51" | O |
| IV | Laguna | Sentiz | España | 42º 46' 38" | N | 05º 12' 42" | O |
| IV | Laguna | Tremedosa | España | 41º 32' 11" | N | 03º 58' 23" | O |
| IV | Laguna | Valdemorgate | España | 42º 36' 13" | N | 05º 04' 28" | O |
| IV, V | Laguna | Villadangos | España | 42º 44' 29" | N | 05º 46' 48" | O |
| IV | Laguna | Villaflores | España | 41º 18' 20" | N | 05º 16' 20" | O |
| IV | Laguna | Zarza | España | 41º 03' 46" | N | 06º 19' 25" | O |
| IV | Laguna | Zuares | España | 42º 33' 09" | N | 05º 40' 53" | O |
| III | Laguna | Arkaute | España | 42º 51' 33" | N | 02º 37' 57" | O |
| III | Laguna | Betoño | España | 42º 51' 25" | N | 02º 38' 42" | O |
| V | Laguna | Dystrup | Dinamarca | 56º 27' 02" | N | 10º 37' 43" | E |
| V | Laguna | En | Dinamarca | 55º 10' 00" | N | 11º 40' 60" | E |
| V | Laguna | Schousbye | Dinamarca | 56º 10' 01" | N | 09º 37' 01" | E |
| V | Laguna | Søbygård | Dinamarca | 56º 15' 19" | N | 09º 48' 27" | E |
| V | Laguna | Stigsholm | Dinamarca | 55º 58' 40" | N | 09º 29' 22" | E |
| V | Laguna | Væng | Dinamarca | 56º 06' 34" | N | 09º 52' 30" | E |
| V | Laguna | Harku | Estonia | 59º 24' 54" | N | 24º 37' 03" | E |
| V | Laguna | Kaiavere | Estonia | 58º 36' 10" | N | 26º 40' 35" | E |
| V | Laguna | Kirikumäe | Estonia | 57º 41' 01" | N | 27º 14' 58" | E |
| V | Laguna | Maardu | Estonia | 59º 26' 40" | N | 24º 59' 46" | E |
| V | Laguna | Prossa | Estonia | 58º 39' 03" | N | 26º 34' 32" | E |
| V | Laguna | Vörtsjärvi | Estonia | 58º 18' 37" | N | 26º 00' 41" | E |
| V | Laguna | Matjarvi | Finlandia | 61º 06' 01" | N | 25º 17' 18" | E |
| V | Laguna | Pukalajärvi | Finlandia | 61º 09' 42" | N | 25º 19' 57" | E |
| V | Laguna | Sylvöjarvi | Finlandia | 60º 57' 54" | N | 26º 01' 41" | E |
| V | Laguna | Alter Weiher | Alemania | 47º 56' 10" | N | 09º 31' 52" | E |
| V | Laguna | Flappach Weher | Alemania | 47º 44' 55" | N | 09º 37' 46" | E |

Tabla 1. Localización y coordenadas geográficas de los puntos de muestreo (cont.).

| Artículo | Tipo | Punto | País | Latitud | | Longitud | |
|---|---|---|---|---|---|---|---|
| V | Laguna | Langensee | Alemania | 53° 48' 36" | N | 12° 01' 55" | E |
| V | Laguna | Lengenweiler See | Alemania | 47° 52' 12" | N | 09° 26' 00" | E |
| V | Laguna | Rohrsee | Alemania | 47° 52' 27" | N | 09° 50' 17" | E |
| V | Laguna | Atedaun | Irlanda | 52° 56' 41" | N | 09° 02' 44" | O |
| V | Laguna | Ballyquirke | Irlanda | 53° 19' 37" | N | 09° 09' 16" | O |
| V | Laguna | Bunny | Irlanda | 53° 01' 05" | N | 08° 55' 46" | O |
| V | Laguna | Maumwee | Irlanda | 53° 28' 34" | N | 09° 32' 30" | O |
| V | Laguna | Mullagh | Irlanda | 54° 10' 56" | N | 08° 38' 53" | O |
| V | Laguna | Ramor | Irlanda | 53° 49' 19" | N | 07° 04' 21" | O |
| V | Laguna | Velauemeer | Holanda | 52° 22' 42" | N | 05° 39' 36" | E |
| V | Laguna | Zwemlust | Holanda | 52° 11' 59" | N | 05° 04' 16" | E |
| V | Laguna | Dagstorpssjörn | Suecia | 55° 59' 18" | N | 13° 29' 32" | E |
| V | Laguna | Hymenjaure | Suecia | 68° 26' 51" | N | 18° 27' 13" | E |
| V | Laguna | Krankesjön | Suecia | 55° 41' 55" | N | 13° 28' 41" | E |
| V | Laguna | Lassejaure | Suecia | 65° 33' 18" | N | 18° 06' 21" | E |
| V | Laguna | Västra Ringsön | Suecia | 55° 53' 37" | N | 13° 28' 10" | E |
| V | Laguna | Bugeilyn | Reino Unido | 52° 30' 54" | N | 03° 44' 15" | O |
| V | Laguna | Filby Broad | Reino Unido | 52° 39' 44" | N | 01° 38' 10" | E |
| V | Laguna | Little Budwort Mere | Reino Unido | 53° 10' 55" | N | 02° 37' 52" | O |
| V | Laguna | Little Mere | Reino Unido | 53° 19' 53" | N | 02° 24' 09" | O |
| V | Laguna | South Walsham Broad | Reino Unido | 52° 40' 20" | N | 01° 29' 58" | E |
| V | Laguna | Baña | España | 42° 14' 58" | N | 06° 45' 01" | O |
| V | Laguna | Laguna Grande | España | 42° 22' 13" | N | 05° 07' 52" | O |
| V | Laguna | Redos | España | 42° 41' 48" | N | 05° 13' 29" | O |
| V | Laguna | Villaverde | España | 42° 34' 33" | N | 05° 11' 18" | O |
| V | Laguna | Zotes | España | 42° 16' 25" | N | 05° 44' 18" | O |
| I | Río | Norwalk | EEUU | 41° 09' 49" | N | 73° 25' 11" | O |
| I | Río | Tormes | España | 40° 57' 37" | N | 05° 38' 42" | O |
| I | ? | Oldesloe | Alemania | 53° 48' 15" | N | 10° 22' 55" | E |

es de 513,9 mm, de los cuales 359,7 se pierden por evapotranspiración. Las lagunas se encuentran, concretamente, en la depresión endorreica de la cuenca, compuesta por sedimentos lacustres y continentales del Terciario y el Cuaternario con inclusiones evaporíticas. Los muestreos se realizaron durante el verano de 2003, excepto en el caso de Muña, que se muestreó en 2004.

Se realizó un estudio a escala continental en el contexto del proyecto paneuropeo ECOFRAME, cuyo objetivo el evaluar la calidad ecológica de lagos someros. Se estudiaron 44 lagunas situadas en 9 países europeos. Las lagunas están situadas en la Zona Templada Norte, es decir, entre el trópico de Cáncer y el Círculo Polar Ártico, a excepción de Hymenjaure (Suecia) que se encuentra en el Ártico. Los muestreos se realizaron en los veranos de 2000 o 2001.

Figura 1. Localización de los puntos de muestreo: a) Europa, b) Estados Unidos.



## Protocolos de muestreo de diatomeas

Con respecto al material lectotipo de *Fragilaria construens* var. *subsalina* **[Artículo I]** no se dispone de información sobre el sustrato muestreado ni sobre el protocolo de muestreo empleado.

En los sistemas lóticos se recogieron muestras epilíticas. Para las muestras recolectadas en el río Norwalk **[Artículo I]** se siguió el método desarrollado por el NAWQA (Fitzpatrick *et al.*, 1998; Moulton *et al.*, 2002). Las muestras del río Tormes y las de la cuenca del Loira-Bretaña se recogieron siguiendo el método descrito en la norma estándar europea (European Standard EN 13946, 2003) **[Artículos I y II]**. Estos protocolos se basan en la recolección de epiliton sobre piedras sumergidas en la zona fótica, en zonas de corriente usando

un cepillo de dientes. Las muestras se conservan con formaldehido al 4% v/v.

En los sistemas leníticos se siguió el método de muestreo de diatomeas epífitas en lagunas para la aplicación de la Directiva Marco del Agua propuesto en Blanco & Bécares (2006) [Artículos III, IV y V]. Los sustratos muestreados fueron Carex riparia Curtis, Iris pseudacorus L., Veronica anagallis-aquatica L. [Artículo III], Scirpus lacustris L., Typha latifolia L., Myriophyllum alterniflorum D.C. [Artículo IV] y Phragmites australis (Cav.) Trin. ex Steud. [Artículo V]. El método se basa en el muestreo sobre matas de helófitos monoespecíficas, lejos de la orilla y en zonas iluminadas evitando zonas demasiado someras y con sedimento resuspendido. Para recoger muestras de tallos se utilizan muestreadores Kornijów (Kornijów & Kairesalo, 1994) con el fin de minimizar la agitación y la presencia de fitoplancton. El número de tallos a muestrear depende del diámetro de éstos. Si los tallos son finos como en Eleocharis o Juncus se recogen 10 tallos por punto de muestreo, si son muy gruesos como Typha se recoge uno y en casos intermedios como Scirpus o Phragmites se recogen 3 tallos. Las muestras se conservan con formaldehido al 4% v/v. Para separar el epifiton de los tallos se siguió el método de Zimba & Hopson (1997) que asegura la recuperación del 90% del epifiton y consiste en llenar parcialmente los botes con agua y agitar enérgicamente durante 2 minutos.

## Protocolos de preparación de muestras y recuento de diatomeas

Para la obtención de suspensiones de frústulos libres de materia orgánica se siguió la norma estándar europea (European Standard EN 13946, 2003). Para ello se toma una alícuota de la muestra y se somete a un proceso de oxidación a 90° C durante 6 horas con peróxido de hidrógeno (30% v/v),

cuyo volumen será el doble de volumen de muestra. Con el fin de deshacer los agregados de carbonato cálcico, cuando la muestra está fría se le añade ácido clorhídrico 1M gota a gota hasta que no exista reacción. La muestra se somete a tres lavados con agua destilada mediante decantación o centrifugación y posterior eliminación del sobrenadante para eliminar el peróxido de hidrógeno **[Artículos I-V]**.

Para la obtención de preparaciones permanentes para su observación al microscopio óptico (MO) se siguió la norma estándar europea (European Standard EN 13946, 2003). Consiste en poner la muestra sobre un cubreobjetos redondo sobre una placa calefactora a temperatuta suave o dejándola secar al aire. Cuando ésta se evapora se monta sobre el porta objetos con Naphrax®, una resina sintética con un índice de refracción óptica de 1,7 **[Artículos I-V]**.

Para la obtención de preparaciones permanentes para su observación al microscopio electrónico de barrido (MEB), alícuotas de material oxidado procedente de Schleswig-Holstein y Salamanca se montaron en *stubs* y se cubrieron con una capa de oro de 40-nm de grosor con un sistema modular de recubrimiento de alto vacío (BAL-TEC MED 020). La muestra de South Wilton recogida en 1998 se cubrió con oro-paladio usando un Metalizador Polaron ® **[Artículo I]**.

Las fotografías de microscopía óptica se tomaron con un MO Leica® DM-RB equipado con una cámara Leica® DC500. Las fotografías de microscopía electrónica se tomaron con un MEB Leica® Stereoscan 430i operando a 20 kV para las muestras de Schleswig-Holstein y Salamanca y un MEB Leo-Zeiss 982-DSM (Zeiss) para la de South Wilton de 1988 **[Artículo I]**.

La realización de inventarios de diatomeas según el protocolo de la norma estándar europea (European Standard EN 14407, 2004) consiste en contar en cada preparación un mínimo de cuatrocientas valvas que son identificadas hasta el menor nivel taxonómico posible (especie, subespecie, variedad, forma) **[Artículos II-V]**.

Para la identificación de los ejemplares en los recuentos se ha seguido la taxonomía y nomenclatura propuestas en las colecciones Susswasserflora von Mitteleuropa (Krammer & Lange-Bertalot, 1986-1991), Iconographia Diatomologica (Lange-Bertalot, 1995-2007) y Diatoms of Europe (Lange-Bertalot, 2000-2002), aplicando las últimas combinaciones disponibles y legítimas según el Código Internacional de Nomenclatura Botánica (McNeill *et al.*, 2006) **[Artículos II-V]**.

La terminología morfológica seguida para la descripción de las especies en el **artículo I** fue Hendey (1964), Anonymous (1975), Cox & Ross (1981) y Krammer & Lange-Bertalot (2000).

### *Protocolos muestreo de Físico-Química*

Las variables físico-químicas (Tabla 2) se midieron *in situ* o en el laboratorio siguiendo los protocolos estandarizados U.S. Geological Survey (Variously dated) **[Artículo I]**, APHA (1995) **[Artículo II]**, APHA (1998) **[Artículos III, IV y V]**.

La abundancia de macrófitos, expresada como el porcentaje de volumen infestado (PVI), se midió haciendo una estimación visual de la cobertura y la altura de las plantas a intervalos de 10 m a lo largo de transectos desde la orilla hasta el centro de la laguna. En los casos en los que la visibilidad no permitió este método, se tomaron muestras aleatorias con un rastrillo en cada punto **[Artículo IV y V]**.

Tabla 2. Variables ambientales medidas para cada estudio.

| Artículo | Variables |
| --- | --- |
| II, IV | $[O_2]$ |
| IV | $[O_2]$ % saturación |
| I, II, IV | $[NO_3^-]$ |
| II | $[NO_2^-]$ |
| II, III, IV | $[NH_4^+]$ |
| I, V | NT |
| II, III | NTK |
| I, II | $[PO_4^{3-}]$ |
| I, IV | Orto-P |
| II, III, IV, V | PT |
| II | DBO |
| I | $DBO_5$ |
| II | DQO |
| II | COD |
| IV | COT |
| IV, V | Chl a |
| I, IV, V | pH |
| I, II, IV, V | Conductividad |
| IV | Temperatura |
| II, IV | Turbidez |
| IV, V | Profundidad del disco Secchi |
| IV | Color |
| IV | PVI |
| IV, V | Profundidad media |
| IV, V | Área |
| IV, V | Altitud |

### Tratamientos estadísticos

Para evaluar si los taxones discriminados en base a la información obtenida a partir de MO y MEB [Artículo I], representaban especies bien definidas, se emplearon técnicas basadas en geometría morfométrica. Esta técnica permite la cuantificación de características morfológicas que se pueden usar como criterio taxonómico (Bookstein, 1991) debido a que los datos se pueden someter a análisis multimétricos para la detección de diferencias significativas entre grupos preestablecidos. Para el análisis de la forma de las valvas de diatomeas, los contornos individuales fueron modelados como una configuración geométrica de seis puntos de referencia (puntos claramente identificables en el grupo de especímenes investigados). La variación morfológica dentro de una muestra de configuraciones de puntos de referencia se describe en términos de parámetros que recogen estas variaciones y se pueden analizar mediante métodos de análisis multivariantes (Beszteri et al., 2005). En este estudio, se situaron seis puntos de referancia en ciertos puntos de la valva y se digitalizaron mediante el programa tpsDig2 (Rohlf, 2004). Los puntos se seleccionaron con el fin de cubrir la máxima variabilidad morfológica dentro del rango de los individuos estudiados. Se situaron cuatro puntos en posiciones fijas en los polos y en los puntos de máxima anchura de la valva. Para estudiar la longitud y la separación entre estrías, se situaron dos puntos más en los extremos interiores de de las estrías centrales. Las coordenadas cartesianas de los puntos de referencia se alinearon (se trasladaron, rotaron y reescalaron) mediante el análisis ortogonal generalizado de deformación por superposición de mínimos cuadrados (Rohlf & Slice, 1990). Se realizó un escalamiento multidimensional no métrico (NMDS) a partir de las coordenadas normalizadas resultantes con el programa PAST versión 1.78 (Hammer et al., 2001). Para visualizar el tamaño y la forma de la nube de puntos para cada grupo predefinido, se añadieron elipses de confianza al gráfico resultante con el programa PAST. Las elipses de confianza son áreas de las nubes de puntos que establecen intervalos de confianza (generalmente a $\alpha = 0,95$) que contienen todas las observaciones no extremas. Finalmente, se realizó un test no paramétrico de similitud (test de permutación ANOSIM, Clarke, 1993) entre las coordenadas cartesianas de los

Tabla 3. Listado de índices autoecológicos y de diversidad.

| Abreviatura | Nombre | Referencia |
|---|---|---|
| %PT | % de taxones tolerantes a la contaminación | Kelly & Whitton, 1995 |
| IDAP | Índice Diatomológico de Artois-Picardie | Prygiel *et al.*, 1996 |
| IBD | Índice Biológico de Diatomeas | Lenoir & Coste, 1996 |
| CEE | Índice de la Comunidad Económica Europea | Descy & Coste, 1991 |
| DESCY | Índice de Descy | Descy, 1979 |
| DI_CH | Índice de Hurlimann | Hurlimann & Niederhauser, 2002 |
| EPI-D | Índice de Eutrofización- Polución | Dell'Uomo, 2004 |
| Eq | Equitabilidad | - |
| IDG | Índice Genérico de Diatomeas | Rumeau & Coste, 1988 |
| Ha | Índice de diversidad de Shannon | Shannon & Weaver, 1949 |
| ILM | Índice de Leclerq & Maquet | Leclerq & Maquet, 1987 |
| LOBO | Índice de Lobo | Lobo *et al.*, 2002 |
| IDP | Índice de Diatomeas de la Pampa | Gómez & Licursi, 2001 |
| S | Riqueza de especies | - |
| SHE | Índice de Steinberg & Schiefele | Steinberg & Schiefele, 1988 |
| SID | Índice Sapróbico de Rott | Rott *et al.*, 1997 |
| SLA | Índice de Sládeček | Sládeček, 1986 |
| IPS | Índice de Poluosensibilidad Específica | Coste en Cemagref, 1982 |
| IDT | Índice Trófico de Diatomeas | Kelly & Whitton, 1995 |
| TID | Índice Trófico de Rott | Rott *et al.*, 1997 |
| DAIpo | Índice de comunidades de diatomeas | Watanabe *et al.*, 1988 |

grupos resultantes del NMDS, tomando la distancia Euclídea como medida de similitud. Para el análisis se utilizaron 100 fotografías de MO del material tipo de Fragilaria construens var. subsalina, y 35 de individuos pertenecientes a las poblaciones de España y EEUU.

Para evaluar la aplicabilidad de los índices de diversidad de diatomeas en bioindicación, a partir de los inventarios florísticos obtenidos [Artículo II] se calcularon índices comunes de diversidad (Tabla 3) con el programa PAST versión 1.66 (Hammer et al., 2001) y se correlacionaron con variables ambientales (Tabla 2) calculando coeficientes de correlación mediante el test no paramétrico de correlación de Spearman ($\rho S$). Adicionalmente, el uso

de tratamientos estadísticos ordinales permite la detección de correlaciones entre variables independientemente de la linearidad de sus relaciones, ya que pueden presentar respuestas logarítmicas (Blanco et al., 2007). Se construyeron diagramas de frecuencia-abundancia, rango-abundancia y frecuencia de distribución siguiendo las especificaciones de Magurran (2004). Los datos se ajustaron a las curvas optimizadas mediante el algoritmo de regresión no linear de Levenberg-Marquardt (Moré, 1977) y se analizaron las diferencias significativas entre las líneas de regresión resultantes mediante el análisis no paramétrico de varianza de una vía de Kruskal-Wallis. Se verificó la bondad de ajuste mediante tests $\chi 2$. Todos los análisis estadísticos se hicieron

con el programa estadístico STATISTICA v. 8.0 software (StatSoft, Inc., 2007).

Los inventarios florísticos obtenidos [Artículos III, IV y V] tras los recuentos de diatomeas al MO se han tratado con el programa informático OMNIDIA versión 5.1 (Lecointe et al., 1993, 1999) que calcula 18 índices diatomológicos (Tabla 3) cuyos valores están relacionados con el estado ecológico de las masas de agua. Para poder comparar los datos con mayor facilidad, utiliza la misma escala para la mayoría de ellos, estando los valores comprendidos entre 1 y 20 (Tabla 4) para facilitar la interpretación de los datos. Además, el programa calcula tres índices de diversidad (Tabla 3). Muchos de los índices diatomológicos (IDAP, EPI-D, IBD, SHE, IPS, DESCY, ILM, IDG e IDP) se basan en la fórmula de Zelinka & Marvan (1961), donde Aj es la abundancia relativa del taxón j, Sj, el valor de sensibilidad a la contaminación del taxón j y Vj, el valor indicador o grado de estenoecia del taxón j.

$$ID = \Sigma\,(A_i \cdot S_i \cdot V_i)\,/\,(\Sigma\,A_i \cdot V_i)$$

Las diferencias principales entre los diferentes índices radican en la precisión taxonómica que requieren, el número de taxones incluidos en su cálculo y en los valores autoecológicos considerados. El IDG utiliza taxonomía a nivel de género e incluye todos los géneros de agua dulce. El IDAP considera 91 especies y 45 géneros. El resto, requieren determinación a nivel específico y se diferencian principalmente en el número de especies implicadas en

Tabla 4. Clases de calidad propuesta para la DMA.

| CALIDAD | RANGO |
|---|---|
| Mala | 1 − 5 |
| Pobre | 5 − 9 |
| Moderada | 9 − 13 |
| Buena | 13 − 17 |
| Excelente | 17 − 20 |

el cálculo (IBD: 209, DESCY: 106, ILM: 210, SLA: 323, IPS: todas las especies conocidas). El índice CEE se calcula a partir de una tabla de doble entrada con valores autoecológicos de 208 especies comunes. El %PT funciona como un suplemento del IDT (indicador del efecto de los nutrientes inorgánicos) e indica la proporción de la muestra compuesta por taxones tolerantes a la contaminación orgánica.

Con el fin de evaluar las relaciones existentes entre las variables físico-químicas medidas (Tabla 2) y los índices calculados (Tabla 3) se calcularon los coeficientes de correlación de Spearman [Artículos III y IV].

Para determinar qué índice diatomológico se adecúa mejor a la zona de estudio [Artículo IV] se calculó el índice de correlación CI (Blanco et al., 2007) entre los 18 índices diatomológicos y las 18 variables ambientales. La fórmula aplicada fue:

$$CI = [\Sigma\,(\rho_S{}^2 \cdot S)] \cdot n^{-2}$$

donde $\rho_S$ es el coeficiente de correlación de Spearman; S es el número de correlaciones estadísticamente significativas (α = 0,95) y n es el número de variables estudiadas. CI oscila entre 0 y 1, indicando el mínimo y el máximo teórico de las relaciones entre las variables.

Las lagunas del **Artículo IV** se clasificaron en tres niveles tróficos (Bajo: < 60, Medio: 60-140, Alto: > 140 µg TP L⁻¹) en función de la concentración media en verano de fósforo total (PT), tomando como base la clasificación para lagos someros de Moss *et al.* (2003).

Con el fin de identificar las diferencias significativas entre los valores resultantes de los índices diatomológicos para las diferentes especies de plantas hospedadoras, se realizó un ANOVA no paramétrico de una vía (Kruskal-Wallis) **[Artículo III]** y una ANOVA de dos vías **[Artículo IV]**. Además, el

análisis de varianza de una vía (Kruskal-Wallis) se utilizó a su vez para buscar diferencias en cuanto a la calidad ecológica entre lagunas **[Artículo III]** y la ANOVA de dos vías para comprobar la capacidad de los índices de diatomeas para diferenciar los niveles tróficos establecidos **[Artículo IV]**. A su vez, para analizar la influencia del macrófito hospedador sobre la composición de la comunidad de diatomeas asociada, se realizó un ANOSIM, a partir de datos de abundancia relativa de diatomeas **[Artículos III y IV]**. Este último test se utilizó también para evaluar las diferencias a nivel de comunidad entre los niveles tróficos establecidos **[Artículo IV]**. Como complemento a estos análisis sobre la composición de las comunidades se realizó un análisis de coordenadas principales (PCO) a partir de las abundancias relativas de los taxones identificados **[Artículo IV]**. Los supuestos necesarios para realizar la ANOVA se verificaron mediante los test de Kolmogorov y Levene.

Los test de correlación de Spearman [Artículos III y IV], el análisis de varianza de una vía (Kruskal-Wallis) [Artículo III] y la ANOVA de dos vías [Artículo IV] se realizaron con el programa STATISTICA (StatSoft, Inc., 2007). El test de permutación ANOSIM con el programa Community Analysis Package version 3.11 (Pisces Conservation Ltd, 2004) [Artículos III y IV], y el PCO con el PAST versión 1.78 (Hammer et al., 2001) [Artículo IV].

En el Artículo V se hizo una clasificación trófica de las lagunas en cinco niveles en función de su concentración de PT (Stenger-Kovacs et al., 2007), por medio del algoritmo "k-means" (Hartigan & Wong, 1979). Los factores abióticos se clasificaron en 3 grupos: químicos (pH, conductividad, profundidad del disco de Secchi, PT, NT y clorofila a), morfométricos (área y profundidad media) y geográficos (latitud y altitud).

Se estudiaron las respuestas de las comunidades de diatomeas a estos grupos mediante un análisis multivariante de distancia para un modelo linear usando selección aditiva (DISTLM, Anderson, 2003). Este análisis realiza una regresión multivariante múltiple en base a una medida de distancia dada, que resulta en una selección aditiva de los conjuntos de variables predictivas con tests de permutaciones aleatorias (McArdle & Anderson, 2001). La medida de distancia usada para el DISTLM fue la distancia de Manhattan, por ser una función de valores absolutos de distancia y robusta frente a valores extremos (Lear & Lewis, 2009). Se realizó también un análisis de correspondencias canónicas (CCA) y un análisis de regresión paso a paso (SRA) para explorar la dependencia de las abundancias relativas de las diatomeas sobre las características químicas del agua. Para evaluar las diferencias significativas entre los grupos de las lagunas y la ordenación resultante del CCA, se realizó un test de similitud (ANOSIM). Los análisis estadísticos se hicieron con los programas STATISTICA versión 8.0 (StatSoft, Inc., 2007) y PAST versión 2.04 (Hammer et al., 2001).

En los casos en los que las variables medidas no siguieron distribuciones normales (test de Kolmogorov-Smirnov, $p < 0,05$), se aplicaron análisis estadísticos no paramétricos [Artículos II y III].

## 4. DISCUSIÓN GENERAL

### Apuntes taxonómicos

El género *Pseudostaurosira*, originalmente descrito por Williams & Round (1987), se caracteriza por presentar un número reducido de areolas por estría, esternón ancho, velo con *volae* normalmente ramificado, ausencia de rimopórtula y campo apical de poros reducido o ausente (Round *et al.*, 1990). La presencia y posición de las espinas es variable dentro del género. La mayoría de los taxones tiene las espinas situadas en los *vimines*, aunque en algunos otros se sitúan en los *virgae* (e.g. *P. pseudoconstruens*) o están ausentes (e.g. *P. parasitica* o *P. microstriata*). En *P. laucensis* las espinas están situadas tanto en los *vimines* como en los *virgae*, formando una línea continua a lo largo del borde de la cara valvar.

En base a las características arriba mencionadas, los tres taxones analizados en este estudio se ajustan al género *Pseudostaurosira*. En los tres taxones las espinas están situadas en los *vimines*. A diferencia de muchos taxones pertenecientes a *Pseudostaurosira*, *P. americana* tiene el esternón estrecho. La disminución en tamaño de las areolas hacia el área axial de la valva es menos pronunciada en *P. americana* que en *P. alvareziae*. Este carácter está presente en muchos otros representantes de *Pseudostaurosira*, aunque su variabilidad aun no ha sido analizada en profundidad. Las proyecciones redondeadas y aplanadas que cubren la areola (presentes en *P. americana* y *P. alvareziae*) son cada vez más frecuentes en representantes de *Pseudostaurosira* (Morales, 2001; Morales & Edlund, 2003; Siver *et al.*, 2005). Las espinas serradas están presentes en *P. trainorii* (Morales, 2001), un taxón ampliamente extendido en Norte América, y aparentemente también presente en Europa (E.A. Morales, obs. pers.). La frecuencia de

espinas serradas en *Pseudostaurosira* aún se desconoce y son necesarios más estudios para determinar si es un carácter ampliamente extendido.

Desde su descripción, *Fragilaria construens* var. *subsalina* ha sido sometida a varios cambios nomenclaturales no basados en observaciones de MEB. Sin embargo, algunos autores han presentado fotografías de MEB de taxones mal identificados como *Fragilaria construens* var. *subsalina* (e.g. Fabri & Leclercq, 1984; Vyverman, 1991; Witak, 2001), ya que las espinas estaban situadas en los *virgae* y no en los *vimines* como en el material lectotípico.

*P. alvareziae* se parece a *P. subsalina* en muchas características como la posición y forma de las espinas y las placas del manto, la densidad de estrías, el número de areolas por estría y la forma y orientación de los *volae*. Sin embargo, ambas especies difieren en varios caracteres: a) en *P. alvareziae* las valvas son más pequeñas y estrechas que en *P. subsalina*; b) los ápices son subrostrados en *P. subsalina* y ampliamente redondeados en *P. alvareziae*; c) el esternón en *P. subsalina* es lanceolado y más ancho, mientras que en *P. alvareziae* es más estrecho y lanceolado, pudiendo ser a veces linear o irregular; y d) la disminución hacia el área axial en el diámetro areolar es gradual en *P. alvareziae* y más abrupto en *P. subsalina*. A pesar de estas diferencias diagnósticas, probablemente se ha confundido a menudo *P. alvareziae* con *P. subsalina* (e.g. Blanco *et al.*, 2008).

*P. americana* fue inicialmente incorrectamente identificada como *P. subsalina* en Morales (2005), basándose en la similitud con los especímenes del material lectotípico de *P. subsalina*

ilustrado en Simonsen (1987) y Krammer & Lange-Bertalot (1991). En este estudio, se presentan imágenes adicionales de MEB de la población tipo (Figs 112-115 en el artículo I) y fotografías de MO de especímenes de una muestra adicional de la localidad tipo recogida seis años después (Figs 74-93 en el artículo I).

Los individuos más grandes de *P. americana* se parecen a *P. subsalina*. Sin embargo, *P. subsalina* nunca tiene un verdadero esternón linear y tiene menor densidad de estrías. *P. americana* se diferencia de *P. alvareziae* por tener ápices cuneados y esternón linear. Además, *P. americana* tiene mayor densidad de estrías (16-18 en 10 μm comparada con 13-15 en 10 μm de *P. alvareziae*).

Los resultados del test ANOSIM mostraron que no existen diferencias significativas entre *P. subsalina* sensu Morales 2005 y *P. americana*. El tamaño relativo de las elipses de confianza en la ordenación multivariante, da una idea de la amplitud morfológica de los taxones, que es bastante mayor en el caso del material tipo analizado de *F. construens* var. *subsalina*.

El estudio del material tipo de especies que pueden ser confundidas fácilmente en MO es de crucial importancia dado que las diatomeas se usan de forma rutinaria en el monitoreo de la calidad del agua. La identificación equivocada de especies pequeñas, poco conocidas pero, a menudo, bastante comunes, puede derivar en evaluaciones de calidad del agua imprecisas. Esto es especialmente relevante cuando las especies involucradas tienen perfiles autoecológicos muy diferentes, como la especie salobre *P. subsalina* que puede confundirse fácilmente con *P. alvareziae* (Blanco *et al.*, 2008) o *P. americana* (Morales, 2005, como *P. subsalina*), taxones que se encuentran en aguas con bajo y moderado contenido

en electrolitos, respectivamente. Por lo tanto, es importante revisar el material tipo de especies que se tipificaron por medio de dibujos como es el caso, por ejemplo, de *P. elliptica* (Edlund *et al.*, 2006), *P. perminuta* (Grunow) Sabbe & Vyverman (Sabbe & Vyverman, 1995) y *P. subsalina* **[Artículo I]**.

## Elección de la métrica más adecuada en bioindicación.

Dada la variedad de métricas utilizadas en el campo de la bioindicación (Ector & Rimet, 2005), es importante determinar la validez de éstas a la hora de aplicarlas en diferentes ecosistemas y a diferentes comunidades de organismos. Una parte del estudio analiza la validez de las medidas de diversidad frente a los índices diatomológicos **[Artículo II]**. Por otra parte, dentro del amplio espectro de índices basados en la autoecología de las diatomeas, se han identificado los índices más adecuados para cada estudio **[Artículos III y IV]**.

### *Aplicabilidad de los índices de diversidad*

Las medidas de diversidad se han utilizado tanto para estudiar la estructura de las comunidades de organismos presentes en un ecosistema, como para evaluar los cambios en los factores abióticos que afectan al ecosistema. Nuestros resultados **[Artículo II]** sugieren que los índices de diversidad difieren en su sensibilidad al aumento en las concentraciones de nutrientes, a pesar de que ninguno de los índices estudiados presentó relaciones claras y consistentes con las variables limnológicas indicadoras de eutrofización. Entre estos métodos, los más usados son el índice de Shannon y la riqueza de especies, los cuales parecen explicar gran parte de la estructura de las muestras biológicas. Los resultados indican que estas variables presentan una respuesta al aumento de las concentraciones de nutrientes en forma de curva unimodal, cuyo máximo se

encuentra a niveles de PT relativamente altos. Numerosos trabajos, entre los que se incluyen varias investigaciones sobre comunidades de diatomeas, se han enfocado a estudiar la relación entre diversidad y fertilidad. Los enfoques clásicos adoptan un patrón de baja diversidad en aguas enriquecidas, con índices de diversidad correlacionados significativamente con los nutrientes (Archibald, 1972; Patrick, 1973). Moss (1973) sugirió que las especies mejor adaptadas al enriquecimiento en nutrientes presentarían una situación ventajosa bajo condiciones eutróficas, resultando en una distribución irregular de los individuos entre los diferentes taxones. Sin embargo, las relaciones positivas entre diversidad y fertilidad son frecuentes en la literatura. Vilbaste & Truu (2003) y Lavoie *et al.* (2008) encontraron que a medida que aumenta la eutrofia del agua, el índice de Shannon tiende también a aumentar. Marcus (1980), Maznah & Mansor (2002) o Bergfur (2007) encontraron resultados análogos. Por otra parte, la hipótesis de perturbación intermedia plantea que la diversidad presenta su máximo bajo una intensidad de perturbación intermedia (Connell, 1978). Esta hipótesis se ha confirmado en muchos estudios de campo sobre diatomeas bentónicas (Lobo *et al,.* 1995; Biggs & Smith, 2002). Esto concuerda con la actual idea generalizada de que la productividad determina la máxima riqueza específica. Según Cox *et al.* (2006), para un ecosistema dado, hay un máximo que sitúa el límite superior de coexistencia de especies a lo largo del gradiente trófico. Sin embargo, Molis (2002) plantea que estas respuestas unimodales dependen del indicador de perturbación elegido y del parámetro de diversidad. Además, no son persistentes en sucesión, debido a que la diversidad llega a su máximo rápidamente durante la colonización (Oemke & Burton, 1986). Chase & Leibold (2002) y Harrison *et al.* (2006) propusieron que la relación entre productividad y diversidad de especies

es habitualmente positiva a escalas regionales, pero a menudo, unimodal, negativa o inexistente a escalas espaciales locales. Sin embargo, Gillman & Wright (2006), en una revisión bibliográfica exhaustiva, sólo confirmaron relaciones positivas tanto en estudios que usaron datos de extensión global o continental como en la mayoría de los estudios de extensión regional o local. Soininen (2009) ha observado recientemente en diatomeas de arroyos que la relación diversidad-productividad muestra una gran variabilidad entre diferentes escalas espaciales, sugiriendo la presencia de otras variables a parte de los nutrientes, como factores determinantes de la diversidad de diatomeas. Además, las limitaciones históricas y biogeográficas pueden explicar estos patrones mejor que las condiciones ambientales actuales (Vyverman *et al.*, 2007). Esta compleja variabilidad puede dar cuenta, hasta cierto punto, del hallazgo habitual de correlaciones no significativas o débiles, entre parámetros de diversidad y productividad en estudios diatomológicos (Wu & Kow, 2002; Bellinger *et al.*, 2006; Simkhada *et al.*, 2006).

Las técnicas tradicionales de regresión son insuficientes para caracterizar las relaciones productividad-diversidad (Cox *et al.*, 2006), por lo que el estudio se ha complementado con análisis gráficos de frecuencia/abundancia de taxones de diatomeas a lo largo del gradiente de PT, lo que ha dado resultados contradictorios. Las curvas de acumulación de especies sugirieron una correlación negativa entre la riqueza de especies y las concentraciones de PT, mientras que el diagrama de rango/abundancia se adecúa mejor con una respuesta unimodal de productividad-diversidad.

Una cuestión importante abordada en este estudio, es la dependencia de la frecuencia de ocurrencia sobre el estado trófico. Los datos muestran que

la pendiente de la relación ocurrencia/abundancia es significativamente menor en comunidades que viven en aguas con concentraciones de PT por debajo de 100 µg·L⁻¹. Las relaciones positivas entre abundancia y ocupación son uno de los patrones más generalizados en ecología, pero rara vez se han relacionado con factores ambientales. Se ha demostrado que la ocupación regional está relacionada positivamente con la amplitud del nicho (Heino & Soininen, 2006), por lo que nuestros datos sugerirían amplitudes ecológicas más estrechas en las especies indicadoras de niveles tróficos bajos, una hipótesis que requiere ser más investigada.

### *Índices autoecológicos de diatomeas*

Tras estudiar la adecuación de los índices autoecológicos a cada ecosistema, se comprobó que los índices que obtuvieron mejores correlaciones con las variables ambientales fueron SID para las lagunas Arkaute y Betoño **[Artículo III]** y CEE, IPS e IBD para las lagunas de la depresión del Duero **[Artículo IV]**. En la cuenca del Duero, el IPS obtiene mejores resultados en estudios de evaluación ecológica en ríos (Blanco *et al.*, 2007) mientras que nuestros resultados muestran que es el CEE el más adecuado en el caso de las lagunas. Las principales diferencias entre los índices autoecológicos radican en el espectro florístico (la lista de especies junto con sus valores autoecológicos) considerado por cada método. Por lo tanto, se pueden encontrar resultados opuestos aplicando diferentes índices al mismo conjunto de datos (Blanco *et al.*, 2007; Feio *et al.*, 2009; Martín *et al.*, 2010). En general, la correlación entre los índices de diatomeas y las variables ambientales depende del porcentaje de individuos considerado por cada índice (Blanco *et al.*, 2007).

### *Evaluación del estado trófico*

El estudio sobre la capacidad de diagnosis de los diferentes índices autoecológicos reflejó que ninguno de los índices autoecológicos **[Artículo IV]** diferenció entre los niveles de fósforo Bajo-Medio, pero sí entre Bajo-Alto. La interpretación de los datos sobre niveles tróficos depende, en gran medida, del modelo de clasificación utilizado. Por ejemplo, un lago profundo con una concentración de PT de 30 µg L⁻¹, se consideraría mesotrófico mientras que un lago somero con la misma concentración de PT se clasificaría como ultraoligotrófico. Por este motivo, nuestra clasificación está basada en la de Moss *et al.* (2003) para lagos someros. El CEE **[Artículo IV]** se correlacionó ligeramente mejor con las variables ambientales que el IPS, probablemente debido a que los valores autoecológicos (S y V) asignados a cada taxón son más precisos en su base de datos. El índice SID **[Artículo III]** sólo se correlacionó significativamente con las variables químicas cuando el macrófito muestreado fue *Iris pseudacorus*, a pesar de que los resultados del test de Kruskal-Wallis demostraron que no hay diferencias significativas con respecto a los valores de los índices entre plantas.

Al comparan la influencia sobre las comunidades de diatomeas epífitas de tres grupos de variables (químicas, morfométricas y geográficas) **[Artículo V]** los resultados evidenciaron que las comunidades de diatomeas epífitas responden principalmente a las variables químicas (clorofila *a*, conductividad y PT). Además, el CCA demostró la concordancia existente entre la clasificación biológica de las lagunas basada en diatomeas epífitas y la clasificación basada en los niveles de PT. Diversos autores han encontrado que las concentraciones de nutrientes son las variables principales que determinan la composición y estructura de las comunidades de diatomeas epífitas (Pan *et al.*, 2000; Della Bella *et al.*, 2007), aunque en lagos ácidos la relación entre las comunidades de epíftas y la concentración de PT puede ser menor (Denys, 2007).

Por otra parte, factores como la latitud y la altitud pueden determinar las características de las lagunas modificando la respuesta de las comunidades epífitas frente al estado trófico. En este estudio, la calidad del agua de las lagunas parece estar menos influenciada por los nutrientes en latitudes altas que en latitudes más bajas. Las inferencias ambientales a partir de epífitas, pueden estar sujetas a variaciones espaciales (Denys, 2007). Sin embargo, diversos estudios (Donohue *et al.*, 2009; McElarney & Rippey, 2009) encontraron poca variación en comunidades biológicas de agua dulce, siendo más importantes los efectos locales que las características a gran escala. Todo esto sugiere que las diatomeas pueden ser utilizadas como bioindicadores en un rango muy amplio de condiciones morfométricas y localizaciones geográficas.

### *Hipótesis del sustrato neutro.*

Esta hipótesis supone que la comunidad perifítica asociada a un sustrato, no se ve afectada por la naturaleza del mismo. Muchos estudios han analizado la influencia del sustrato en la calidad del agua inferida a partir del análisis de comunidades perifíticas (Cattaneo & Amireault, 1992; Poulícková *et al.*, 2004). Por ejemplo, en un estudio de calidad en el río Plaine (Francia), Besse-Lototskaya *et al.* (2006) observaron que 8 de las 17 métricas estudiadas (incluyendo CEE) variaron dependiendo del tipo de sustrato muestreado (macrófitos, piedras o sedimento) y encontraron que el sustrato más adecuado para el CEE fueron los macrófitos, sin embargo, el IPS y el IBD no resultaron afectados por la elección del sustrato. Poulícková *et al.* (2004) compararon comunidades de diatomeas desarrolladas sobre piedras, sedimento y tallos de juncos y encontraron que la composición específica difiere entre sustratos y que la evaluación del estado trófico se ve afectada por estas diferencias. Potapova & Charles (2005) encontraron menor riqueza específica,

diversidad y biovolumen de diatomeas en sustratos duros (roca y madera) que en sedimentos (arena y limo), sin embargo, la relación entre estos atributos y los factores ambientales fue similar independientemente del tipo de sustrato. En nuestro estudio **[Artículos III y IV]** la capacidad de diagnóstico de los índices CEE, IPS y SID no se vio afectada por la identidad del macrófito hospedador, mientras que los valores del IBD difirieron entre especies. Los resultados obtenidos por Eminson & Moss (1980) sugieren que las diferencias encontradas en las comunidades de epifiton están más influenciadas por las variaciones en el ambiente que por el macrófito hospedador. En la misma línea, Caput & Plenković-Moraj (2000) concluyen que la similitud entre las comunidades diatomológicas desarrolladas en tallos vivos y muertos de *Cladium mariscus* Phol. se debe probablemente a la uniformidad de las condiciones físico-químicas. Sin embargo, hemos observado **[Artículo III]** que la relación entre comunidades de diatomeas epífitas y las variables limnológicas puede ser más débil en algunas plantas como *Carex riparia* y *Veronica anagallis-aquatica*.

Los análisis de similitud relacionados con la abundancia y composición específica de las comunidades de diatomeas a lo largo de los niveles tróficos **[Artículo IV]** confirman que las comunidades epífitas reflejan cambios estructurales relacionados con el ambiente, proporcionando así, una base para su implementación como bioindicadores en lagos someros. Se han encontrado diferencias análogas comparando diferentes plantas sustrato. Las diferencias estructurales en las comunidades perifíticas desarrolladas sobre diferentes sustratos han sido estudiadas por diversos autores. Kitner & Poulícková (2003) observaron diferencias significativas en la riqueza específica de diatomeas desarrolladas en distintos sustratos (epiliton, epipelon y epifiton).

Por el contrario, Winter & Duthie (2000) no encontraron diferencias estructurales consistentes en comunidades de diatomeas epilíticas, epipélicas y epífitas de ríos, a pesar de que algunos taxones fueron más abundantes en ciertos hábitats. Yallop *et al.* (2009) tampoco encontraron diferencias significativas en la evaluación del estado trófico (TDI), la riqueza de especies, la diversidad o la abundancia relativa de taxones mótiles entre comunidades de diatomeas epilíticas y epífitas.

En nuestro estudio, hemos encontrado diferencias significativas en la composición específica de las comunidades de diatomeas desarrolladas en *Scirpus lacustris*, *Typha latifolia* y *Myriophyllum alterniflorum* **[Artículo IV]** pero no entre las desarrolladas en *Carex riparia*, *Iris pseudacorus* y *Veronica anagallis-aquatica* **[Artículo III]**.

Las diferencias a nivel de comunidad han sido estudiadas también para distintos macrófitos con resultados diferentes. Por ejemplo, Townsend & Gell (2005) encontraron que las comunidades eran similares entre *Juncus* y *Chara* y, sin embargo, las comunidades desarrolladas en *Vallisneria* estaban empobrecidas en especies. Las diferencias intraespecíficas en el epifiton macrofítico pueden afectar también a parámetros funcionales como la biomasa algal o el contenido de Clorofila *a* (Laugaste & Reunanen, 2005). Tales diferencias pueden no estar relacionadas con la similitud morfológica entre plantas, por ejemplo, Blindow (1987) encontró diferencias mayores, con respecto a la composición del epifiton, entre *Chara globularis* Thuill. y *Chara tormentosa* L. que entre esta última y *Nitellopsis obtusa* (Desv.) J. Groves. Además, encontró diferencias en la densidad del epifiton desarrollado sobre distitntos macrófitos, con algunas especies de diatomeas más abundantes en ciertas plantas (e.g. *Cocconeis placentula* Ehrenberg

presentó gran afinidad por *Potamogeton pectinatus* L.). Esta preferencia por un sustrato concreto ha quedado recogida en muchos artículos (e.g. Caput & Plenković-Moraj, 2000; Townsend & Gell, 2005; Messyasz & Kuczyńska-Kippen, 2006). En este estudio **[Artículo IV]** se han encontrado este tipo de preferencias (e.g. *Gomphonema parvulum* (Kützing) Kützing y *Halamphora veneta* (Kützing) Z. Levkovpor *T. latifolia* L., *Encyonopsis subminuta* Krammer & Reichardt por *M. alterniflorum* DC. in Lam. & DC., o *Cocconeis euglypta* Ehrenberg que parece desarrollarse mejor sobre *S. lacustris* L.), junto con una mayor riqueza específica en las comunidades desarrolladas sobre *M. alterniflorum*. Se han considerado varias hipótesis alternativas para explicar las diferencias estructurales del epifiton entre macrófitos hospedadores, por ejemplo, factores estocásticos asociados con la colonización de las diatomeas (Townsend & Gell, 2005) o la liberación de sustancias alelopáticas por parte de los macrófitos (Gross, 2003), a pesar de que el epifiton es menos sensible a estas sustancias que el fitoplancton (Hilt, 2006).

Los datos mostrados en este estudio indican que los índices más adecuados para la evaluación de la calidad ecológica fueron SID, para el humedal de Salburua **[Artículo III]** y CEE para las lagunas de la depresión del Duero **[Artículo IV]**. A pesar de que el CEE no discriminó adecuadamente entre los niveles Bajo- Medio, fue el mejor correlacionado y discrimina adecuadamente entre diferentes niveles de calidad independientemente de la planta sustrato, con lo que se podría aplicar a una gran variedad de lagos someros independientemente de su vegetación.

Las comunidades de diatomeas responden, principalmente, a las condiciones físico-químicas del agua, por encima de factores fisiográficos

o geográficos, por lo tanto, queda demostrada su universalidad como bioindicadores en sistemas leníticos, independientemente de la planta sustrato, la profundidad y extensión del lago y su ubicación geográfica.

# 5. CLONCLUSIONES

1. *P. alvareziae* queda definida como una especie diferenciada de *P. subsalina* por su menor tamaño, por presentar ápices ampliamente redondeados, esternón más estrecho y una disminución hacia el área axial en el diámetro areolar más gradual que en *P. subsalina*.

2. *P. americana* queda definida como una nueva especie diferenciada de *P. subsalina* por presentar esternón linear y mayor densidad de estrías. Los índices de diversidad de diatomeas no son adecuados para inferir los niveles de PT en ríos. Estudios posteriores con datos adicionales de diferentes regiones permitirán evaluar la utilidad de estas métricas como indicadores de calidad del agua a escalas espaciales y temporales diferentes.

3. La correlación significativa entre el índice SID y las variables químicas prueba la efectividad de las comunidades de diatomeas epífitas como indicadores ecológicos potenciales en sistemas leníticos de acuerdo a las pautas establecidas en la DMA.

4. El índice CEE discrimina adecuadamente entre diferentes niveles de calidad independientemente de la planta sustrato, con lo que se podría aplicar a una gran variedad de lagos someros independientemente de su vegetación.

5. La composición de las comunidades de diatomeas epífitas varió en función del macrófito hospedador y el nivel trófico de la laguna. Sin embargo, la inferencia de la calidad del agua no se vio afectada por el macrófito hospedador muestreado. Por lo tanto, las diatomeas epífitas se pueden usar como bioindicadores del estado trófico en una amplia variedad de sistemas lacustres independientemente de la composición específica de la comunidad de macrófitos presente. Las comunidades de diatomeas epífitas responden principalmente a variables químicas, pudiendo ser utilizadas en bioindicación bajo un amplio rango de condiciones morfométricas y ubicaciones geográficas.

## 6. ABSTRACT

This work is based on five research papers concerning different aspects of bioindication from periphytic diatom assemblages growing on both lotic and lenthic systems.

The taxonomy, ecology and systematic position of *Fragilaria construens* var. s*ubsalina* has been clarified and two morfologically related taxa are here presented as two new species to science (*Pseudostaurosira alvareziae* and *Psedostaurosira americana*). To that end, the type material of the three taxa have been studied by means of light and scanning electron microscopy. A morphometric analysis of the valve shape supports the morphological separation between the three species.

The reliability of diversity indices based on diatom communities as biomonitoring metrics was studied. For that purpose, common diversity indices were calculated from epilithic diatom communities from 640 stations spread over the Loire-Bretagne National Network (France) and compared against abiotic factors. Sampling stations were classified according to their trophic status (TP concentration). Several theoretical predictions about the relationship between community structural parameters and limnological variables were tested. Diversity indices exhibited poor linear correlations with environmental factors indicating ecological status. No clear patterns were found concerning species accumulation curves, occurrence-abundance, frequency-abundance and frequency distribution of diatom taxa between different trophic levels. Therefore, the use of diatom diversity indices in biomonitoring studies is discouraged.

The applicability in lenthic systems of diatom autoecological indices developed for lotic systems was also tested. To that

end, epiphytic diatom communities from two shallow lakes, Arkaute and Betoño, located in the Salburúa wetland and nineteen shallow lakes located in the depression of the Duero basin were studied. Correlation among indices an environmental variables were analyzed, obtaining better results for SID index in Arkaute and Betoño and for CEC, SPI and BDI in the shallow lakes from Duero depression. Furthermore, the influence of plant substrata on the structure of the diatom communities and on the water quality inferred from them was studied by means of variance, similarity and multivariate analysis. The diagnostic capacities of CEC, SPI and SID were unaffected by macrophyte species identity, whereas BDI values did differ among species. Significant differences were found in the specific composition of diatom assemblages developed on *Scirpus lacustris*, *Typha latifolia* and *Myriophyllum alterniflorum* but not on the ones developed on *Carex riparia*, *Iris pseudacorus* and *Veronica anagallis-aquatica*. Analysis of similarity relating to species abundance and composition of diatom assemblages throughout trophic levels confirm that epiphytic communities show environment-related structural changes, thereby providing a basis for their implementation as bioindicators for shallow lakes.

Finally, the effects of lake chemical, morphometric and geographical parameters on epiphytic diatom assemblages from 44 shallow lakes located in different European countries, covering a wide range of geographical regions and limnological conditions. Distance-based multivariate analysis, canonical correspondence analyses and stepwise regression analyses were performed. Results show higher influence of chemical variables than morphometric and geographical variables. This demonstrates the applicability of

epiphytic diatom communities in lenthic systems, on a wide range of morphometric conditions and geographical locations.

In conclusion, diatom communities respond mainly to physical and chemical variables, overriding the effects of morphometric and geographical factors. Thus, it has been demonstrated the universality of diatoms as bioindicators in lenthic systems, independently of plant substrata, lake depth, size and location.

## 7. RESUMEN

El presente trabajo está basado en cinco artículos de investigación sobre diferentes aspectos referentes a la bioindicación a partir de comunidades de diatomeas perifíticas desarrolladas tanto en sistemas lóticos como leníticos.

Se ha clarificado la taxonomía, ecología y posición sistemática de *Fragilaria construens* var. s*ubsalina* y de dos taxones morfológicamente similares presentados aquí como dos nuevas especies para la ciencia (*Pseudostaurosira alvareziae* y *Psedostaurosira americana*). Para ello, se ha estudiado el material tipo de los tres taxones mediante microscopía óptica y electrónica. Un análisis morfométrico de las valvas confirma la separación morfológica entre las tres especies.

Se ha estudiado la fiabilidad en bioindicación de los índices de diversidad basados en comunidades de diatomeas. Para ello se calcularon varios índices de diversidad a partir de comunidades de diatomeas epilíticas pertenecientes a 640 estaciones a lo largo de la cuenca del Loira-Bretaña, y se relacionaron con factores abióticos. Las estaciones se clasificaron en diferentes niveles tróficos de acuerdo a su concentración de PT. Se analizaron varias hipótesis teóricas relativas a la relación entre los parámetros estructurales de la comunidad y las variables limnológicas. Los índices de diversidad mostraron correlaciones lineares débiles con los factores ambientales indicadores del estado ecológico. Además, no se encontraron patrones claros en relación a las curvas de acumulación de especies, de ocurrencia-abundancia, de frecuencia-abundancia y frecuencia de distribución de los taxones de diatomeas entre los diferentes niveles tróficos. Por lo tanto, se desaconseja el uso de los índices de diversidad basados en diatomeas para estudios de bioindicación.

Se estudió también la aplicabilidad en sistemas leníticos de los índices autoecológicos basados en comunidades de diatomeas desarrollados para sistemas lóticos. Para ello se estudiaron las comunidades de diatomeas epífitas de las lagunas de Arkaute y Betoño, situadas en el humedal de Salburua, y de diecinueve lagunas localizadas en la depresión de la cuenca del Duero. Se analizaron las correlaciones entre los índices y las variables ambientales, obteniendo mejores resultados con el índice SID para las lagunas Arkaute y Betoño y con los índices CEE, IPS e IBD para las lagunas de la depresión del Duero. A su vez, se estudió la influencia de la planta-sustrato sobre la estructura de las comunidades de diatomeas y sobre la calidad del agua inferida a partir de éstas, mediante análisis de varianza, de similitud y análisis multimétricos. La capacidad de diagnóstico de los índices CEE, IPS y SID no se vio afectada por la identidad del macrófito hospedador, mientras que los valores del IBD difirieron entre especies. Se encontraron diferencias significativas en la composición específica de las comunidades de diatomeas desarrolladas en *Scirpus lacustris*, *Typha latifolia* y *Myriophyllum alterniflorum* pero no entre las desarrolladas en *Carex riparia*, *Iris pseudacorus* y *Veronica anagallis-aquatica*. Los análisis de similitud relacionados con la abundancia y composición específica de las comunidades de diatomeas a lo largo de los niveles tróficos confirman que las comunidades epífitas reflejan cambios estructurales relacionados con el ambiente, sentando así, las bases para su implementación como bioindicadores en lagos someros.

Por último, se ha evaluado la influencia de parámetros químicos, morfométricos y geográficos sobre las comunidades de diatomeas epífitas desarrolladas en 44 lagunas localizadas en diferentes países europeos, cubriendo un rango de condiciones geográficas y limnológicas muy amplio. Se realizaron

análisis multivariantes de distancia, de correspondencias canónicas, análisis de regresión paso a paso y test de similitud. Los resultados mostraron una mayor influencia de los factores químicos frente a los morfométricos y geográficos, lo que demuestra la aplicabilidad de las comunidades de diatomeas epífitas en sistemas leníticos, en un amplio rango de condiciones morfométricas y localizaciones geográficas.

En conclusión, las comunidades de diatomeas responden, principalmente, a las condiciones físico-químicas del agua, por encima de factores morfométricos o geográficos, por lo tanto, queda demostrada su universalidad como bioindicadores en sistemas leníticos, independientemente de la planta sustrato, la profundidad y extensión del lago y su ubicación geográfica.

## 8. REFERENCIAS

Anderson M.J. 2003. *DISTLM forward: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model using forward selection.* Department of Statistics, University of Auckland, New Zealand.

Anonymous. 1975. Proposals for the standardization of diatom terminology and diagnoses. *Nova Hedwigia*, Beiheft 53: 323-354.

APHA. 1995. *Standard methods for the examination of water and wastewater.* (19th ed.). American Water Works Association, Washington.

APHA 1998. *Standard methods for the examination of water and wastewater.* (20th ed.). American Water Works Association, Washington.

Archibald R.E.M. 1972. Diversity in some South African diatom associations and its relation to water quality. *Water Research*, 6: 1229-1238.

Bécares E., Conty A., Rodríguez-Villafañe C. & Blanco S. 2004. Funcionamiento de los lagos someros mediterráneos. *Ecosistemas*, 13 (2): 1-14.

Bellinger B., Cocquyt C. & O'Reilly C. 2006. Benthic diatoms as indicators of eutrophication in tropical streams. *Hydrobiologia*, 537: 75-87.

Bergfur J. 2007. Ecological Integrity of Boreal Streams. Assessing Impacts on Community Structure and Function. Ph.D. thesis. Swedish University of Agricultural Sciences, Uppsala. *Acta Universitatis Agriculturae Sueciae*, 50: 1-39. http://tinyurl.com/ygj87cb

Besse-Lototskaya A., Verdonschot P.F.M.

& Sinkeldam J.A. 2006. Uncertainly in diatom assessment: Sampling, identification and counting variation. *Hydrobiologia*, 566: 247-260.

Beszteri B., Ács É. & Medlin L. 2005. Conventional and geometric morphometric studies of valve ultrastructural variation in two closely related *Cyclotella* species (Bacillariophyta). *European Journal of Phycology*, 40: 89-103.

Biggs B.J.F. & Smith R.A. 2002. Taxonomic richness of stream benthic algae: Effects of flood disturbance and nutrients. *Limnology and Oceanography*, 47: 1175-1186.

Blanco S., Ector L. & Bécares E. 2004. Epiphytic diatoms as water quality indicators in Spanish shallow lakes. *Vie et Milieu - Life and Environment*, 54: 71-79.

Blanco S. & Bécares E. 2006. Método de muestreo de diatomeas epífitas en lagunas para la aplicación de la Directiva Marco del Agua. *Tecnología del Agua*, 277: 42-47.

Blanco S., Bécares E., Cauchie H.M., Hoffmann L. & Ector L. 2007. Comparison of biotic indices for water quality diagnosis in the Duero Basin (Spain). *Archiv für Hydrobiologie, Supplement Large Rivers*, 17: 267-286.

Blanco S., Ector L., Huck V., Monnier O., Cauchie H.M., Hoffmann L. & Bécares E. 2008. Diatom assemblages and water quality assessment in the Duero basin (NW Spain). *Belgian Journal of Botany*, 141: 39-50, S1-S6.

Blindow I. 1987. The composition and density of epiphyton on several species of submerged macrophytes.

The neutral hypothesis tested. *Aquatic Botany*, 29: 157-168.

Bonn A., Storch D. & Gaston K.J. 2004. Structure of the species-energy relationship. *Proceedings of the Royal Society of London Series B*, 271: 1685-1691.

Bookstein F.L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, New York.

Canning-Clode J., Kaufmann M., Molis M., Lenz M. & Wahl M. 2008. Influence of disturbance and nutrient enrichment on early successional fouling communities in an oligotrophic marine system. *Marine Ecology*, 29: 115-124.

Caput K. & Plenković-Moraj A. 2000. Epiphytic diatoms on sawgrass (*Cladium mariscus*) in the karstic Plitvice Lakes, Croatia. *Biologia, Bratislava*, 55: 343-350.

Cattaneo A. & Amireault M.C. 1992. How artificial is artificial substrata for periphyton? *Journal of the North American Benthological Society*, 11: 244-256.

Cattaneo A., Méthot G., Pinel-Alloul B., Niyonsenga T. & Lapierre L. 1995. Epiphyte size and taxonomy as biological indicators of ecological and toxicological factors in lake Saint-François (Québec). *Environmental Pollution*, 87: 357-372.

Cemagref. 1982. Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Q. E. Lyon-A. F. Bassin Rhône-Méditerranée-Corse.

Chase J.M. & Leibold M.A. 2002. Spatial scale dictates the productivity-diversity relationship. *Nature*, 416: 427-430.

Clarke K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18: 117-143.

Connell J.H. 1978. Diversity in tropical rain forests and coral reefs. *Science*, 199: 710-723.

Cox S.B., Bloch C.P., Stevens R.D. & Huenneke L.F. 2006. Productivity and species richness in an arid ecosystem: a long-term perspective. *Plant Ecology*, 186: 1-12.

Cox E.J. & Ross R. 1981. *The striae of pennate diatoms*. En: Proceedings of the Sixth Symposium on Recent and Fossil Diatoms (Ed. R. Ross), pp. 267-278. Koeltz, Koenigstein, Germany.

de la Rey P.A., van Rensburg L. & Vosloo A. 2008. On the use of diatom-based biological monitoring. Part 1: A comparison of the response of diversity and autoecological diatom indices to water quality variables in the Marico-Molopo River catchment. *Water SA*, 34: 53-60.

Della Bella V., Puccinelli C., Marcheggiani S., & Mancini L. 2007. Benthic diatom communities and their relationship to water chemistry in wetlands of central Italy. *Annales de Limnologie-International Journal of Limnology*, 43 (2): 88-89.

Dell'Uomo A. 2004. *L'Indice Diatomico de Eutrofizzacione/Polluzione (EPI-D) nel Monitoraggio delle Acque Correnti. Linee Guida*. APAT, Roma.

Denys L. 2007. Water-chemistry transfer functions for epiphytic diatoms in standing freshwaters and a comparison with models based on littoral sediment assemblages

(Flanders, Belgium). *Journal of Paleolimnology*, 38: 97-116.

Descy J.P. 1979. A new approach to water quality estimation using diatoms. *Nova Hedwigia*, 64: 305-323.

Descy J.P. & Coste M. 1991. A test of methods for assessing water quality based on diatoms. *Verhandlungen - Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 24: 2112-2116.

Donohue I., Donohue L.A., Ní Ainín B. & Irvine K. 2009. Assessment of eutrophication pressure on lakes using littoral invertebrates. *Hydrobiologia*, 633: 105-122.

Drake J.M., Lodge D.M. & Costello C. 2008. Reply to Ricciardi & MacIsaac. *Ecological Applications*, 18: 1323-1324.

Ector L. & Rimet F. 2005. *Using bioindicators to assess rivers in Europe: An overview*. En: Modelling community structure in freshwater ecosystems. (Eds S. Lek, M. Scardi, P.F.M. Verdonschot, J.P. Descy & Y.S. Park), pp. 7-19. Springer-Verlag, Berlin, Heidelberg.

Edlund M.B., Morales E.A. & Spaulding S.A. 2006. *The type and taxonomy of* Fragilaria elliptica *Schumann, a widely misconstrued taxon*. En: Proceedings of the Eighteenth International Diatom Symposium (Ed. A. Witkowski), pp. 53-59. Biopress Limited, Bristol.

Eminson D. & Moss B. 1980. The composition and ecology of periphyton communities in freshwaters. 1. The influence of host type and external environment on community composition. *British Phycological Journal*, 15: 429-446.

European Standard EN 13946. 2003. *Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*. European Committee for Standardization, Brussels.

European Standard EN 14407. 2004. *Water quality - Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters*. European Committee for Standardization, Brussels.

European Union. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities*, L327: 1–73.

Fabri R. & Leclercq L. 1984. Etude écologique des rivières du nord du massif Ardennais (Belgique): flore et végétation de diatomées et physico-chimie des eaux. 1. Contexte mésologique, méthodes, analyses physico-chimiques, synthèse taxonomique, écologique et floristique, iconographie, bibliographie. Univ. Liège, Station Sci. Hautes-Fagnes, Robertville.

Feio M.J., Almeida S.F.P., Craveiro S.C., & Calado A.J. 2009. A comparison between biotic indices and predictive models in stream water quality assessment based on benthic diatom communities. *Ecological Indicators*, 9: 497-507.

Fitzpatrick F.A., Waite I.R., d'Arconte P.J., Meador M.R., Mauplin M.A. & Gurtz M.E. 1998. *Revised methods for characterizing stream habitat in the National Water-Quality Assessment Program*. U.S. Geological Survey Water Resources Investigations

Report 98-4052, Raleigh.

Foody G.M. 2004. Spatial nonstationarity and scale-dependency in the relationship between species richness and environmental determinants for the sub-Saharan endemic avifauna. *Global Ecology and Biogeography*, 13: 315-320.

Gevrey M., Rimet F., Park Y.S., Giraudel J.L., Ector L. & Lek S. 2004. Water quality assessment using diatom assemblages and advanced modelling techniques. *Freshwater Biology*, 49: 208–220.

Gillman L.N. & Wright S.D. 2006. The influence of productivity on the species richness of plants: a critical assessment. *Ecology*, 87: 1234-1243.

Gómez N. & Licursi M. 2001. The Pampean Diatom Index (IDP) for Assessment of Rivers and Streams in Argentina. *Aquatic Ecology*, 35: 173-181.

Grace J.B., Anderson T.M., Smith M.D., Seabloom E., Andelman S.J., Meche G., Weiher E., Allain L.K., Jutila H., Sankaran M., Knops J., Ritchie M. & Willig M.R. 2007. Does species diversity limit productivity in natural grassland communities? *Ecology Letters*, 10: 680-689.

Gross E.M. 2003. Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences*, 22: 313-339.

Hammer Ø., Harper D.A.T. & Ryan P.D. 2001. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4: 1-9. http://folk.uio.no/ohammer/past/.

Harrison S., Safford H.D., Grace J.B., Viers J.H. & Davies F.K. 2006. Regional and local species richness in an insular environment: serpentine plants of California. *Ecological Monographs*, 76: 41-56.

Hartigan J.A., & Wong M.A. 1979. Algorithm AS 136: A k-means Clustering Algorithm. *Applied Statistics*, 28: 100-108.

Heino J. & Soininen J. 2006. Regional occupancy in unicellular eukaryotes: a reflection of niche breadth, habitat availability or size-related dispersal capacity? *Freshwater Biology*, 51: 672-685.

Hendey N.I. 1964. *An introductory account of the smaller algae of British coastal waters: V. Bacillariophyceae (diatoms).* Fishery Investigations Series IV. Her Majesty's Stationery Office, London.

Hilt S. 2006. Allelopathic inhibition of epiphytes by submerged macrophytes. *Aquatic Botany*, 85: 252-256.

Hürlimann J. & Niederhauser P. 2002. *Méthodes d'étude et d'appréciation de l'état de Santé des Cours d'eau : Diatomées – Niveau R (Région).* Office fédéral de l'environnement, des forêts et du paysage, Berne.

Kahlert M., Albert R.-L., Anttila E.-L., Bengtsson R., Bigler C., Eskola T., Gälman V., Gottschalk S., Herlitz E., Jarlman A., Kasperoviciene J., Kokociński M., Luup H., Miettinen J., Paunksnyte I., Piirsoo K., Quintana I., Raunio J., Sandell B., Simola H., Sundberg I., Vilbaste S. & Weckström J. 2009. Harmonization is more important than experience— results of the first Nordic–Baltic diatom intercalibration exercise 2007 (stream monitoring). *Journal of Applied Phycology*, 21: 471–482.

Kelly M.G. & Whitton B.A. 1995. The Trophic Diatom Index, a new index

for monitoring eutrophication in rivers. *Journal of Applied Phycology*, 7: 433-444.

Kelly M.G., Cazaubon A., Coring E., Dell'Uomo A., Ector L., Goldsmith B., Guasch H., Hürlimann J., Jarlman A., Kawecka B., Kwandrans J., Laugaste R., Lindstrøm E.-A., Leitao M., Marvan P., Padisák J., Pipp E., Prygiel J., Rott E., Sabater S., van Dam H. & Vizinet J. 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology*, 10: 215–224.

Kelly M.G. 2001. Use of similarity measures for quality control of benthic diatom samples. *Water Research*, 35 (11): 2784–2788.

Kelly M.G., Bennett C., Coste M., Delgado C., Delmas F., Denys L., Ector L., Fauville C., Ferreol M., Golub M., Jarlman A., Kahlert M., Lucey J., Ni Chathain B., Pardo I., Pfister P., Picinska- Faltynowicz J., Rosebery J., Schranz C., Schaumburg J., van Dam H. & Vilbaste S. 2009. A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: results of an intercalibration exercise. *Hydrobiologia*, 621: 169-182.

King L., Clarke G., Bennion H., Kelly M. & Yallop M. 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *Journal of Applied Phycology*, 18: 15-25.

Kitner M. & Poulícková A. 2003. Littoral diatoms as indicators for the eutrophication of shallow lakes. *Hydrobiologia*, 506-509: 519-524.

Kornijów R. & Kairesalo T. 1994. A simple apparatus for sampling epiphytic communities associated with emergent macrophytes. *Hydrobiologia*, 294: 141-143.

Krammer K. & Lange-Bertalot H. 1986-1991. *Süsswasserflora von Mitteleuropa Bacillariophyceae* Teil 1-4. G. Fisher Verlag, Stuttgart.

Krammer K. & Lange-Bertalot H. 2000. *Bacillariophyceae Part 5*: *English and French translation of the keys* (Ed. B. Büdel, G. Gärtner, L. Krienitz & G.M. Lokhorst). Vol. 2/5. Spektrum Akademischer: Heidelberg & Berlin.

Lange-Bertalot H. (ed) 1995-2009. *Iconographia Diatomologica. Annotated Diatom Micrographs.* Vols. I – XX. Ediciones Gantner Verlag.

Lange-Bertalot H. (ed) 2000-2009. *Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats.* Vols. I-V. Ediciones Gantner Verlag.

Laugaste R. & Reunanen M. 2005. The composition and density of epiphyton on some macrophyte species in the partly meromictic Lake Verevi. *Hydrobiologia*, 547: 137-150.

Lavoie I., Somers K.M., Paterson A.M. & Dillon P.J. 2005. Assessing scales of variability in benthic diatom community structure. *Journal of Applied Phycology*, 17: 509-513.

Lavoie I., Campeau S., Darchambeau F., Cabana G. & Dillon P.J. 2008. Are diatoms good integrators of temporal variability in stream water quality? *Freshwater Biology*, 53: 827-841.

Lavoie I., Dillon P.J. & Campeau S. 2009. The effect of excluding diatom taxa and reducing taxonomic resolution on multivariate analyses and

stream bioassessment. *Ecological Indicators*, 9: 213-225.

Lear G. & Lewis G.D. 2009. Impact of catchment land use on bacterial communities within stream biofilms. *Ecological Indicators*, 9: 848-855.

Leclerq L. & Maquet B. 1987. Deux nouveaux indices chimique et diatomique de qualité d'eau courante. Application au Samson et à ses affluents. (Bassin de la Meuse Belge). Comparaison avec d'autres indices chimiques biocénotiques et diatomiques. *Institut Royal des Sciences Naturelles de Belgique Documents de Travail*, 38: 1-113.

Lecointe C., Coste M. & Prygiel J. 1993. OMNIDIA software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia*, 269/270: 509-513.

Lecointe C., Coste M., Prygiel J. & Ector L. 1999. Le logiciel OMNIDIA version 2, une puissante base de données pour les inventaires de diatomées et pour le calcul des indices diatomiques européens. *Cryptogamie Algologie*, 20: 132-134.

Lenoir A. & Coste M. 1996. *Development of a practical diatom index of overall water quality applicable to the French national water board network*. En: Use of Algae for Monitoring Rivers II. (Eds B.A. Whitton & E. Rott), pp. 29-43. Botany Institute, University of Innsbruck, Innsbruck.

Linares J.E. 2003. *Las diatomeas bentónicas de las lagunas del Parque Nacional de Sierra Nevada. Estudio comparado con las colecciones del herbario de la Universidad de Granada (GDA)*. Tesis Doctoral, Universidad de Granada, Granada.

Lobo E.A., Katoh K. & Aruga Y. 1995. Response of epilithic diatom assemblages to water pollution in rivers in the Tokyo metropolitan area, Japan. *Freshwater Biology*, 34: 191-204.

Lobo E.A., Callegaro V.L.M. & Bender E.P. 2002. *Utilizaçao de Algas Diatomáceas Epilíticas como Indicadores da Qualidade da Água em Rios e Arroios da Regiao Hidrográfica do Guaíba, RS, Brasil*. EDUNISC, Santa Cruz do Sul.

Lobo Urrutia L. & Sesma Ausejo J. (comp.) 2006. Ficha informativa de los humedales de Ramsar (FIR).

Magurran A.E. 2004. *Measuring Ecological Diversity*. Blackwell Publishing, Oxford.

Marcus M.D. 1980. Periphytic community response to chronic nutrient enrichment by a reservoir discharge. *Ecology*, 61: 387-399.

Martín G., Toja J., Sala S.E., de los Reyes Fernández M., Reyes I. & Casco M.A. 2010. Application of diatom biotic indices in the Guadalquivir River Basin, a Mediterranean basin. Which one is the most appropriated? *Environmental Monitoring and Assessment*. DOI 10.1007/s10661-009-1254-5

Maznah W.O. & Mansor M. 2002. Aquatic pollution assessment based on attached diatom communities in the Pinang River Basin, Malaysia. *Hydrobiologia*, 487: 229-241.

McArdle B.H., & Anderson M.J. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, 82(1): 290-297.

McElarney Y.R. & Rippey B. 2009. A comparison of lake classifications

based on aquatic macrophytes and physical and chemical water body descriptors. *Hydrobiologia*, 625: 195-206.

McNeill J., Barrie F.R., Burdet H.M., Demoulin V., Hawksworth D.L., Marhold K., Nicolson D.H., Prado J., Silva P.C., Skog J.E., Wiersema J.H. & Turland N.J. (eds.) 2006. International Code of Botanical Nomenclature (Vienna Code). *Regnum Vegetabile*, 146: 1-568.

Messyasz B., & Kuczyńska-Kippen N. 2006. Periphytic algal communities, a comparison of *Typha angustifolia* L. and *Chara tomentosa* L. beds in three shallow lakes (West Poland). *Polish Journal of Ecology*, 54: 15-27.

Molis M. 2002. *Shallow water fouling assemblages exposed to abiotic disturbance and stress: the structuring role of emersion and UV-radiation*. Tesis Doctoral, Christian-Albrechts University, Kiel. http://tinyurl.com/yzcjqm8

Morales E.A. 2001. Morphological studies in selected fragilarioid diatoms (Bacillariophyceae) from Connecticut waters (U.S.A.). *Proceedings of the Academy of Natural Sciences of Philadelphia*, 151: 105-120.

Morales E.A. 2005. Observations of the morphology of some known and new fragilarioid diatoms (Bacillariophyceae) from rivers in the USA. *Phycological Research*, 53: 113-133.

Morales E.A. & Edlund M.B. 2003. Studies in selected fragilarioid diatoms (Bacillariophyceae) from Lake Hovsgol, Mongolia. *Phycological Research*, 51: 225-239.

Moré J. 1977. Levenberg-Marquardt algorithm: implementation and theory. *Notes in Mathematics*, 630: 105-116.

Moss B. 1973. Diversity in fresh-water phytoplankton. *American Midland Naturalist*, 90: 341-355.

Moss B., Stephen D., Álvarez C., Bécares E., van de Bund W., Collings S.E., van Donk E., de Eyto E., Feldmann T., Fernández-Aláez C., Fernández-Aláez M., Franken R.J.M., García-Criado F., Gross E.M., Gyllström M., Hansson L.-A., Irvine K., Järvalt A., Jensen J.-P., Jeppesen E., Kairesalo T., Kornijów R., Krause T., Künnap H., Laas A., Lill E., Lorens B., Luup H., Miracle M.R., Nõges P., Nõges T., Nykänen M., Ott I., Peczula W., Peeters E.T.H.M., Phillips G., Romo S., Russell V., Salujõe J., Scheffer M., Siewertsen K., Smal H., Tesch C., Timm H., Tuvikene L., Tonno I., Virro T., Vicente E. & Wilson D. 2003. The determination of ecological quality in shallow lakes - a tested system (ECOFRAME) for implementation of the European Water Framework Directive. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 13: 507-549.

Moulton S.R., Kennen J.G., Goldstein R.M. & Hambrook J.A. 2002. *Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program*. Open-file report 02-150. U.S. Department of the Interior, U.S. Geological Survey, Reston.

Ndiritu G.G., Gichuki N.N. & Triest L. 2006. Distribution of epilithic diatoms in response to environmental conditions in an urban tropical stream, Central Kenya. *Biodiversity and Conservation*, 15: 3267-3293.

Ní Chatháin B. & Harrington T.J. 2008. Benthic diatoms of the River Deel:

diversity and community structure. *Biology and Environment: Proceedings of the Royal Irish Academy*, 108B: 29-42.

Oemke M.D. & Burton T.M. 1986. Diatom colonization dynamics in a lotic system. *Hydrobiologia*, 139: 153-166.

Pan Y., Stevenson R.J., Vaithiyanathan P., Slate J. & Richardson C.J. 2000. Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, USA. *Freshwater Biology*, 44: 339-354.

Patrick R. 1973. *Use of Algae, especially diatoms, in the assessment of water quality*. En: Biological Methods for the Assessment of Water Quality. (Eds J. Jr. Cairns & K.L. Dickson), pp. 23-47. American Society for Testing and Materials, Philadelphia.

Pisces Conservation Ltd. 2004. Community Analysis Package version 3.11.

Ponader K.C., Charles D.F., Belton T.J. & Winter D.M. 2008. Total phosphorus inference models and indices for coastal plain streams based on benthic diatom assemblages from artificial substrates. *Hydrobiologia*, 610: 139–152.

Potapova M. & Charles D.F. 2005. Choice of substrate in algae-based water-quality assessment. *Journal of the North American Benthological Society*, 24: 415-427.

Poulíčková A., Duchoslav M. & Dokulil M. 2004. Littoral diatom assemblages as bioindicators of lake trophic status, a case study from perialpine lakes in Austria. *European Journal of Phycology*, 39: 143-152.

Prygiel J., Leveque L., & Iseretant R. 1996. Un nouvel indice diatomique pratique pour l'évaluation de la qualité des eaux en réseau de surveillance. *Revue des Sciences de l'Eau*, 9: 97-113.

Prygiel J., Whitton B.A. & Bukowska J. (eds). 1999. *Use of Algae for Monitoring Rivers III*. Agence de l'Eau Artois-Picardie, Douai.

Reavie E.D., Kireta A.R., Kingston J.C., Sgro G.V., Danz N.P., Axler R.P. & Hollenhorst T.P. 2008. Comparison of simple and multimetric diatom-based indices for great lakes coastline disturbance. *Journal of Phycology*, 44: 787–802.

Rohlf F.J. 2004. TpsDig, digitize landmarks and outlines, version 2.0. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook.

Rohlf F.J. & Slice D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, 39: 40-59.

Rott E. Hofmann G., Pall K., Pfister P. & Pipp E. 1997. *Indikationslisten für Aufwuchsalgen Teil 1, Saprobielle Indikation*. Bundesministerium für Land- und Forstwirtschaft, Wien.

Round F.E., Crawford R.M. & Mann D.G. 1990. *The Diatoms, Biology & Morphology of the Genera*. Cambridge University Press: Cambridge. 747 pp.

Rumeau A. & Coste M. 1988. Initiation à la systématique des diatomées d'eau douce pour l'utilisation pratique d'un indice diatomique générique. *Bulletin Français de la Pêche et de la Pisciculture*, 309: 1-69.

Sabbe K. & Vyverman W. 1995. Taxonomy,

morphology and ecology of some widespread representatives of the diatom genus *Opephora*. *European Journal of Phycology*, 30: 235-249.

Scheffer M. 2001. Alternative attractors of shallow lakes. *Science World*, 1: 254-263.

Scheffer M. & van Nes E.H. 2007. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia*, 584: 455-466.

Shannon C.E. & Weaver W. 1949. *The mathematical Theory of Communication*. Univ. of Illinois Press, Urbana.

Simkhada B., Jüttner I. & Chimonides P.J. 2006. Diatoms in lowland ponds of Koshi Tappu, eastern Nepal – relationships with chemical and habitat characteristics. *International Review of Hydrobiology*, 91: 574-593.

Simonsen R. 1987. *Atlas and catalogue of the diatom types of Friederich Hustedt*. - Stuttgart. Vols 1 -3. J. Cramer, Berlin.

Siver P.A., Hamilton P.B., Stachura-Suchoples K. & Kociolek J.P. 2005. Diatoms of North America. The freshwater flora of Cape Cod, Massachusetts, U.S.A. *Iconographia Diatomologica*, 14: 1-463.

Sládeček V. 1986. Diatoms as indicators of organic pollution. *Acta Hydrochimica et Hydrobiologica*, 14: 555-566.

Soininen J. 2009. Is diatom diversity driven by productivity in boreal streams? *Diatom Research*, 24: 197-207.

StatSoft, Inc. 2007. STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Steinberg C. & Schiefele S. 1988. Biological indication of trophy and pollution of running waters. *Zeitschrift für Wasser und Abwasser Forschung*, 21: 227-234.

Stenger-Kovács C., Buczkó K., Hajnal E. & Padisák J. 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL) developed in Hungary. *Hydrobiologia*, 589: 141-154.

Townsend S.A. & Gell P.A. 2005. The role of substrate type on benthic diatom assemblages in the Daly and Roper Rivers of the Australian wet/dry tropics. *Hydrobiologia*, 548: 101-115.

Trobajo R. 2003. *Ecological analysis of periphytic diatoms in Mediterranean coastal wetlands (Empordà wetlands, NE Spain)*. Tesis Doctoral, Universitat de Girona, Girona.

U.S. Geological Survey. Variously dated. *National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations*, book 9, chaps. A1-A9. http://pubs.water.usgs.gov/twri9A.

Vilbaste S. & Truu J. 2003. Distribution of benthic diatoms in relation to environmental variables in lowland streams. *Hydrobiologia*, 493: 81-93.

Vyverman W. 1991. Diatoms from Papua New Guinea. *Bibliotheca Diatomologica*, 22: 1-224.

Vyverman W., Verleyen E., Sabbe K., Vanhoutte K., Sterken M., Hodgson D.A., Mann D.G., Juggins S., van de Vijver B., Jones V., Flower R., Roberts D., Chepurnov V.A., Kilroy

C., Vanormelingen P. & de Wever A. 2007. Historical processes constrain patterns in global diatom diversity. *Ecology*, 88: 1924-1931.

Waide R.B., Willing M.R., Steiner C.F., Mittelbach G., Gough L., Dodson S.I., Juday G.P. & Parmenter R. 1999. The relationship between productivity and species richness. *Annual Review of Ecology and Systematics*, 30: 257-300.

Watanable T., Asai K. & Houki A. 1988. *Numerical water quality monitoring of organic pollution using diatom assemblages*. En: Proceedings of the 9th International Diatom Symposium 1986. (Ed. F.E. Round), pp. 123-141. Koeltz Science Books, Königstein.

Wetzel R.G. 1990. Land-water interfaces: Metabolic and limnological regulators. *Verhandlungen Internationalen Vereinigung Limnologie*, 24: 6-24.

Williams D.M. & Round F.E. 1987. Revision of the genus *Fragilaria*. *Diatom Research*, 2: 267-288.

Winter J.G. & Duthie H.C. 2000. Stream epilithic, epipelic and epiphytic diatoms: habitat fidelity and use in biomonitoring. *Aquatic Ecology*, 34: 345-353.

Witak M. 2001. *A Late Glacial diatom flora from lacustrine sediments of Puck Bay, Southern Baltic Sea, Poland*. En: Lange-Bertalot-Festschrift. Studies on diatoms. Dedicated to Prof. Dr. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th birthday (Ed. R. Jahn, J.P. Kociolek, A. Witkowski & P. Compère), pp. 457-475. Gantner Verlag, Ruggell.

Wu J. & Kow L. 2002. Applicability of a generic index for diatom assemblages to monitor pollution in the tropical River Tsanwun, Taiwan. *Journal of Applied Phycology*, 14: 63-69.

Yallop M., Hirst H., Kelly M., Juggins S., Jamieson J. & Guthrie R. 2009. Validation of ecological status concepts in UK rivers using historic diatom samples. *Aquatic Botany*, 90: 289-295.

Zelinka M. & Marvan P. 1961. Zur Präzisierung der biologischen Klassifikation der Reinheit fliessender Gewässer. *Archiv für Hydrobiologie*, 57: 389-407.

Zimba P.V. & Hopson M.S. 1997. Quantification of epiphyte removal efficiency from submersed aquatic plants. *Aquatic Botany*, 58: 173-179.

## PUBLICACIONES

Álvarez I., Cejudo C., Blanco S. & Bécares E. 2006. *Convenio específico de colaboración entre la Confederación Hidrográfica del Duero y la Universidad de León dentro del convenio marco de colaboración general entre el Ministerio de Medio Ambiente y la Universidad de León para el análisis y la aplicación de índices de diatomeas en la cuenca del Duero. Estudio de la situación actual de la calidad de las aguas superficiales mediante índices de diatomeas en las estaciones de la Red de Control Biológico. Campaña 2005.* Confederación Hidrográfica del Duero, Valladolid.

Álvarez-Blanco I., Cejudo-Figueiras C., Bécares E. & Blanco S. 2010. Spatiotemporal changes in diatom ecological profiles: implications for biomonitoring. *Limnology*. DOI 10.1007/s10201-010-0333-1

Álvarez I., Escudero A., Cejudo C., Pérez M., Blanco S. & Bécares E. 2010. *Convenio específico de colaboración entre la Confederación Hidrográfica del Duero y la Universidad de León dentro del convenio marco de colaboración general entre el Ministerio de Medio Ambiente y la Universidad de León para la elaboración de un atlas de diatomeas bentónicas de la cuenca del Duero (Fase III de III); El control y seguimiento del alga invasora* Didymosphenia geminata *y análisis de muestras de diatomeas y fitoplancton.Control y seguimiento del alga invasora* Didymosphenia geminata. Confederación Hidrográfica del Duero, Valladolid.

Blanco S., Álvarez I. & Cejudo C. 2008. A test on different aspects of diatom processing techniques. *Journal of Applied Phycology*, 20: 445-450.

Blanco S., Cejudo C., Álvarez I. & Bécares E. 2007. *Convenio específico de colaboración entre la Confederación Hidrográfica del Duero y la Universidad de León dentro del Convenio Marco de Colaboración General entre el Ministerio de Medio Ambiente y la Universidad de León para para: 1- El asesoramiento y la determinación de índices biológicos 2- La elaboración de un atlas de diatomeas bentónicas de la Cuenca del Duero (fase I). Análisis comparativo de indicadores ecológicos de la calidad del agua. Campaña biológica de primavera-verano de 2007.* Confederación Hidrográfica del Duero, Valladolid.

Blanco S., Cejudo C., Álvarez I. & Bécares E. 2009. *Convenio específico de colaboración entre la Confederación Hidrográfica del Duero y la Universidad de León dentro del Convenio Marco de Colaboración General entre el Ministerio de Medio Ambiente y la Universidad de León para para: 1- El asesoramiento y la determinación de índices biológicos 2- La elaboración de un atlas de diatomeas bentónicas de la Cuenca del Duero (fase I). Análisis y aplicación de índices de diatomeas. Campaña biológica de verano de 2009.* Confederación Hidrográfica del Duero, Valladolid.

Blanco S., Cejudo-Figueiras C., Álvarez-Blanco I., Bécares E., Hoffmann L. & Ector L. 2010. *Atlas de diatomeas de la cuenca del Duero /Diatom Atlas of the Duero Basin*. Área de publicaciones de la Universidad de León, León.

Cejudo C., Álvarez I., Blanco S. & Bécares E. 2006. *Convenio específico de colaboración entre la Confederación Hidrográfica del Duero y la*

*Universidad de León dentro del convenio marco de colaboración general entre el Ministerio de Medio Ambiente y la Universidad de León para el análisis y la aplicación de índices de diatomeas en la cuenca del Duero. Estudio de la situación actual de la calidad de las aguas superficiales mediante índices de diatomeas en las estaciones de la Red de Control Biológico. Campaña 2004.* Confederación Hidrográfica del Duero, Valladolid.

## COMUNICACIONES

Álvarez I., Cejudo C., Blanco S., Hernández N. & Bécares E. 2007. Análisis de calidad de las aguas de los ríos de la cuenca hidrográfica del Duero utilizando índices diatomológicos (panel). XVI Simposio de botánica Criptogámica. Libro de resúmenes, pp. 32. León. 19-2/9/2007.

Álvarez-Blanco I., Cejudo-Figueiras C., Blanco S., Hernández N. & Bécares E. 2009. Autoecology of epilithic diatoms in the Duero rivers Basin. *Diatomededelingen* 33: 33.

Blanco S., Álvarez-Blanco I., Cejudo-Figueiras C., Hernández N. & Bécares E. 2010. Atlas de las diatomeas de la Cuenca del Duero (panel). XV Congreso de la Asociación Española de Limnología y VII Congreso Ibérico de Limnología. Libro de resúmenes, pp. 79. Ponta Delgada (Portugal). 5-9/7/2010.

Blanco S., Cejudo C., Álvarez I., Bécares E., Sáenz de Buroaga M. & Lobo L. 2008. Water quality assessment in two Spanish shallow lakes using epiphytic diatoms (panel). Shallow lakes conference "Structure and Function of World Shallow Lakes". Punta del Este (Uruguay). 23-28/11/2008.

Blanco S., Cejudo-Figueiras C., Tudesque L., Bécares E., Hoffmann L. & Ector L. 2009. Are diatom diversity indices reliable monitoring metrics? (panel). International Symposium "Use of Algae for Monitoring Rivers". Abstracts book, pp. 73. Belvaux (Luxemburgo). 23-25/11/2009.

Blanco S., Escudero A., Cejudo-Figueiras C., Álvarez-Blanco I., Seisdedos P. & Bécares E. 2009. *Didymosphenia geminata* en la cuenca del Duero: caracterización preliminar (panel).

3º Congreso Nacional sobre Especies Exóticas Invasoras. Libro de resúmenes pp. 78. Zaragoza. 24-27/11/2009.

Blanco S., Escudero A., Cejudo-Figueiras C., Álvarez-Blanco I., Seisdedos P. & Bécares E. 2010. *Didymosphenia geminata*: a new biological invasion in the duero basin (presentación oral). XV Congreso de la Asociación Española de Limnología y VII Congreso Ibérico de Limnología. Libro de resúmenes, pp. 148. Ponta Delgada (Portugal). 5-9/7/2010.

Cejudo-Figueiras C., Blanco S. & Bécares E. 2007. Diatomeas epífitas en lagos europeos: diversidad florística y relación con factores ambientales (panel). XVI Simposio de Botánica Criptogámica. Libro de resúmenes, pp. 33. León. 19-22/9/2007.

Cejudo C., Álvarez I., Blanco S., Hlúbiková D., Bécares E., Hernández N., Hoffmann L. & Ector L. 2008. Flore des diatomées du bassin hydrographique du Duero (Espagne): résultats préliminaires (presentación oral). 27ème Colloque de l'Association des Diatomistes de Langue Française. Programa científico, pp. 44. Dijon (Francia). 1-4/9/2008.

Cejudo C., Álvarez I., Blanco S. & Bécares E. 2008. Estudio comparativo de las diatomeas de las lagunas de la Depresión del Duero. XIV congreso de la Asociación Ibérica de Limnología. Libro de resúmenes. Huelva. 8-12/09/2008.

Cejudo-Figueiras C., Morales E.A., Wetzel C., Blanco S., Hoffmann L. & Ector L. 2009. The taxonomy of *Fragilaria construens* var. *subsalina* (Hustedt) and two morphologically related new taxa. *Diatomededelingen* 33: 44

Cejudo-Figueiras C. 2009. Las diatomeas bentónicas como bioindicadores: muestreo, procesado de las muestras y cálculo de índices. Curso "Aqua Qalidad", Asociación Medioambiental Oro Verde. Cubillas de Arbas. 23-24/05/2009.

Cejudo-Figueiras C., Blanco S., Álvarez-Blanco I., Bécares E., Sáenz de Buroaga M., Lobo L. & Ector L. 2009. Utilisation des diatomées épiphytes pour l'évaluation de la qualité de l'eau dans les lagunes (Province d'Alava, Nord de l'Espagne). 28ème Colloque de 'Association des Diatomistes de Langue Française. Programa científico, pp. 61. Banyuls (Francia). 7-10/09/2009.

Ector L., Hlúbiková D., Cejudo-Figueiras C., Hoffmann L. & Blanco S. 2009. Rare and exotic diatoms in the Duero River basin (NW Spain), with notes on their ecology and world distribution. *Scripta Botanica Belgica*, 45: 34
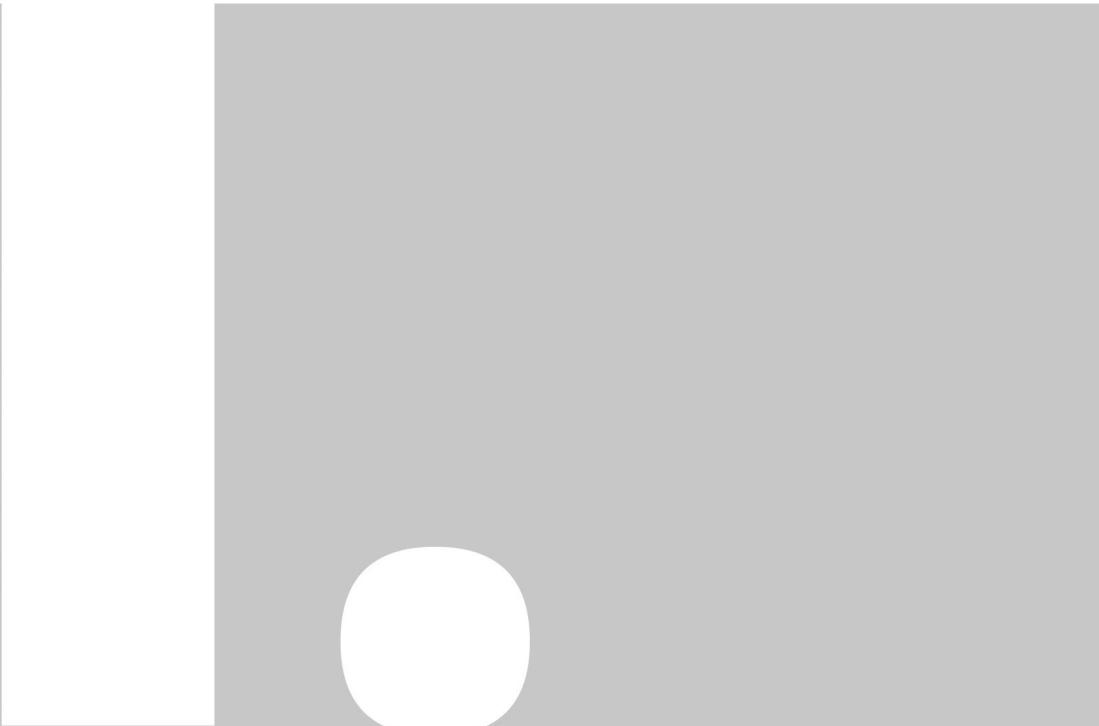
# Analysis of the type of *Fragilaria construens* var. *subsalina* (Bacillariophyceae) and description of two morphologically related taxa from Europe and the United States

Cristina Cejudo-Figueiras[1,2]*, Eduardo A. Morales[3,4,5], Carlos E. Wetzel[2,6], Saúl Blanco[1,2], Lucien Hoffmann[2] and Luc Ector[2]

[1]*Área de Ecología, Universidad de León, E-24071 León, Spain*
[2]*Department of Environment and Agro-biotechnologies, Public Research Centre–Gabriel Lippmann. 41, rue du Brill, L-4422 Belvaux, Grand-Duchy of Luxembourg*
[3]*Herbario Criptogámico, Universidad Católica Boliviana San Pablo. Av. Gral. Galindo No. 5381, Cochabamba, Bolivia*
[4]*Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA 19103, USA*
[5]*Unidad de Limnología y Recursos Acuáticos, Universidad Mayor de San Simón, P.O. Box 992, Cochabamba, Bolivia*
[6]*Instituto de Botânica, Seção de Ecologia. Av. Miguel Stéfano 3687, CEP 04301-012, São Paulo, SP, Brazil*

*Fragilaria construens* var. *subsalina* is a common diatom found in a wide variety of aquatic systems worldwide. It was described by Hustedt (1925) from brackish waters in Oldesloe, Germany; a lectotype specimen was established by Simonsen (1987). We analysed the ultrastructure of specimens from the lectotype material by means of light (LM) and scanning electron microscopy (SEM) and show that this taxon should be placed at the species level within the genus *Pseudostaurosira* (as *Pseudostaurosira subsalina*). A similar but distinct taxon from the United States, found in waters with low electrolyte content, is proposed as a new species of *Pseudostaurosira*, namely *Pseudostaurosira americana* sp. nov. We also describe a second new species from Spain (*Pseudostaurosira alvareziae* sp. nov.), found in waters with moderate conductivity, which was misidentified as *P. subsalina* in the past. A morphometric analysis of the valve shape supports the morphological separation between the three species. The present study clarifies the taxonomy, ecology and systematic position of these three taxa and provides a comparison with other, similar taxa.

Key Words: Bacillariophyta, Diatoms, Fragilariales, Lectotype, Morphometry, New species, *Pseudostaurosira*

## INTRODUCTION

During the last three decades, diatom taxonomists have paid increasing attention to the taxonomy of small araphid species (Hallegraeff & Burford 1996; Le Cohu 1999; Morales 2001, 2002, 2003, 2005; Van de Vijver & Beyens 2002; Morales & Edlund 2003; Edlund *et al.* 2006; Williams 2006). Studies concentrating on the ultrastructure of the cell wall resulted in the establishment of several new genera, such as *Pseudostaurosira* D.M. Williams & Round 1987, *Punctastriata* D.M. Williams & Round 1987, *Staurosirella* D.M. Williams & Round 1987, *Nanofrustulum* Round, Hallsteinsen & Paasche 1999, *Pseudostaurosiropsis* E.A. Morales 2001 and *Sarcophagodes* E.A. Morales 2002. The valve structure of *Fragilaria construens* var. *subsalina* Hustedt and related taxa has not yet been thoroughly studied and compared in light microscopy (LM) and scanning electron microscopy (SEM), with negative impacts on for example bioassessment protocols using diatom indices, which are highly dependent on taxonomic accuracy (e.g. Besse-Lototskaya *et al.* 2006). Since *F. construens* var. *subsalina* has a supposedly broad distribution and is frequently listed in studies throughout the world, there is

an obvious need to confirm the identity of the specimens from these reports and differentiate the diagnostic criteria of this taxon from those of morphologically similar taxa.

*Fragilaria construens* var. *subsalina* was described by Hustedt (1925) from samples collected from a brackish habitat in Oldesloe (Holstein, Germany). Simonsen (1987) lectotypified this taxon by choosing slide '144/4. Oldesloe, Holstein. I,2. 15.7.22', on the basis of the fact that the name of the taxon was annotated by Hustedt on the record sheet for that slide. Simonsen also reported that 'the taxon occurs in great quantities'.

Hustedt presented three drawings of the variety *subsalina* in his 1925 publication (Fig. 1). The length of the valves in these drawings ranges from 19 to 29 μm and the width from 3 to 4 μm, the longer specimen being narrower. The stria density is constant (14 in 10 μm) in all three valves. Hustedt also presented a colony in girdle view, with some specimens showing girdle bands that are distinctly curved and narrower near the apices. The brief description given by Hustedt (1925) does not include measurements; the taxon was said to differ from the nominate variety [*Fragilaria construens* (Ehrenberg) Grunow var. *construens*] by its more linear shape, becoming more curved in smaller valves. Hustedt described the apices as attenuated, a feature observed in valves of all sizes. Hustedt (1925) also mentions

* Corresponding author (ccejf@unileon.es).

67
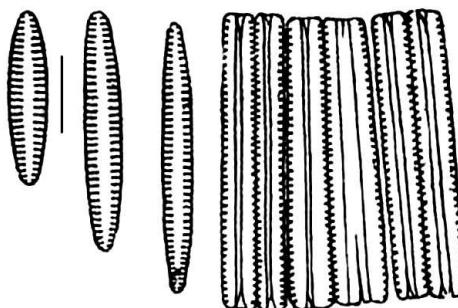
**Fig. 1.** Hustedt's drawings of *F. construens* var. *subsalina* presented in his 1925 publication.

that this variety intergrades with smaller elliptical *Fragilaria construens* var. *venter* Grunow and attributes the origin of the variety *subsalina* to the increased salinity of the water.

Krammer & Lange-Bertalot (2004) proposed the combination *Staurosira subsalina* (Hustedt) Lange-Bertalot and later Morales (2005) provided the combination *Pseudostaurosira subsalina* (Hustedt) E.A. Morales on the basis of the ultrastructure of a population from the Norwalk River (Connecticut, USA) that resembled the lectotype material presented in Simonsen (1987) and Krammer & Lange-Bertalot (1991).

A study of the type of *Pseudostaurosira elliptica* (Schumann) Edlund, E.A. Morales & S. Spaulding (formerly *Fragilaria elliptica* Schumann) yielded new insights into the taxonomy of the species group around *Pseudostaurosira brevistriata* (Grunow) D.M. Williams & Round, pointing to the need for revision of this group on the basis of detailed studies of type materials and careful LM and SEM analyses (Edlund *et al.* 2006). The present study aims at resolving the taxonomy of *F. construens* var. *subsalina*, a taxon morphologically related to *P. brevistriata*, by studying its type material. Two new, morphologically similar taxa, one from the Duero Basin, Spain and another from the northeastern United States [misidentified in Morales (2005) as *P. subsalina*], are also described in detail using LM and SEM.

## MATERIAL AND METHODS

Four samples from three different sites were analysed (Schleswig-Holstein, Germany; Connecticut, USA; Salamanca, Spain). Lectotype material of *P. subsalina* was collected on 15 July 1922 (BRM 144/04). No accurate information about the type of substratum and sampling process was found for this material. The sample from the United States consists of epilithic material collected from the Norwalk River at South Wilton (Connecticut, USA) on 9 September 1998 following the method developed by the National Water Quality Assessment Program (NAWQA) (Fitzpatrick *et al.* 1998; Moulton *et al.* 2002). LM and SEM analyses of this sample were already presented in Morales (2005), and we make reference to these data in this manuscript. An additional epilithic sample, also from the

Norwalk River at South Wilton, but collected on 10 August 2004, was observed under LM during the present study. This latter sample is housed in the Academy of Natural Sciences of Philadelphia (GS137711, ANSP, Phycology Section). The fourth sample corresponds to epilithic material collected from the Tormes River, Duero Basin, Salamanca, Spain on 5 August 2004 by scrubbing several boulders with a toothbrush, following the method described in AENOR (2004, Spanish official version of the European Standard UNE-EN 13946).

Subsamples of material from the three localities were cleaned by oxidation with hot hydrogen peroxide 30% v/v and further digested with hot nitric acid. Each sample was then rinsed using distilled water, and air-dried aliquots were mounted on permanent glass slides using Naphrax®. For SEM analyses, aliquots of oxidized materials from Germany and Spain were mounted on stubs and covered with gold (coating 40-nm thick) with a modular high-vacuum coating system (BAL-TEC MED 020). The sample from the United States collected in 1998 was coated with gold–palladium using a Polaron sputter coater. LM images were taken with a Leica® DM-RB light microscope equipped with a Leica® DC500 camera. SEM photographs were obtained using a Leica® Stereoscan 430i operated at 20 kV for the samples from Germany and Spain and a Leo-Zeiss 982-DSM electron microscope (Zeiss) was used for the sample from the United States collected in 1998. Variation in valve dimensions was assessed by measuring up to 50 valves of each taxon using LM and SEM images. Morphological terminology follows Hendey (1964), Anonymous (1975), Cox & Ross (1981) and Krammer & Lange-Bertalot (2000).

Geometric morphometry was used to assess if the taxa distinguished on the basis of LM and SEM information represented well-circumscribed variants. Geometric morphometry is an exploratory technique that allows the quantification of morphological characteristics that can be used as taxonomic criteria (Bookstein 1991). Resulting data can then be analysed by means of multivariate analysis to test for significant differences between pre-established groups. For diatom valve shape analysis, valve morphology is modelled as a geometric configuration of landmarks (reliably identifiable points in the set of specimens investigated). Shape variation within a sample of landmark configurations is described in terms of parameters that describe these variations and can be analysed using multivariate analytical methods (Beszteri *et al.* 2005). In the present study, six landmarks were placed at certain points on the valve (see below) and digitized using tpsDig2 software (Rohlf 2004). These points were selected to maximally cover morphological variability within the range of individuals studied. Four landmarks were placed in fixed positions on the valve poles and on the widest points of the frustule. Two more landmarks were placed on the inner extremities of the central striae to account for their length and separation. The Cartesian coordinates of the landmarks were aligned (translated, rotated and scaled) by the Procrustes generalized orthogonal least-squared superimposition procedure (Rohlf & Slice 1990). A nonmetric multidimensional scaling (NMDS) multivariate analysis was carried out on the resulting normalized coordinates by

means of the software Past version 1.78 (Hammer *et al.* 2001). To visualize the size and shape of the scatter plot for each predefined group, confidence ellipses were added in the resulting graphic using Past software. Confidence ellipses are scatter-plot areas establishing confidence intervals (generally at $\alpha = 0.95$) that contain all nonoutlier observations. Finally, a similarity test [analysis of similarity (ANOSIM)] was performed between the Cartesian coordinates of resulting groups in the NMDS using the Euclidean distance measure. A total of 100 LM photographs taken from the type material of *F. construens* var. *subsalina* and 35 individuals belonging to populations from Spain and the United States were also used for this analysis.

## RESULTS

### *Pseudostaurosira subsalina* (Hustedt) E.A. Morales excl. descr. and figs in Morales (2005)

Figs 2–33, 94–99, 107, 109, 111

BASIONYM: *Fragilaria construens* var. *subsalina* Hustedt 1925.

NOMENCLATURAL SYNONYMS: *Fragilaria construens* f. *subsalina* (Hustedt) Hustedt 1957, *Staurosira construens* f. *subsalina* (Hustedt) Bukhtiyarova 1995, *Staurosira construens* var. *subsalina* (Hustedt) N.A. Andresen, Stoermer & Kreis 2000, *Staurosira subsalina* (Hustedt) Lange-Bertalot 2004.

MATERIAL INVESTIGATED: Hustedt lectotype material (E3688) from Oldesloe, Holstein, Germany (BRM 144/04).

HABITAT OF THE TYPE MATERIAL: Brackish waters (Hustedt 1925).

SPECIES DESCRIPTION: Frustules rectangular in girdle view, forming chainlike colonies (Fig. 14) with the aid of interlocking spines (Fig. 98). Valves isopolar, narrowly lanceolate with substrate apices (Figs 2–13, 15–33). Length: 10–36 µm; width: 4.0–5.3 µm; stria density: 13–14 in 10 µm ($n$ = 31). Striae punctate, uniseriate, alternate and parallel to slightly radiate toward the apices, composed of one to four areolae on the valve face, highly variable among specimens. A single row of areolae is present on the valve mantle (Figs 94, 109). Areolae square to transapically elongated with rounded corners, with usually an abrupt decrease in size between the first (the nearest to the valve face margin) and the second areola, and a more gradual decrease toward the sternum; smaller areolae are often rounded (Figs 94, 97, 109, 111). Volae opposite, projecting from the virgae (Fig. 95). Linking spines spatulate with a lateral projection at the base (Fig. 98), situated on the vimines at the valve face margin (Figs 94, 97, 98, 109, 111). The transition between the valve and the mantle is rather gradual (Figs 94, 109, 111). Central sternum is variable in width, from broadly to narrowly lanceolate (Figs 2–32, 94, 96, 97, 107, 109, 111). Mantle plaques present (Fig. 98). Apical pore fields are present on each apex at the valve face/valve mantle junction (e.g. Figs 95, 111). Girdle bands open, ligulate and lacking perforations (Figs 98, 99). Valvocopula wider than the other girdle elements (Fig. 99). Rimoportulae absent (Figs 94, 96, 97, 107). Plastid structure unknown.

### *Pseudostaurosira alvareziae* Cejudo-Figueiras, E.A. Morales & Ector *sp. nov.*

Figs 34–73, 100–105, 106, 108, 110

DESCRIPTION: *Frustula aspectu cingulari rectangularia in catenis. Valvae isopolares lanceolatae apicibus late rotundatis, 10–18 µm longae, 3.6–5.0 µm latae. Striae 13–15 in 10 µm, uniseriatae alternae parallelae vel leviter radiantes ad apices. Una ad quatuor areolae in quoque stria. Areolae quadratae angulis rotundatis gradiatim decrescentes versus margines. Sternum lanceolatum vel irregulare. Volae projecturis ramosis ex areolarum parietis. Spinae spatulatae. Area porellarum ad apices. Rimoportulae nullae.*

HOLOTYPE: Slide BR-4141 (National Botanical Garden, Meise, Belgium).

ISOTYPES: Slide BRM-ZU7/06 (Hustedt Collection, Bremerhaven, Germany); slide BM 101395 (Natural History Museum, London, England).

TYPE LOCALITY: Tormes River, Salamanca, Spain; 40°57′37″N, 5°38′42″W.

ETYMOLOGY: The specific epithet is dedicated to the Spanish diatomist Irene Álvarez-Blanco.

HABITAT OF THE HOLOTYPE: This taxon was found in oligosaprobic (biological oxygen demand over 5 days $< 2$ mg·l$^{-1}$), mesoeutrophic [(PO$_4$$^{3-}$-P) 0.23 µg·l$^{-1}$, (NO$_3$-N) 3.01 µg·l$^{-1}$] and slightly alkaline (pH 7.2) water with low electrolyte content (conductivity 97 µS·cm$^{-1}$).

SPECIES DESCRIPTION: Frustules rectangular in girdle view, forming chainlike colonies (Fig. 73) with the aid of spines (Figs 101, 102). Valves isopolar, narrowly elliptical with broadly rounded apices (Figs 34–72). Length: 10–18 µm; width: 3.6–5.0 µm; stria density: 13–15 in 10 µm ($n$ = 39). Striae punctate, uniseriate, alternate, parallel or slightly radiate toward apices (Figs 34–72). Areolae one to four, number highly variable even within same valve (Figs 100, 103–106, 108, 110). A single row of areolae is located on valve mantle (Figs 106, 108, 110), sometimes covered by flattened projections (Fig. 100). Areolae square with rounded corners, usually gradually decreasing in size toward the sternum; smaller areolae usually rounded (Figs 103–106, 108, 110). Volae opposite, projecting from the virgae (Figs 103, 106, 108, 110). Linking spines spatulate with a lateral projection visible at the base of spine (Figs 100, 101); the spines are situated on the vimines at the valve face margin (Figs 100, 103, 104, 106, 108, 110). Central sternum is variable in width, from broadly to narrowly lanceolate (Figs 34–45, 47–72, 103–106, 108, 110), rarely linear (Fig. 46). Mantle plaques present (Figs 100–102). Apical pore fields located on each apex at the valve face/valve mantle junction and composed of pores that are sometimes covered by flattened projections (Figs 100, 103, 106, 110). Girdle band structure unclear, possibly open and ligulate (cf. Fig. 102); they lack areolae, although this feature was not clearly seen (Fig. 102). Valvocopula wider than the other girdle elements (Fig. 102). Rimoportulae absent (Figs 100, 105). Plastid structure unknown.
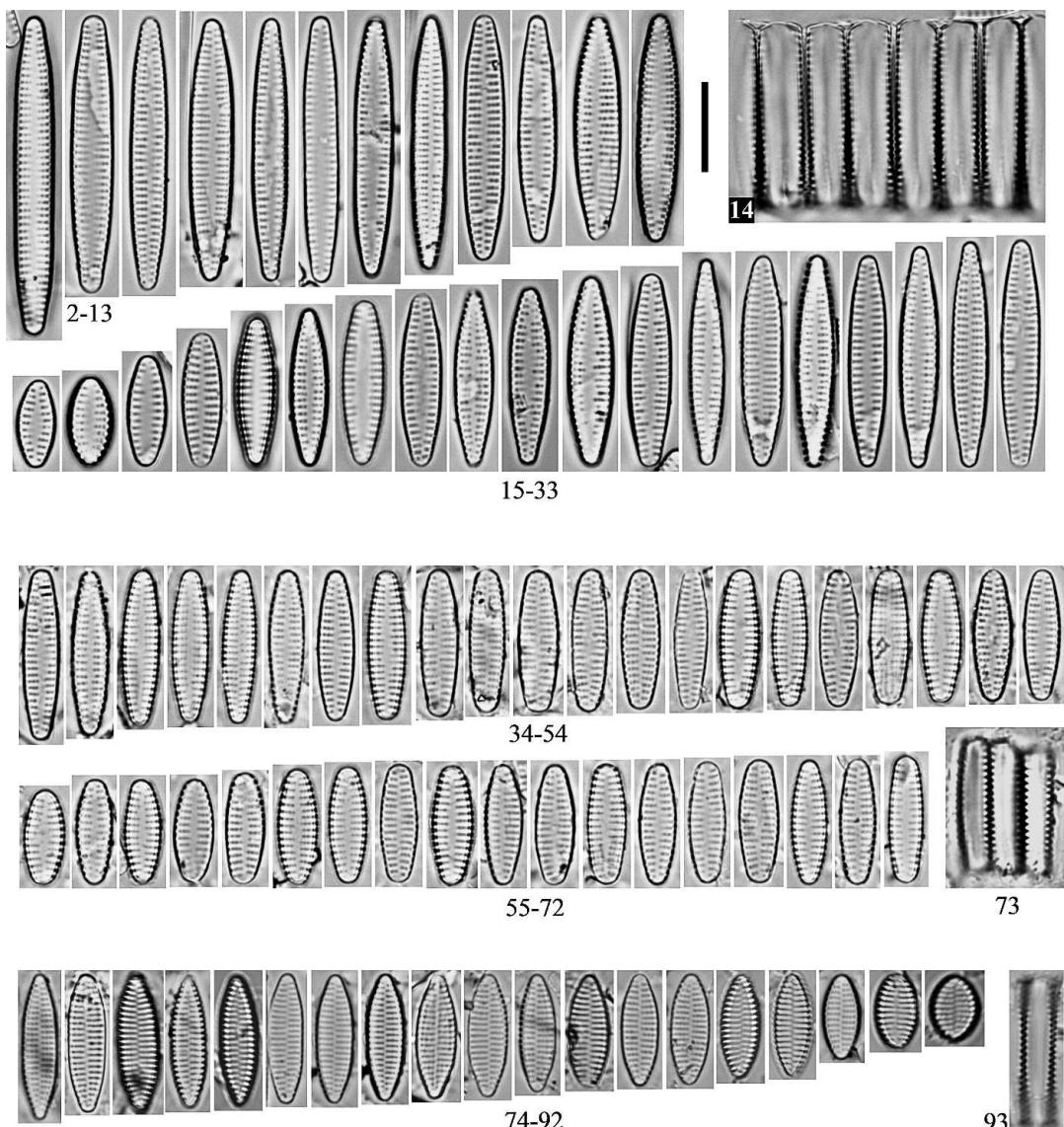
**Figs 2–93.** Cleaned valves (LM). Scale bar = 10 μm.
**Figs 2–33.** *Pseudostaurosira subsalina.* Hustedt lectotype material (E3688) from Oldesloe, Holstein, Germany (BRM 144/04).
**Figs 34–73.** *Pseudostaurosira alvareziae.* Holotype population from Tormes River at Salamanca, Spain. Slide BR-4141, National Botanical Garden, Meise, Belgium.
**Figs 74–93.** *Pseudostaurosira americana.* Population from Norwalk River at South Wilton, Fairfield County, Connecticut, Academy of Natural Sciences of Philadelphia (ANSP), GS137711, collected on 10 August 2004.

### *Pseudostaurosira americana* E.A. Morales *sp. nov.*

figs 1–20, 8–85 in Morales 2005

Figs 74–93, 112–115

DESCRIPTION: *Frustula aspectu cingulari rectangularia in catenis. Valvae isopolares lanceolatae apicibus cuneatis, 6–22 μm longae, 4.5–5.0 μm latae. Striae 16–18 in 10 μm, uniseriatae alternae parallelae. Duae ad quinque areolae in quoque stria. Sternum angustum lineare. Volae projecturis ramosis ex areolarum parietis. Spinae spatulatae. Area*
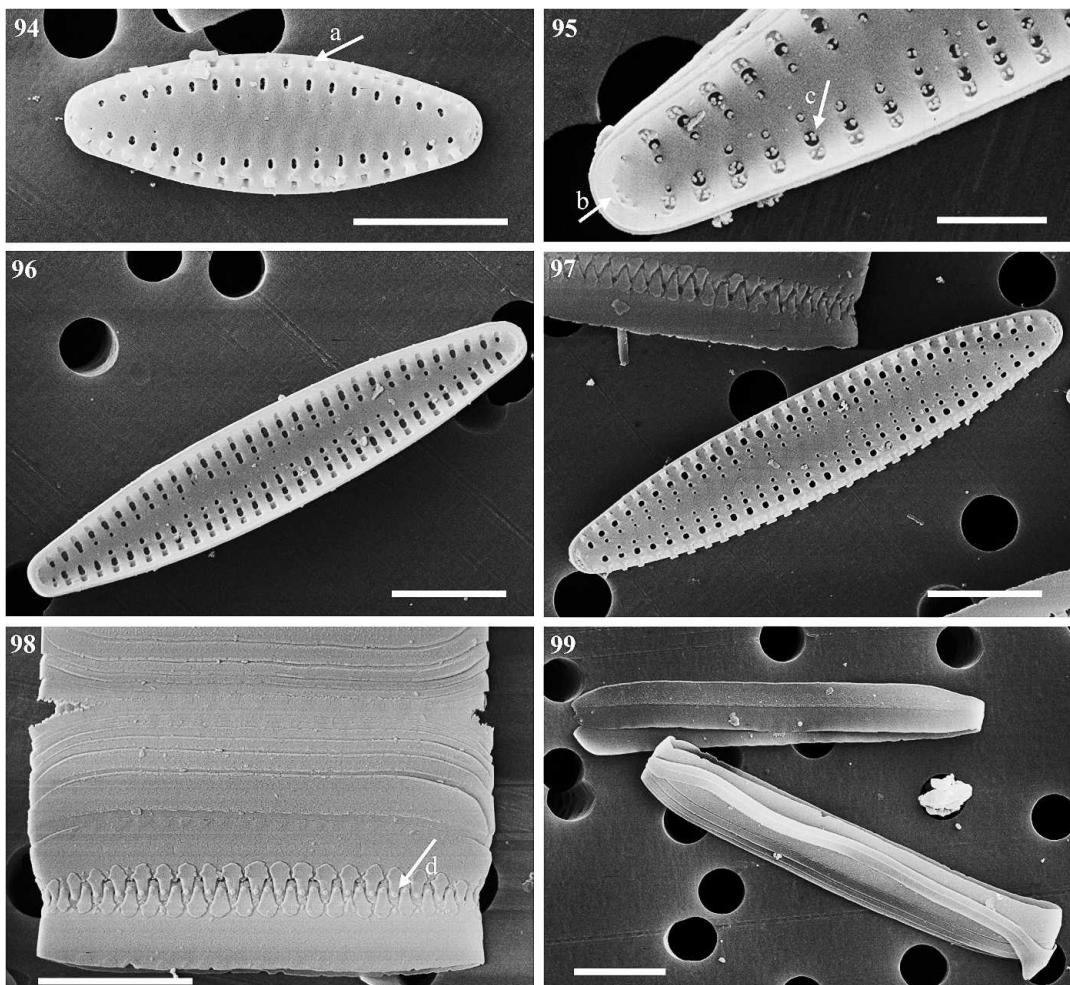
**Figs 94–99.** *Pseudostaurosira subsalina*. Hustedt lectotype material (E3688) from Oldesloe, Holstein, Germany. Cleaned valves (SEM). Scale bars = 5 µm (Figs 94, 96–99), 2 µm (Fig. 95). In Fig. 99 the valvocopula is the isolated copula. Arrows = a: single row of areolae present on the valve mantle; b: apical pore field; c: volae; d: lateral projection at the base of the spine.

*porellarum ad apices cum duabus seriebus poris. Rimoportulae nullae.*

HOLOTYPE: Circled specimen on slide ANSP G.C. 106191 (Academy of Natural Sciences of Philadelphia, USA).

TYPE LOCALITY: Norwalk River at South Wilton, Fairfield County, Connecticut. 41°09′49″N, 73°25′11″W.

HABITAT OF THE HOLOTYPE: This taxon was found in circumneutral waters (pH 7.5) with low to medium concentrations of orthophosphate–phosphorus [($PO_4^{3-}$-P) 0.024 mg·l$^{-1}$] and total N [($NO_3$-N) 0.490 mg·l$^{-1}$], and moderate electrolyte content (conductivity 238 µS·cm$^{-1}$) (Morales 2005).

SPECIES DESCRIPTION: Frustules rectangular in girdle view, forming chainlike colonies (Fig. 93, fig. 20 in Morales 2005) with the aid of spines (Fig. 115, figs 83, 84 in Morales 2005). Valves isopolar, lanceolate with cuneate apices (Figs 74–92, figs 1–19 in Morales 2005). Length: 6–22 µm. Width: 4.5–5.0 µm. Striae density: 16–18 in 10 µm ($n = 50$). Striae punctate, uniseriate, alternate, parallel or slightly radiate toward apices (Figs 74–92, figs 1–19 in Morales 2005). Areolae roundish (Figs 112, 114, 115, figs 80–85 in Morales 2005). One, rarely two, rows of areolae are located on valve mantle (Figs 114, 115, figs 81, 82, 84, 85 in Morales 2005). Volae two or more, projecting from the virgae or entire inner areolar periphery (Fig. 115, figs 82, 85 in Morales 2005). Linking spines spatulate, with serrated
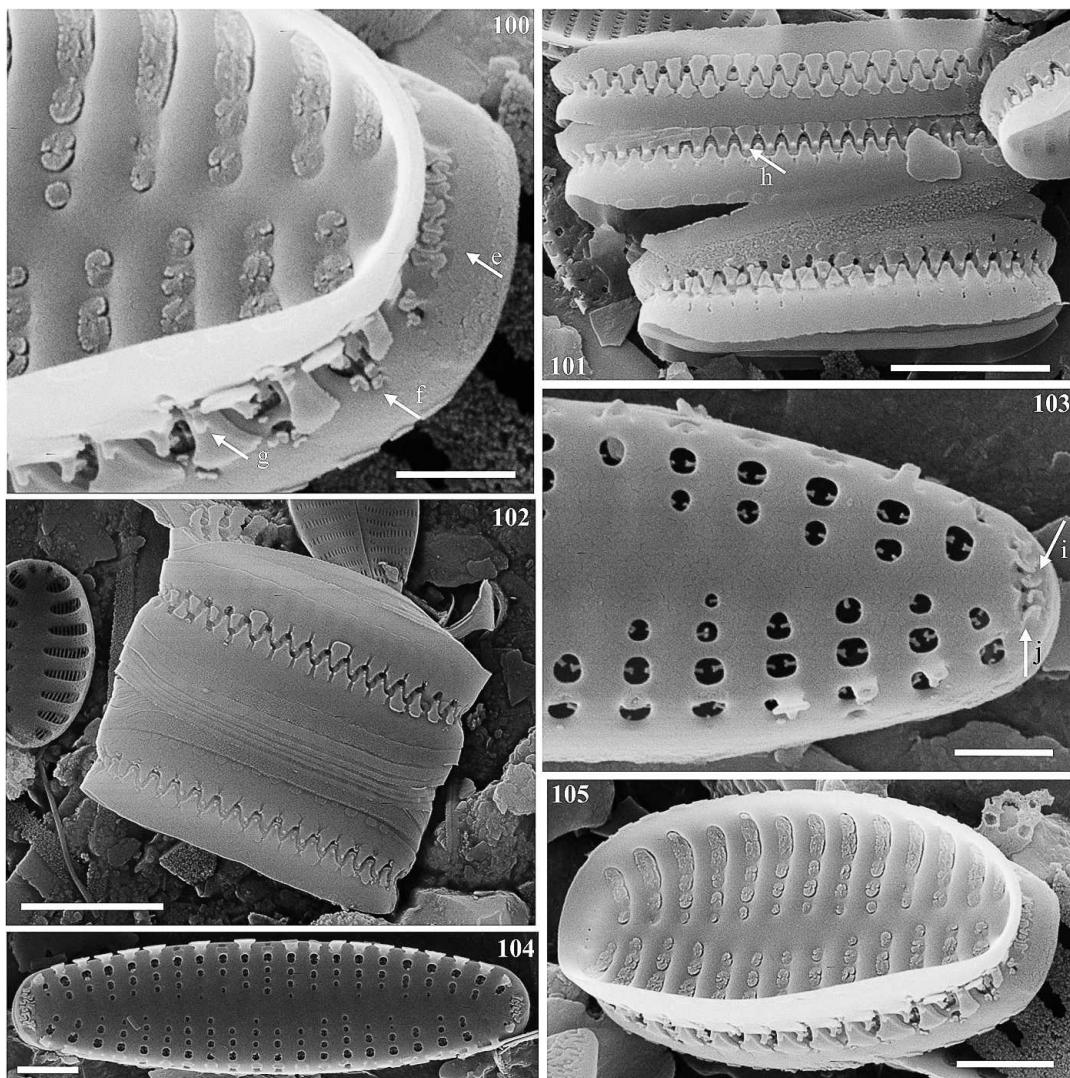
**Figs 100–105.** *Pseudostaurosira alvareziae.* Holotype population from Tormes River at Salamanca, Spain. Cleaned valves (SEM). Scale bars = 5 µm (Figs 101, 102), 2 µm (Figs 104, 105), 1 µm (Figs 100, 103). Arrows = e, i: flattened projections covering the apical pore field; f: flattened projections covering areola; g, h: lateral projection on the base of the spine; j: apical pore field.

margins, apparently with a softer core (inner part erodes more rapidly than outer part), situated on the vimines at the valve face margin (Figs 113–115, figs 80–82, 84, 85 in Morales 2005). A flattened, rounded projection subtends each spine and covers the valve mantle areola immediately below the spine (Figs 113–115, figs 84, 85 in Morales 2005). Another flattened projection may also be present and originates from the same areola and opposite to the flattened projection at the base of the spine (fig. 85 in Morales 2005); both structures may thus cover the entire opening of the areola. Central sternum linear and very narrow (Figs 74–92, figs 1–19, 80 in Morales 2005). Mantle plaques present (Figs 113–115, figs 81, 84, 85 in Morales 2005). Apical pore fields typically composed of two rows of round pores, positioned at valve face/mantle junction (figs 81, 83 in Morales 2005). Girdle bands without areolae; it is not visible whether they are open or closed (Figs 113 and 114). Valvocopula wider (Fig. 113). Rimoportulae absent (Fig. 112, fig. 83 in Morales 2005). Plastid structure unknown.
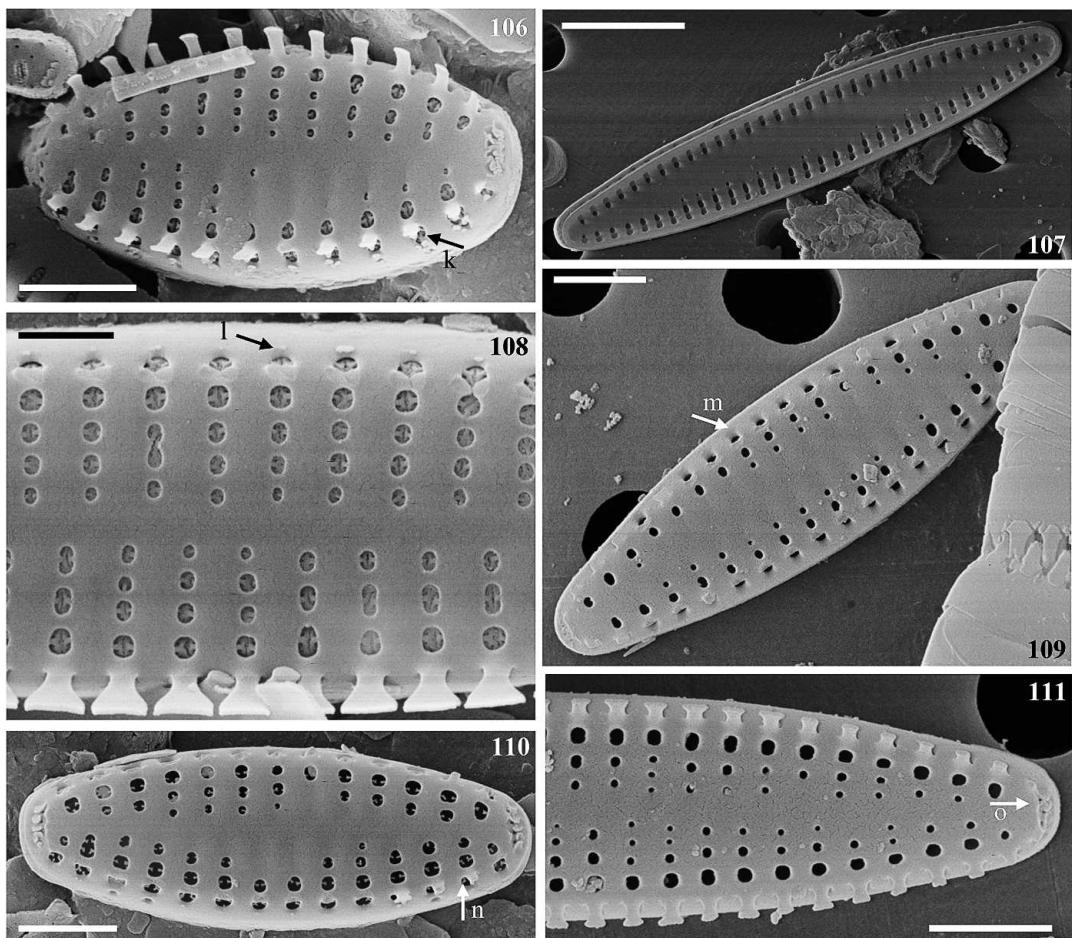
**Figs 106, 108, 110.** *Pseudostaurosira alvareziae*. Holotype population from Tormes River at Salamanca, Spain. Slide BR-4141 (National Botanical Garden, Meise, Belgium).
**Figs 107, 109, 111.** *Pseudostaurosira subsalina*. Hustedt lectotype material (E3688) from Oldesloe, Holstein, Germany. Cleaned valves (SEM). Scale bars = 5 μm (Fig. 107), 2 μm (Figs 106, 109–111), 1 μm (Fig. 108). Arrows = k, l, m, n: single row of areolae present on the valve mantle; o: apical pore field.

### Geometric morphometrics

Morphological differences between the analysed groups were evidenced by means of valve shape analysis (Fig. 116). Similarity tests revealed statistically significant differences between the three analyzed taxa on the basis of the normalized geometric coordinates of the morphological landmarks (Table 1).

### DISCUSSION

At present, 23 taxa are placed in the genus *Pseudostaurosira* (Table 2), a genus originally described by Williams & Round (1987). Some taxa have been transferred to the genus *Pseudostaurosira* without any taxonomic argumentation or consultation of the type materials, such as *P. brevistriata* var. *binodis*, *P. brevistriata* var. *capitata*, *P. brevistriata* var. *elliptica*, *P. brevistriata* var. *inflata* and *P. brevistriata* var. *nipponica* (Edlund 1994; Andresen *et al.* 2000; Kingston 2000; Mayama *et al.* 2002). Common features in *Pseudostaurosira* are the reduced number of areolae per stria, the wide central sternum, the shape of the velum (with volae usually branched), the lack of rimoportulae and the small size (or absence) of the apical pore fields (Round *et al.* 1990). The presence and the position of spines are variable within the genus. The majority of taxa have spines situated on the vimines, but in some others they are on the virgae (e.g. *Pseudostaurosira pseudoconstruens*) or absent (e.g. *Pseudostaurosira parasitica* or *Pseudostaurosira*
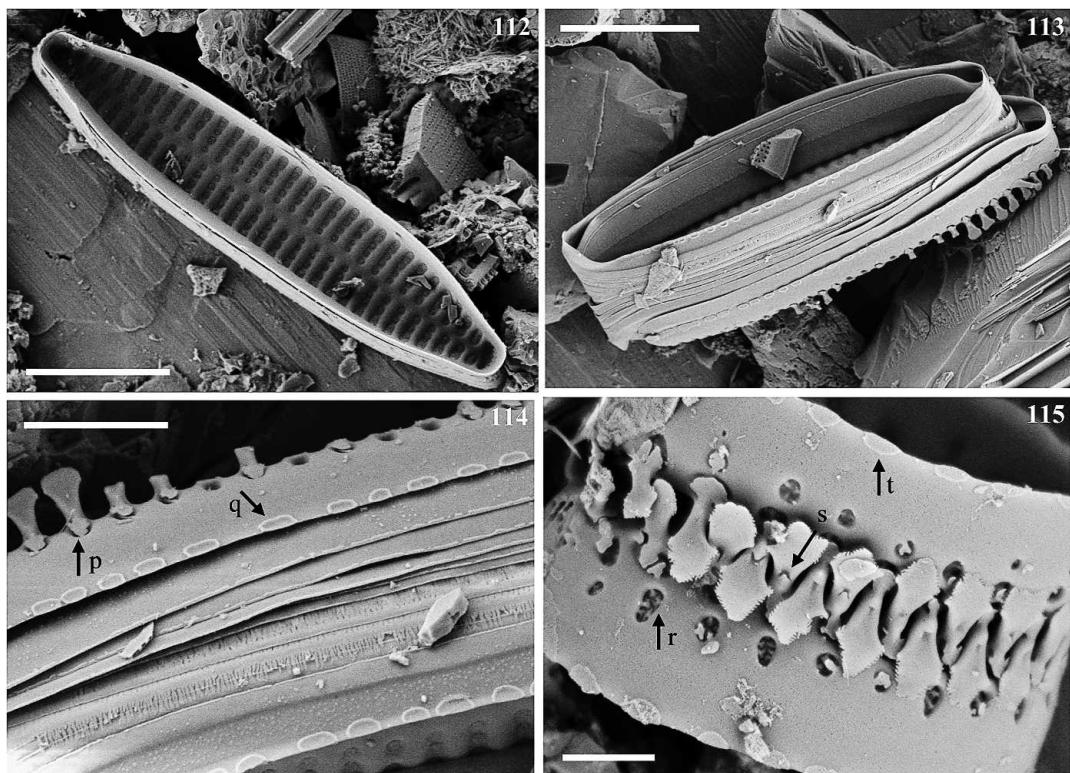
**Figs 112–115.** *Pseudostaurosira americana*. Holotype population from the Norwalk River at South Wilton, Fairfield County, Connecticut, Academy of Natural Sciences (ANSP) G.C. 106191a. Cleaned valves (SEM). Scale bars = 5 µm (Figs 112, 113), 2 µm (Fig. 114), 1 µm (Fig. 115). Arrows = p: flattened projection subtending each spine and covering the valve mantle areola; q: mantle plaques; r: volae; s: lateral projection on the base of the spine; t: mantle plaques.

*microstriata*). In *Pseudostaurosira laucensis* spines are present on both the vimines and virgae, forming a continuous row along the valve face margin.

The three taxa analysed in the present study conform to the genus *Pseudostaurosira* on the basis of the presence of the aforementioned features. In all three taxa the spines are situated on the vimines. In contrast with many other *Pseudostaurosira* taxa, *Pseudostaurosira americana* typically has a narrow central sternum. The decrease in size of the areolae toward the axial area of the valve is less pronounced in *P. americana* than in *P. alvareziae*. This feature is present in many other *Pseudostaurosira* representatives, but its variability has not yet been properly assessed. Flattened, rounded projections covering the areolae (present in *P. alvareziae* and *P. americana*) are increasingly reported in representatives of *Pseudostaurosira* (Morales 2001; Morales & Edlund 2003; Siver *et al.* 2005). Serrate spines have been reported in *Pseudostaurosira trainorii* (Morales 2001), a taxon that is widespread in North America and apparently also present in Europe (E.A. Morales, pers. obs). The extent of the occurrence of serrate spines in *Pseudostaurosira* is unknown, and further studies are needed to determine if this is a widespread character.

**Pseudostaurosira subsalina: comparison with morphologically related taxa**

Since its description, *F. construens* var. *subsalina* has been subjected to several nomenclatural changes that were not based on SEM observations. Furthermore, some authors have presented SEM photographs of taxa that were misidentified as *F. construens* var. *subsalina* (e.g. Fabri & Leclercq 1984; Vyverman 1991; Witak 2001), as taxa are illustrated with the spines situated on the virgae, not on the vimines as in the lectotype material.

*Pseudostaurosira subsalina* resembles some taxa of the ‘*brevistriata*’ group such as *Pseudostaurosira polonica* or *Pseudostaurosira tenuis*, but these taxa differ by usually having only one areola per stria on the valve face (figs 39–41, 43, 44, 45–50 in Morales & Edlund 2003). Moreover, in *P. polonica* the areolae are elliptical and larger, with a higher number of volae (figs 45, 46, 49, 50 in Morales & Edlund 2003) than in *P. subsalina* (Figs 95, 111). In *P. tenuis* the areolae are similar to those in *P. subsalina* but have volae projecting from the vimines (figs 39, 40, 43, 44 in Morales & Edlund 2003), not the virgae as in *P. subsalina* (Fig. 95). Both *P. polonica* and *P. tenuis* have closed girdle
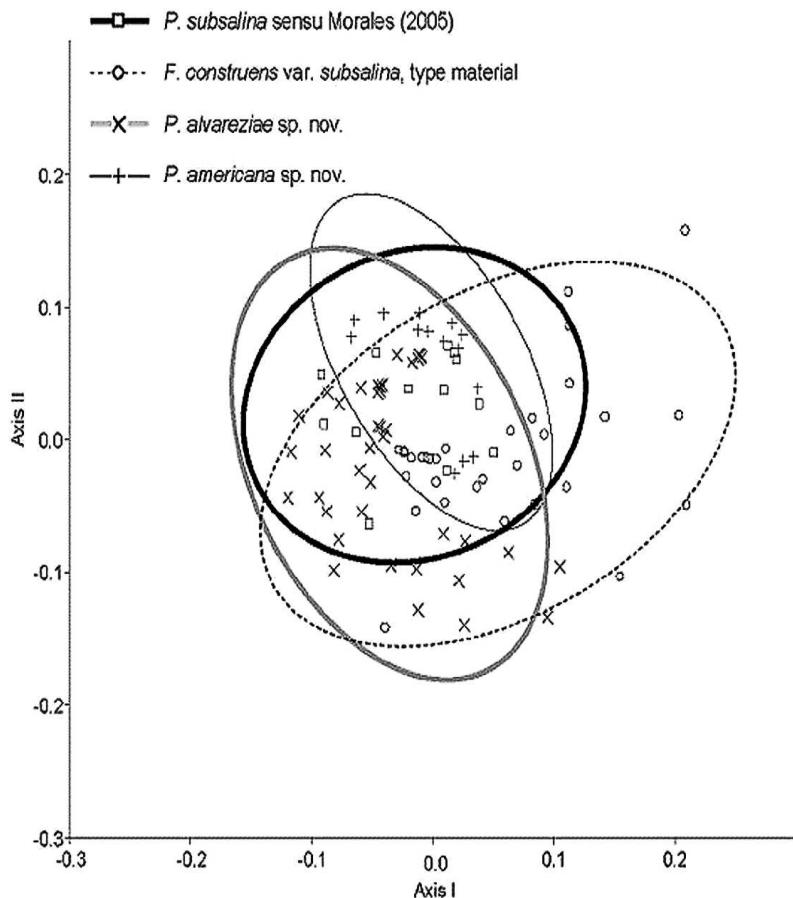
**Fig. 116.** Nonparametric multidimensional scaling plot of normalized coordinates for the morphological landmarks digitized on LM images of selected populations of *Pseudostaurosira*. See text for details. Confidence ellipses (α = 0.95) shown.

bands (Morales & Edlund 2003), whereas *P. subsalina* has open girdle elements (Fig. 99).

### *Pseudostaurosira alvareziae*: comparison with morphologically related taxa

*Pseudostaurosira alvareziae* resembles *P. subsalina* in many features such as the position and shape of the spines and mantle plaques, stria density, number of areolae per stria and the shape and orientation of the volae. However, both species differ in several features: (1) in *P. alvareziae* valves are on average shorter and narrower than in *P. subsalina*; (2) the apices are subrostrate in *P. subsalina* and broadly rounded in *P. alvareziae*; (3) the sternum in *P. subsalina* is lanceolate and wider, whereas in *P. alvareziae* it can be lanceolate and narrower and sometimes linear or irregular and (4) the decrease in the areolar diameter toward the axial area is more gradual in *P. alvareziae* and more abrupt in *P.*

**Table 1.** ANOSIM test performed on the Cartesian coordinates of resulting groups in the NMDS using the Euclidean distance measure. *P* values shown. Significant differences at α = 0.95 are marked in bold.

|  | *Pseudostaurosira alvareziae* sp. nov. | *Fragilaria construens* var. *subsalina*, lectotype material | *Pseudostaurosira americana* sp. nov. |
|---|---|---|---|
| *Pseudostaurosira subsalina sensu* Morales 2005 | **0.0112** | **0.0001** | 0.3737 |
| *P. alvareziae* sp. nov. |  | **< 0.0001** | **0.0004** |
| *F. construens* var. *subsalina*, lectotype material |  |  | **< 0.0001** |

**Table 2.** List of the 23 taxa placed in the genus *Pseudostaurosira.*

| Taxon name |
| --- |
| *P. binodis* (Ehrenberg) Edlund in Edlund *et al.* 2001 |
| *P. brevistriata* (Grunow in Van Heurck) D.M. Williams & Round 1987 |
| *P. brevistriata* var. *binodis* (Pantocsek) N.A. Andresen, Stoermer & Kreis 2000 |
| *P. brevistriata* var. *capitata* (Héribaud) N.A. Andresen, Stoermer & Kreis 2000 |
| *P. brevistriata* var. *elliptica* (Héribaud) J.C. Kingston 2000 |
| *P. brevistriata* var. *inflata* (Pantocsek) Edlund 1994 |
| *P. brevistriata* var. *nipponica* (Skvortsov) H. Kobayasi in Mayama *et al.* 2002 |
| *P. clavatum* E.A. Morales 2002 |
| *P. elliptica* (Schumann) Edlund, E.A. Morales & S. Spaulding 2004 |
| *P. laucensis* (Lange-Bertalot & Rumrich) E.A. Morales & Vis 2007 |
| *P. laucensis* var. *vulpina* (Lange-Bertalot & Rumrich) E.A. Morales 2007 |
| *P. microstriata* (Marciniak) Flower 2005 |
| *P. microstriata* var. *spinosa* Flower 2005 |
| *P. naveana* (Le Cohu) E.A. Morales & Edlund 2003 |
| *P. neoelliptica* (Witkowski) E.A. Morales 2002 |
| *P. parasitica* (W. Smith) E.A. Morales 2003 |
| *P. perminuta* (Grunow) Sabbe & Vyverman 1995 |
| *P. polonica* (Witak & Lange-Bertalot) E.A. Morales & Edlund 2003 |
| *P. pseudoconstruens* (Marciniak) D.M. Williams & Round 1987 |
| *P. robusta* (Fusey) D.M. Williams & Round 1987 |
| *P. tenuis* E.A. Morales & Edlund 2003 |
| *P. trainorii* E.A. Morales 2001 |
| *P. zeilleri* (Héribaud) D.M. Williams & Round 1987 |

*subsalina.* Despite these diagnostic differences, *P. alvareziae* has probably often been confused with *P. subsalina* (e.g. Blanco *et al.* 2008).

In LM, *P. alvareziae* also resembles *Pseudostaurosira neoelliptica*: both taxa have similar valve shape, wide and lanceolate sterna and punctuate striae. However, *P. neoelliptica* is smaller than *P. alvareziae* (Witkowski 1994). In SEM, the differences are distinct; *P. alvareziae* presents one areola per stria on the valve mantle (Figs 106, 108, 110), whereas *P. neoelliptica* presents two and sometimes three areolae (pl. 3, figs 1–6 in Morales 2002). The other noticeable difference between these taxa lies in the apical pore fields, composed by one row of pores in *P. alvareziae* (Figs 103, 106, 110) and by several rows in the case of *P. neoelliptica* (pl. 3, fig. 4 in Morales 2002). Both *P. subsalina* and *P. neoelliptica* are brackish-water species, whereas *P. alvareziae* was found in waters with low electrolyte content.

### *Pseudostaurosira americana*: comparison with morphologically related taxa

*Pseudostaurosira americana* was initially incorrectly identified as *P. subsalina* in Morales (2005) on the basis of similarity with specimens of the lectotype material of *P. subsalina* illustrated in Simonsen (1987) and Krammer & Lange-Bertalot (1991). In the present study, additional SEM images of the type population (Figs 112–115) and LM photographs of specimens for an additional sample from the type locality, but collected 6 years later, are presented (Figs 74–93).

The largest individuals of *P. americana* resemble *P. subsalina.* However, *P. subsalina* never has a truly linear sternum and has a lower stria density. *Pseudostaurosira americana* differs from *P. alvareziae* in having cuneate apices and a linear sternum. In addition, *P. americana* has higher stria density (16–18 in 10 µm compared with the 13–15 in 10 µm of *P. alvareziae*). *Pseudostaurosira americana* also resembles *P. neoelliptica*, but the first is wider (4.5–5 µm as opposed to 3–4 µm in *P. neoelliptica*) and has a higher stria density (12–15 in 10 µm in *P. neoelliptica*).

As expected, ANOSIM test results (Table 1) show that there are no statistically significant differences between *P. subsalina sensu* Morales 2005 and *P. americana*. The relative size of confidence ellipses in the multivariate plot (Fig. 116), which gives an idea of the morphological amplitude of the taxa, is quite broader in the case of the analysed type material of *F. construens* var. *subsalina*.

The study of the type materials of species that can easily be confused in LM is of crucial importance because diatoms are routinely used in water-quality monitoring. The misidentification of small, poorly known, but often quite common, species can lead to inaccurate water-quality assessments. This is especially relevant when the species involved have very different autoecological profiles, such as the brackish-water species *P. subsalina*, which can easily be confused with *P. alvareziae* (Blanco *et al.* 2008) or *P. americana* (Morales 2005, as *P. subsalina*), taxa found in waters with low and moderate electrolyte content respectively. It is therefore critical to review the type materials of species that were typified only by means of drawings, as was for example done for *P. elliptica* (Edlund *et al.* 2006), *Pseudostaurosira perminuta* (Grunow) Sabbe & Vyverman (Sabbe & Vyverman 1995) and *P. subsalina* (this study).

### REFERENCES

AENOR. 2004. *Norma española UNE-EN 13946:2004. Calidad del agua. Guía para el muestreo en rutina y el pretratamiento de diatomeas bentónicas de ríos.* AENOR, Madrid. 16 pp.

ANDRESEN N.A., STOERMER E.F. & KREIS R.G. JR. 2000. New nomenclatural combinations referring to diatom taxa which occur in the Laurentian Great Lakes of North America. *Diatom Research* 15: 413–418.

ANONYMOUS. 1975. Proposals for the standardization of diatom terminology and diagnoses. *Nova Hedwigia, Beiheft* 53: 323–354.

BESSE-LOTOTSKAYA A., VERDONSCHOT P.F.M. & SINKELDAM J.A. 2006. Uncertainty in diatom assessment: sampling, identification and counting variation. *Hydrobiologia* 566: 247–260.

BESZTERI B., ÁCS É. & MEDLIN L. 2005. Conventional and geometric morphometric studies of valve ultrastructural variation in two closely related *Cyclotella* species (Bacillariophyta). *European Journal of Phycology* 40: 89–103.

BLANCO S., ECTOR L., HUCK V., MONNIER O., CAUCHIE H.M., HOFFMANN L. & BÉCARES E. 2008. Diatom assemblages and water quality assessment in the Duero basin (NW Spain). *Belgian Journal of Botany* 141: 39–50, S1–S6.

BOOKSTEIN F.L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, New York. 435 pp.

COX E.J. & ROSS R. 1981. The striae of pennate diatoms. In: *Proceedings of the Sixth Symposium on Recent and Fossil Diatoms* (Ed. by R. Ross), pp. 267–278. Koeltz, Koenigstein, Germany.

EDLUND M.B. 1994. Additions and confirmations to the algal flora of Itasca State Park II. Diatoms from Chambers Creek. *Journal of the Minnesota Academy of Science* 59: 10–21.

EDLUND M.B., MORALES E.A. & SPAULDING S.A. 2006. The type and taxonomy of *Fragilaria elliptica* Schumann, a widely misconstrued taxon. In: *Proceedings of the Eighteenth International Diatom Symposium* (Ed. by A. Witkowski), pp. 53–59. Biopress Limited, Bristol, UK.

FABRI R. & LECLERCQ L. 1984. *Etude écologique des rivières du nord du massif Ardennais (Belgique): flore et végétation de diatomées et physico-chimie des eaux. 1. Contexte mésologique, méthodes, analyses physico-chimiques, synthèse taxonomique, écologique et floristique, iconographie, bibliographie*. Univ. Liège, Station Sci. Hautes-Fagnes, Robertville, Belgium. 379 pp.

FITZPATRICK F.A., WAITE I.R., D'ARCONTE P.J., MEADOR M.R., MAUPLIN M.A. & GURTZ M.E. 1998. *Revised methods for characterizing stream habitat in the National Water-Quality Assessment Program*. U.S. Geological Survey Water Resources Investigations Report 98-4052. Raleigh. 67 pp.

HALLEGRAEFF G.M. & BURFORD M.A. 1996. Some new or little known nanoplankton diatoms cultured from tropical and subtropical Australian shelf waters. *Nova Hedwigia, Beiheft* 112: 329–342.

HAMMER Ø., HARPER D.A.T. & RYAN P.D. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 1–9.

HENDEY N.I. 1964. *An introductory account of the smaller algae of British coastal waters: V. Bacillariophyceae (diatoms)*. Fishery Investigations Series IV. Her Majesty's Stationery Office. London. 317 pp.

HUSTEDT F. 1925. Bacillariales aus den Salzgewässern bei Oldesloe in Holstein. *Mitteilungen der Geographischen Gesellschaft und des Naturhistorischen Museums in Lübeck, 2. Reihe, Heft* 30: 84–121.

KINGSTON J.C. 2000. New combinations in the freshwater Fragilariaceae and Achnanthidiaceae. *Diatom Research* 15: 409–411.

KRAMMER K. & LANGE-BERTALOT H. 1991. *Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae*. In: *Süsswasserflora von Mitteleuropa* (Ed. by H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer). Vol. 2/3. G. Fischer, Stuttgart & New York. 576 pp.

KRAMMER K. & LANGE-BERTALOT H. 2000. *Bacillariophyceae Part 5: English and French translation of the keys* (Ed. by B. Büdel, G. Gärtner, L. Krienitz & G.M. Lokhorst). Vol. 2/5. Spektrum Akademischer, Heidelberg & Berlin. 311 pp.

KRAMMER K. & LANGE-BERTALOT H. 2004 (2nd edition). *Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae*. In: *Süsswasserflora von Mitteleuropa* (Ed. by H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer). Vol. 2/3. Spektrum Akademischer Verlag, Heidelberg & Berlin. 599 pp.

LE COHU R. 1999. Révision des principales espèces de Fragilariales (Bacillariophyta) des îles Kerguelen. *Canadian Journal of Botany* 77: 821–834.

MAYAMA S., IDEI M., OSADA K. & NAGUMO T. 2002. Nomenclatural changes for 20 diatom taxa occurring in Japan. *Diatom* 18: 89–91.

MORALES E.A. 2001. Morphological studies in selected fragilarioid diatoms (Bacillariophyceae) from Connecticut waters (U.S.A.). *Proceedings of the Academy of Natural Sciences of Philadelphia* 151: 105–120.

MORALES E.A. 2002. Studies in selected fragilarioid diatoms of potential indicator value from Florida (USA) with notes on the genus *Opephora* Petit (Bacillariophyceae). *Limnologica* 32: 102–113.

MORALES E.A. 2003. On the taxonomic status of the genera *Belonastrum* and *Synedrella* proposed by Round and Maidana (2001). *Cryptogamie, Algologie* 24: 277–288.

MORALES E.A. 2005. Observations of the morphology of some known and new fragilarioid diatoms (Bacillariophyceae) from rivers in the USA. *Phycological Research* 53: 113–133.

MORALES E.A. & EDLUND M.B. 2003. Studies in selected fragilarioid diatoms (Bacillariophyceae) from Lake Hovsgol, Mongolia. *Phycological Research* 51: 225–239.

MOULTON S.R., KENNEN J.G., GOLDSTEIN R.M. & HAMBROOK J.A. 2002. *Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program*. Open-file report 02-150. U.S. Department of the Interior, U.S. Geological Survey, Reston. 75 pp.

ROHLF F.J. 2004. *TpsDig, digitize landmarks and outlines, version 2.0*. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook.

ROHLF F.J. & SLICE D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* 39: 40–59.

ROUND F.E., CRAWFORD R.M. & MANN D.G. 1990. *The diatoms. Biology & morphology of the genera*. Cambridge University Press, Cambridge, UK. 747 pp.

SABBE K. & VYVERMAN W. 1995. Taxonomy, morphology and ecology of some widespread representatives of the diatom genus *Opephora*. *European Journal of Phycology* 30: 235–249.

SIMONSEN R. 1987. *Atlas and catalogue of the diatom types of Friederich Hustedt*. J. Cramer, Berlin - Stuttgart. Vol. 1 (catalogue). 525 pp. Vol. 2, 597 pp., pls 1–395; Vol. 3, 619 pp., pls 396–772.

SIVER P.A., HAMILTON P.B., STACHURA-SUCHOPLES K. & KOCIOLEK J.P. 2005. Diatoms of North America. The freshwater flora of Cape Cod, Massachusetts, U.S.A. *Iconographia Diatomologica* 14: 1–463.

VAN DE VIJVER B. & BEYENS L. 2002. *Staurosira jolinae* sp. nov. and *Staurosira circula* sp. nov. (Bacillariophyceae), two new fragilarioid diatoms from Subantarctica. *Nova Hedwigia* 75: 319–331.

VYVERMAN W. 1991. Diatoms from Papua New Guinea. *Bibliotheca Diatomologica* 22: 1–224.

WILLIAMS D.M. 2006. Some notes on the classification of *Fragilaria*, *Synedra* and their sub-groups. *Nova Hedwigia, Beiheft* 130: 17–34.

WILLIAMS D.M. & ROUND F.E. 1987. Revision of the genus *Fragilaria*. *Diatom Research* 2: 267–288.

WITAK M. 2001. A Late Glacial diatom flora from lacustrine sediments of Puck Bay, Southern Baltic Sea, Poland. In: *Lange-Bertalot-Festschrift. Studies on diatoms. Dedicated to Prof. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th birthday* (Ed. by R. Jahn, J.P. Kociolek, A. Witkowski & P. Compère), pp. 457–475. Gantner Verlag, Ruggell, Liechtenstein.

WITKOWSKI A. 1994. Recent and fossil diatom flora of the Gulf of Gdańsk, Southern Baltic Sea. Origin, composition and changes of diatom assemblages during the Holocene. *Bibliotheca Diatomologica* 28: 1–313.

# Are diatom diversity indices reliable monitoring metrics?

**Saúl Blanco • Cristina Cejudo-Figueiras • Loïc Tudesque •**
**Eloy Bécares • Lucien Hoffmann • Luc Ector**

**Abstract** A biological survey was carried out in 640 stations spread over the Loire-Bretagne National Network (France) between 1996 and 2000. Epilithic diatom inventories were obtained following standard methods. A total of 934 diatom taxa were identified. Common diversity indices (species richness, Shannon's diversity, equitability, dominance, etc.) were calculated and compared against abiotic factors in order to verify their reliability as biomonitoring metrics. Sampling stations were classified according to their trophic status (TP concentration). Several theoretical predictions about the relationship between community structural parameters and limnological variables were tested. In general, diversity indices exhibited poor linear correlations with environmental factors indicating ecological status. No clear patterns were found concerning species accumulation curves, occurrence-abundance, frequency-abundance and frequency distribution of diatom taxa between different trophic levels. In general, assemblages from stations with lower TP levels were characterized by relatively high dominances of certain taxa, mainly *Achnanthidium minutissimum*. In the light of these findings, the use of diatom diversity indices in biological quality surveillance protocols in continental waters is discouraged. Results are compared and discussed with similar studies.

**Keywords** Bioassessment • Rank/abundance • Shannon's index • Species accumulation • Species richness • Trophic level

S. Blanco (✉) • C. Cejudo-Figueiras • E. Bécares
Department of Biodiversity and Environmental Management, University of León, 24071 León, Spain
e-mail: sblal@unileon.es

S. Blanco • C. Cejudo-Figueiras • L. Hoffmann • L. Ector
Public Research Centre - Gabriel Lippmann, Department of Environment and Agro-Biotechnologies (EVA), 41 rue du Brill, 4422 Belvaux, Grand-Duchy of Luxembourg

L. Tudesque
Laboratoire Evolution et Diversité Biologique, UMR 5174 (UPS/CNRS), Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex 9, France

## Introduction

Diatom-based water quality monitoring has become a routine practice in many aquatic environments worldwide. The structure of periphytic diatom communities usually exhibits a strong dependence on many abiotic factors, especially those reflecting the "biological quality" of the water mass, a term that gathers many different aspects such as nutrient concentrations, habitat disturbances or presence of micropollutants. Hence, the abundance of many "indicator" taxa correlates with important limnological variables, this being the basis for the implementation

of diatom indices for the diagnosis and surveillance of freshwater ecosystem health. The use of such metrics in environmental monitoring programs during the last decades, particularly in lotic systems, has been thoroughly assessed in the scientific literature (see this and previous volumes of the *Proceedings of International Symposium Use of Algae for Monitoring Rivers*).

In this context, many attempts have been carried out to assess the comparative performance of diatom-based methods (e.g. Blanco et al., 2007). In general, two main groups of metrics using diatom communities have been historically proposed: autoecological indices and diversity indices. The first ones are frequently based on the average of sensitivity values of the taxa present in the samples, weighted by their relative frequencies and their ecological amplitudes, making use of the niche requirements and habitat preferences of the individual species or higher taxonomic groupings (Ector & Rimet, 2005; de la Rey et al., 2008). On the other hand, the use of diversity measures for water quality assessment is based on the assumption that impairment (e.g. pollution, eutrophication, etc.) causes a decline in diversity, as the abundance of certain intolerant taxa decreases while tolerant species outcompete the others (Archibald, 1972; Patrick, 1973). However, fertilization may also enhance community diversity by favouring the establishment and growth of several species (Canning-Clode et al., 2008). In fact, one of the most fruitful theoretical paradigms in current biology is the relationship between diversity and productivity in both terrestrial and aquatic ecosystems. Several meta-studies (Waide et al., 1999; Bonn et al., 2004; Gillman & Wright, 2006) reported monotonic, unimodal or nonsignificant relationships between these variables depending on the spatial scale covered (Foody, 2004; Drake et al., 2008) and, secondarily, on the presence of competitive feedbacks and complex environmental influences (Molis, 2002; Grace et al., 2007). This variability has made diversity indices unsuitable for evaluating ecological conditions, although they are frequently used in bioassessment studies, especially since a high species diversity of

diatoms usually reflects a high diversity of other forms of life (John, 2003).

**Table 1** Theoretical predictions on the relationship between benthic diatom diversity and trophic status; based on Magurran (2004) and references therein.

| Diagram | Response to increasing TP levels |
| --- | --- |
| TP/Diversity | Linear, negative and significant correlation |
| Species accumulation curves | Lower species richness in eutrophic sites |
| Rank-abundance curves | Higher dominancy in eutrophic sites |
| Frequency distribution curves | Histograms tend to follow geometric and not lognormal distributions |
| Abundance/occurrence | Unimodal response of occupancy |

This paper presents a statistical exploration of the relationships between a set of physical and chemical variables and selected structural parameters of phyto-benthic diatom assemblages collected in the Loire-Bretagne River Basin (France), focused on the trophic status (measured as water total phosphorus concentration [TP]) as a major environmental factor contributing to variation in algal communities at the basin-scale (Foerster et al., 2004). Our aim is to assess the usefulness of diatom diversity indices as biomonitoring metrics for lotic habitats, testing the following predictions: i) a linear, negative and significant correlation between TP and diversity, as found by many authors (e.g. Ní Chatháin & Harrington, 2008; Ndiritu et al., 2006), ii) significantly different patterns in the species accumulation, frequency-abundance, rank-abundance and frequency distribution diagrams for diatom communities appearing under different trophic levels according to the hypotheses described in Magurran (2004) (Table 1), and iii) higher occupancy values for taxa appearing in assemblages under mesotrophic conditions, since

generalist species that tolerate a wide range of environmental conditions occur at most sites, whereas specialist species are restricted to a few sites where their more stringent requirements are met (Heino & Soininen, 2006).

## Methods

A biological survey was carried out in 640 stations spread over the Loire-Bretagne National Network (France) between 1996 and 2000. Common water physical and chemical parameters were measured *in situ* or determined in the laboratory following standard methods (APHA, 1995). Sampling sites were classified according to their trophic status into four categories: [TP] < 100 μg·L$^{-1}$, [TP] 100-200 μg·L$^{-1}$, [TP] 200-300 μg·L$^{-1}$ and [TP] > 300 μg·L$^{-1}$. Diatom samples were taken and processed following European standards (UNE EN 13946 and UNE-EN 14407).

**Table 2** Spearman's rank correlation indices between diversity indices and environmental variables; n = 640; significant values (p < 0.05) in bold

| | Turbidity | [O$_2$] | DOC | Conductivity | [NO$_3$] | [NO$_2$] | [NH$_4^+$] | [PO$_4^{3-}$] | BOD | COD | NKJ | TP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Berger-Parker's index | -0.06 | 0.01 | **-0.08** | -0.03 | **-0.11** | **-0.09** | -0.05 | -0.06 | -0.03 | 0.11 | -0.06 | -0.06 |
| Fisher's index | 0.00 | **-0.12** | **0.09** | 0.04 | 0.05 | -0.04 | -0.07 | -0.02 | -0.03 | -0.11 | -0.06 | **-0.08** |
| Equitability | 0.03 | -0.07 | **0.11** | **0.10** | **0.19** | **0.13** | **0.08** | **0.12** | 0.06 | -0.09 | **0.11** | **0.12** |
| Margalef's index | 0.00 | **-0.12** | **0.08** | 0.04 | 0.05 | -0.04 | -0.07 | -0.02 | -0.03 | -0.11 | -0.06 | **-0.08** |
| Menhinick's index | 0.00 | **-0.12** | **0.08** | 0.03 | 0.05 | -0.04 | -0.06 | -0.03 | -0.03 | -0.11 | -0.06 | **-0.08** |
| Evenness | 0.03 | -0.06 | **0.09** | **0.09** | **0.19** | **0.14** | **0.10** | **0.13** | 0.06 | -0.09 | **0.12** | **0.14** |
| Simpson's index | 0.03 | -0.05 | **0.09** | 0.06 | **0.15** | **0.11** | 0.06 | **0.08** | 0.03 | -0.12 | 0.07 | 0.08 |
| Shannon's index | 0.02 | **-0.10** | **0.12** | **0.10** | **0.17** | **0.10** | 0.05 | **0.09** | 0.04 | -0.10 | 0.07 | -0.07 |
| Dominance | -0.03 | 0.05 | **-0.09** | -0.06 | **-0.15** | **-0.11** | -0.06 | **-0.08** | -0.03 | 0.12 | -0.07 | -0.08 |
| Species richness | 0.00 | **-0.12** | **0.08** | 0.04 | 0.04 | -0.05 | -0.07 | -0.02 | -0.03 | -0.11 | -0.06 | -0.08 |

Epilithon was sampled on submerged stones in the flow, euphotic zone of each stream, using a toothbrush, and preserved in 4% v/v formaldehyde. Clean frustule suspensions were obtained by oxidizing organic matter with hot hydrogen peroxide 30% v/v. Carbonate inclusions were removed adding a few drops of hydrochloric acid. Permanent microscopic slides were mounted using a refractive resin (Naphrax®). At least 400 valves were identified and counted under 1000x light microscopy. Taxonomy and nomenclature followed Krammer & Lange-Bertalot (1986-1991) and other usual reference works. Common diversity indices were obtained using PAST software (Hammer et al., 2007) and correlated to environmental variables by calculating pairwise correlation coefficients. Despite trying several transformations, measured variables did not follow normal distributions (Kolmogorov-Smirnov test, p < 0.05); therefore nonparametric Spearman rank correlation tests ($\rho_S$) were calculated. Additionally, the use of ordinal statistics allows the detection of correlations between variables irrespective of the linearity of their relationship, as long as these can fit rather logarithmic curves (Blanco et al., 2007). Frequency-abundance, rank-abundance and frequency distribution diagrams were constructed following the specifications of Magurran (2004). Data were adjusted to best-fit curves using the Levenberg-Marquardt nonlinear regression algorithm (Moré, 1977) and significant differences between resulting lines were assessed by means of Kruskal-Wallis nonparametric one-way analysis of variance test. Goodness-of-fit was verified using $\chi^2$ tests. All statistical analyses were performed using STATISTICA v. 8.0 software (StatSoft, Inc., 2008).

## Results

Spearman rank correlation coefficients between diatom diversity indices and environmental variables are shown in Table 2. Evenness was the index with the highest average coefficients, while DOC was the abiotic factor that best correlated with diversity indi-

ces, although in general physical and chemical variables were weakly and inconsistently correlated with diversity indices, with both positive and negative relationships. Figure 1 represents Shannon's index and species richness plotted against TP concentrations. Both variables were negatively correlated to TP, but achieved better fitting coefficients with quadratic than with linear curves (Shannon's index, quadratic: $R = 0.14$, $p < 10^{-5}$, linear: $\rho_S = -0.07$, $p = 0.056$; species richness, quadratic: $R = 0.16$, $p < 10^{-5}$, linear: $\rho_S = -0.08$, $p = 0.056$), suggesting a rather unimodal response of diversity to increasing trophic levels. According to the regression equation, maximal diversity scores appear at relatively high TP levels ([TP] = 409 $\mu g \cdot L^{-1}$).
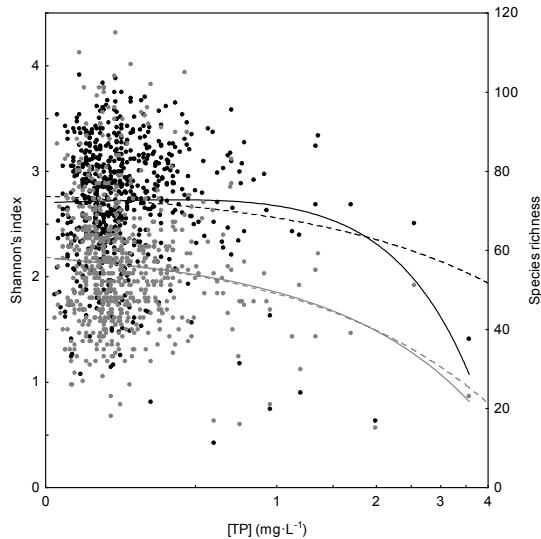


**Fig. 1** Correlation between TP concentration and two benthic diatom diversity indices: Shannon's index (black) and species richness (grey); data fitted to linear (dotted) or quadratic (continuous) curves.

Figure 2 shows species accumulation curves (number of taxa gathered at increasing sampling efforts) for the different trophic levels. The relative position of regression lines indicates that, for a given number of samples, sites with lower TP concentrations tend to have higher species richness (Kruskal-

Wallis test: $H = 12.53$, df = 3, $n = 384$, $p = 0.0058$). Figure 3 presents the relationship between frequency of occurrence in the analysed samples and local abundance of taxa at contrasting TP levels. ANOSIM test results indicate significant differences between regression lines ($p < 0.001$), showing a direct correspondence between TP and regional occupancy.
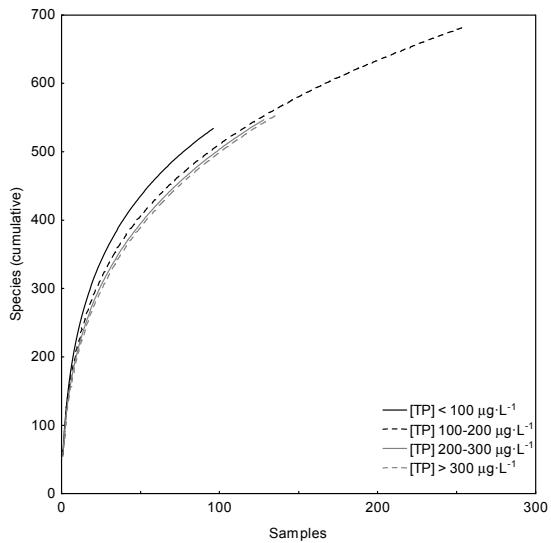


**Fig. 2** Species accumulation curves for samples taken under different trophic levels; data (omitted) fitted to logarithmic curves ($p < 0.001$).

Figure 4 shows a rank/abundance diagram for taxa collected at different trophic levels. This plot represents the number of individuals counted for each species ordered by decreasing number of individuals. The most diverse diatom communities appear under moderate trophic statuses (100-200 $\mu g \cdot L^{-1}$ [TP]), while assemblages from stations with lower TP levels were characterized by relatively high dominances of certain taxa, mainly *Achnanthidium minutissimum* (Kützing) Czarnecki, that represented 17.7% of all the individuals on average. Despite the existence of significant differences among TP classes (rank/abundance: Kruskal-Wallis test: $H = 136.66$, df = 3, $n = 2136$, $p < 0.001$), there was no clear linear tendency in the diversity/TP relationship.

Frequency distributions of diatom species are presented in Figure 5. These diagrams show the number of species found with a certain number of individuals (those represented in the X axis and divided into $\log_2$ classes), providing a visual estimation of evenness. All distributions follow geometric rather than lognormal curves (Table 2), thus indicating high dominances regardless of trophic level (Kruskal-Wallis test: H = 0.85, df = 3, n = 56, p = 0.838). In general, 25% of the diatom species are represented by one single individual in the studied samples irrespective of the concentration of TP.
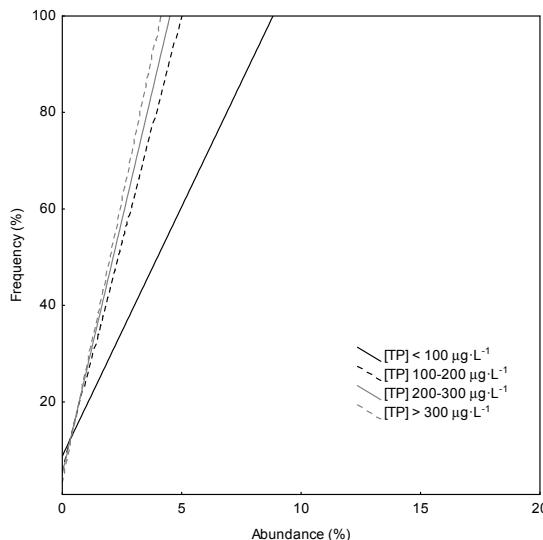


**Fig. 3** Relationship between frequency of occurrence and abundance of taxa collected in samples at different trophic levels; data (omitted) fitted to linear curves (p < 0.001).

**Discussion**

Characterization of environmental condition in rivers requires assessment of the structure of biotic assemblages and their response to changes in the abiotic factors. Diversity measures are often employed for this purpose, providing a rapid and synthetic overview of the ecological status. Several diversity indices have historically been proposed with different

particularities (see Magurran, 2004 for a review). Our data suggest that these metrics differ also in their sensitivity to increasing nutrient concentrations, although none of the studied indices exhibited clear and consistent relationships with limnological variables indicating eutrophication. Within these methods, Shannon's index and species richness appear to capture much of the structure of biological assemblages, being probably the most widely used diversity metrics. Results presented in this study indicate a hump-shaped response curve of these variables to increasing nutrient concentrations, peaking at relatively high water TP levels. Many works have paid attention to the diversity/fertility relationship, including several investigations on diatom communities.
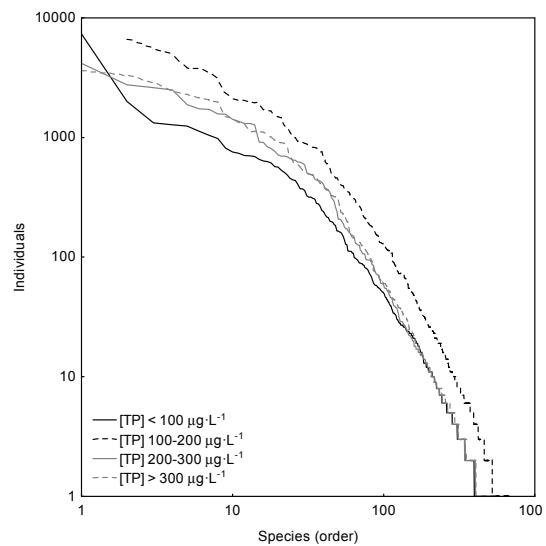


**Fig. 4** Rank/abundance diagram for species appearing at different trophic levels.

Classical approaches present a pattern of reduced diversity in enriched waters, with diversity indices significantly negatively correlated with nutrients (e.g. Archibald, 1972; Patrick, 1973). Moss (1973) suggested that species better adapted to nutrient enrichment would have an advantage under eutrophic conditions, resulting in an uneven distribution of individuals among taxa. However, positive
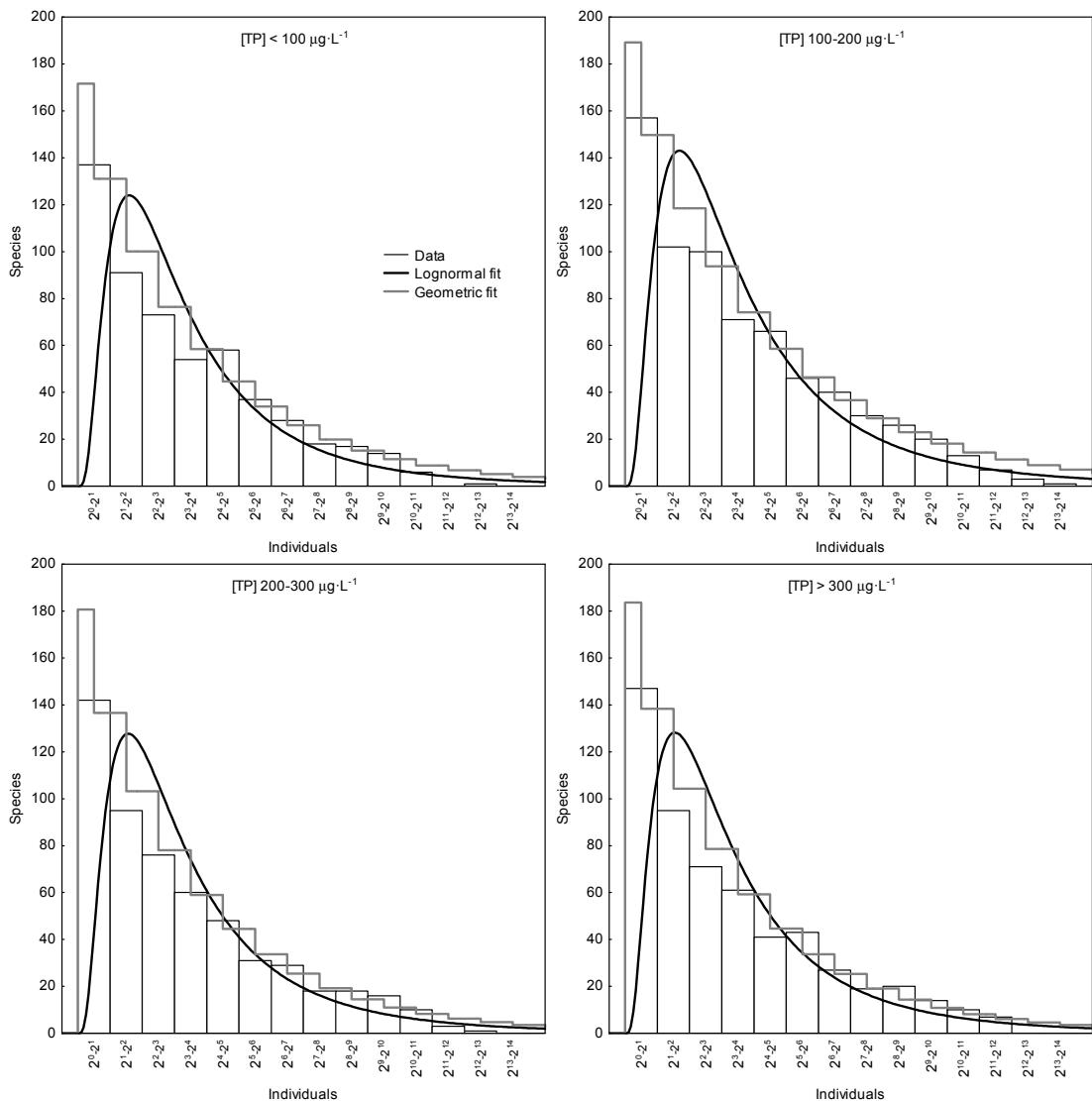
**Fig. 5** Frequency distribution histograms for taxa appearing at different trophic levels; data fitted to geometric or lognormal curves; see Table 3 for details.

relationships are not uncommon in the literature, e.g. Vilbaste & Truu (2003) and Lavoie et al. (2008) reported a tendency to increase Shannon's index with growing water trophy. Analogue results were observed by Marcus (1980), Maznah & Mansor (2002) or Bergfur (2007).

**Table 3** Goodness-of-fit tests for the frequency distribution histograms presented on Figure 5; df = 13; $\chi^2$ values shown.

|  | Geometric fit | Lognormal fit |
|---|---|---|
| $< 100 \ \mu g \cdot L^{-1}$ | 21.88 | 61.75 |
|  | (p = 0.057) | (p < 10$^{-5}$) |
| $100\text{-}200 \ \mu g \cdot L^{-1}$ | 16.07 | 68.41 |
|  | (p = 0.244) | (p < 10$^{-5}$) |
| $200\text{-}300 \ \mu g \cdot L^{-1}$ | 13.70 | 52.53 |
|  | (p = 0.395) | (p < 10$^{-5}$) |
| $> 300 \ \mu g \cdot L^{-1}$ | 16.88 | 59.91 |
|  | (p = 0.205) | (p < 10$^{-5}$) |

On the other hand, the intermediate, disturbance hypothesis, which states that diversity peaks at an intermediate frequency or intensity of disturbance (Connell, 1978), has been confirmed in many field studies concerning benthic diatoms (e.g. Lobo et al., 1995; Biggs & Smith, 2002). This is consistent with the currently generalised idea that productivity determines maximum species richness. According to Cox et al. (2006), for a given ecosystem there is a boundary that places an upper limit on species coexistence across the trophic gradient. However, as Molis (2002) states, these unimodal responses are dependent on the chosen disturbance estimator and diversity parameter. Moreover, they are non-persistent in succession, since diversity peaks quickly during colonization (Oemke & Burton, 1986). Chase & Leibold (2002) and Harrison et al. (2006) proposed that the relationship of productivity to species diversity is usually positive at regional scales, but is often neutral, unimodal or negative at local spatial scales, although Gillman & Wright (2006), in a comprehensive literature review, confirmed only positive relationships in studies that used data of continental to global extent and in most of those of regional or local

extent. Soininen (2009) has recently reviewed this question, observing that, for stream diatoms, the diversity-productivity relationship exhibits a large variability among different spatial scales, suggesting the presence of other variables than nutrients as the main determining factors of diatom diversity. Additionally, historical and biogeographical constraints may explain these patterns better than contemporary environmental conditions (Vyverman et al., 2007). This complex variability may explain to a certain extent the usual finding of weak or nonsignificant correlations between diversity and productivity parameters in diatom studies (e.g. Wu & Kow, 2002; Bellinger et al., 2006; Simkhada et al., 2006).

Traditional regression techniques are insufficient to characterize productivity-diversity relationships (Cox et al., 2006), so we complemented our assessment with graphical analyses of the frequency and abundance of diatom taxa along a TP gradient that, however, lead to contradictory results. Species accumulation curves suggested a negative correlation between species richness and TP concentrations, while the rank/abundance diagram better fits with a unimodal productivity-diversity response.

One important issue addressed in the present study is the dependence of occupancy on trophic status. Data shown indicate that the slope of the occurrence/abundance relationship is significantly lower for assemblages inhabiting waters with TP concentrations below 100 $\mu g \cdot L^{-1}$. Positive relationships between abundance and occupancy are one of the most general patterns in ecology, but have been seldom related to environmental factors. It has been demonstrated that regional occupancy is positively related to niche breadth (Heino & Soininen, 2006), so our data would suggest narrower ecological amplitudes in species indicating low trophic levels, a hypothesis that deserves further investigations.

Data presented here describe productivity-diversity relationships over several years within a relatively large geographical area, while maintaining standard methods and sampling regimes, so that observed patterns are likely not biased by methodological differences (Cox et al., 2006). In the light of

these results, two alternative conclusions can be depicted: i) the TP gradient at the basin scale in the studied rivers was too weak to consistently alter the productivity-diversity relationship or ii) the TP level is indeed a poor predictor of benthic diatom diversity, indicating that diatom diversity indices are not suitable for monitoring stream TP levels. Further testing with additional data from different regions will allow assessing the utility of these metrics as water quality proxies at different spatial and temporal scales.

# References

APHA, 1995. Standard Methods for the Examination of Water and Wastewater. APHA, Washington.

Archibald, R. E. M., 1972. Diversity in some South African diatom associations and its relation to water quality. Water Research 6: 1229-1238.

Bellinger, B., C. Cocquyt & C. O'Reilly, 2006. Benthic diatoms as indicators of eutrophication in tropical streams. Hydrobiologia 537: 75-87.

Bergfur, J., 2007. Ecological Integrity of Boreal Streams. Assessing Impacts on Community Structure and Function: Ph.D. thesis. Swedish University of Agricultural Sciences, Uppsala. Acta Universitatis Agriculturae Sueciae 50: 39 pp. http://tinyurl.com/ygj87cb

Biggs, B. J. F. & R. A. Smith, 2002. Taxonomic richness of stream benthic algae: effects of flood disturbance and nutrients. Limnology and Oceanography 47: 1175-1186.

Blanco, S., E. Bécares, H. M. Cauchie, L. Hoffmann & L. Ector, 2007. Comparison of biotic indices for water quality diagnosis in the Duero Basin (Spain). Archiv für Hydrobiologie, Supplement Large Rivers 17: 267-286.

Bonn, A., D. Storch & K. J. Gaston, 2004. Structure of the species-energy relationship. Proceedings of the Royal Society of London B 271: 1685-1691.

Canning-Clode, J., M. Kaufmann, M. Molis, M. Lenz & M. Wahl, 2008. Influence of disturbance and nutrient enrichment on early successional fouling communities in an oligotrophic marine system. Marine Ecology 29: 115-124.

Chase, J. M. & M. A. Leibold, 2002. Spatial scale dictates the productivity-diversity relationship. Nature 416: 427-430.

Connell, J. H., 1978. Diversity in tropical rain forests and coral reefs. Science 199: 710-723.

Cox, S. B., P. B. Christopher, R. D. Stevens & L. F. Huenneke, 2006. Productivity and species richness in an arid ecosystem: a long-term perspective. Plant Ecology 186: 1-12.

de la Rey, P. A., L. van Rensburg & A. Vosloo, 2008. On the use of diatom-based biological monitoring. Part 1: A comparison of the response of diversity and autecological diatom indices to water quality variables in the Marico-Molopo River catchment. Water SA 34: 53-60.

Drake, J. M., D. M. Lodge & C. Costello, 2008. Response. Ecological Applications 18: 1323-1324.

Ector, L. & F. Rimet, 2005. Using bioindicators to assess rivers in Europe: An overview. In Lek, S., M. Scardi, P. F. M. Verdonschot, J. P. Descy & Y. S. Park (eds), Modelling Community Structure in Freshwater Ecosystems. Springer, Berlin, Heidelberg, New York: 7-19.

Foerster, J., A. Gutowski & J. Schaumburg, 2004. Defining types of running waters in Germany using benthic algae: A prerequisite for monitoring according to the Water Framework Directive. Journal of Applied Phycology 16: 407-418.

Foody, G. M., 2004. Spatial nonstationarity and scale-dependency in the relationship between species richness and environmental determinants for the sub-Saharan endemic avifauna. Global Ecology and Biogeography 13: 315-320.

Gillman, L. N. & S. D. Wright, 2006. The influence of productivity on the species richness of plants: a critical assessment. Ecology 87: 1234-1243.

Grace, J. B., T. M. Anderson, M. D. Smith, E. Seabloom, S. J. Andelman, G. Meche, E. Weiher, L. K. Allain, H. Jutila, M. Sankaran, J. Knops, M. Ritchie & M. R. Willig, 2007. Does species diversity limit productivity in natural grassland communities? Ecology Letters 10: 680-689.

Hammer, Ø., D. A. T. Harper & P. D. Ryan, 2007. PAST PAlaeontological STatistics, ver. 1.66. http://folk.uio.no/ohammer/past/.

Harrison, S., H. D. Safford, J. B. Grace, J. H. Viers & K. F. Davies, 2006. Regional and local species richness in an insular environment: serpentine plants of Califor-

nia. Ecological Monographs 76: 41-56.

Heino, J. & J. Soininen, 2006. Regional occupancy in unicellular eukaryotes: a reflection of niche breadth, habitat availability or size-related dispersal capacity? Freshwater Biology 51: 672-685.

John, J., 2003. Bioassessment on health of aquatic systems by the use of diatoms. In Ambasht, R. S. & N. K. Ambasht (eds), Modern Trends in Applied Aquatic Ecology. Kluwer Academic Publications, New York: 1-20.

Krammer, K. & H. Lange-Bertalot, 1986-1991. Bacillariophyceae. In Ettl, H., J. Gerloff, H. Heynig & D. Mollenhauer (eds), Süßwasserflora von Mitteleuropa. Band 2/1-4. Gustav Fischer Verlag, Stuttgart: 1-2484.

Lavoie, I, S. Campeau, F. Darchambeau, G. Cabana & P. J. Dillon, 2008. Are diatoms good integrators of temporal variability in stream water quality? Freshwater Biology 53: 827-841.

Lobo, E. A., K. Katoh & Y. Aruga, 1995. Response of epilithic diatom assemblages to water pollution in rivers in the Tokyo metropolitan area, Japan. Freshwater Biology 34: 191-204.

Magurran, A. E., 2004. Measuring Ecological Diversity. Blackwell Publishing, Oxford.

Marcus, M. D., 1980. Periphytic community response to chronic nutrient enrichment by a reservoir discharge. Ecology 61: 387-399.

Maznah, W. O. & M. Mansor, 2002. Aquatic pollution assessment based on attached diatom communities in the Pinang River Basin, Malaysia. Hydrobiologia 487: 229-241.

Molis, M., 2002. Shallow water fouling assemblages exposed to abiotic disturbance and stress: the structuring role of emersion and UV-radiation. Ph.D. thesis, Christian-Albrechts University, Kiel. http://tinyurl.com/yzcjqm8

Moré, J., 1977. Levenberg-Marquardt algorithm: implementation and theory. Notes in Mathematics 630: 105-116.

Moss, B., 1973. Diversity in fresh-water phytoplankton. American Midland Naturalist 90: 341-355.

Ndiritu, G. G., N. N. Gichuki & L. Triest, 2006. Distribution of epilithic diatoms in response to environmental conditions in an urban tropical stream, Central Kenya. Biodiversity and Conservation 15: 3267-3293.

Ní Chatháin, B. & T. J. Harrington, 2008. Benthic diatoms of the River Deel: diversity and community structure. Biology and Environment: Proceedings of the Royal Irish Academy 108B: 29-42.

Oemke, M. D. & T. M. Burton, 1986. Diatom colonization dynamics in a lotic system. Hydrobiologia 139: 153-166.

Patrick, R., 1973. Use of Algae, especially diatoms, in the assessment of water quality. In Cairns, J. Jr. & K. L. Dickson (eds), Biological Methods for the Assessment of Water Quality. American Society for Testing and Materials, Philadelphia: 23-47.

Simkhada, B., I. Jüttner & P. J. Chimonides, 2006. Diatoms in lowland ponds of Koshi Tappu, eastern Nepal – relationships with chemical and habitat characteristics. International Review of Hydrobiology 91: 574-593.

Soininen, J., 2009. Is diatom diversity driven by productivity in boreal streams? Diatom Research 24: 197-207.

StatSoft, Inc., 2008. STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Vilbaste, S. & J. Truu, 2003. Distribution of benthic diatoms in relation to environmental variables in lowland streams. Hydrobiologia 493: 81-93.

Vyverman, W., E. Verleyen, K. Sabbe, K. Vanhoutte, M. Sterken, D. A. Hodgson, D. G. Mann, S. Juggins, B. Van de Vijver, V. Jones, R. Flower, D. Roberts, V. A. Chepurnov, C. Kilroy, P. Vanormelingen & A. De Wever, 2007. Historical processes constrain patterns in global diatom diversity. Ecology 88: 1924-1931.

Waide, R. B., M. R. Willing, C. F. Steiner, G. Mittelbach, L. Gough, S. I. Dodson, G. P. Juday & R. Parmenter, 1999. The relationship between productivity and species richness. Annual Review of Ecology and Systematics 30: 257-300.

Wu, J. & L. Kow, 2002. Applicability of a generic index for diatom assemblages to monitor pollution in the tropical River Tsanwun, Taiwan. Journal of Applied Phycology 14: 63-69.

# NUTRIENT MONITORING IN SPANISH WETLANDS USING EPIPHYTIC DIATOMS

*C. CEJUDO-FIGUEIRAS[1,2*], S. BLANCO[1,2], I. ÁLVAREZ-BLANCO[1,2],
L. ECTOR[3], E. BÉCARES[1]*

[1] *Department of Biodiversity and Environmental Management, University of León, E-24071 León, Spain*
[2] *Institute of the Environment, La Serna 58, E-24007 León, Spain*
[3] *Department of Environment and Agro-Biotechnologies (EVA), Public Research Centre-Gabriel Lippmann,
Rue du Brill, 41, L-4422 Belvaux, Grand-duchy of Luxembourg*
* *Corresponding author: ccejf@unileon.es*

BIOASSESSMENT
DIATOMS
MACROPHYTES
MONITORING
SHALLOW LAKES
WATER FRAMEWORK DIRECTIVE

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 bioindicators 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 show 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 applicability 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 105105 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 epiphythic 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 ($NH_4^+$) were measured following APHA (1998). Sampling method follows the protocol for the collection of epiphytic diatoms in shallow lakes described by Blanco & Bécares (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 ($\rho_s^2$) 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 *Achnanthidium minutissimum* (Kütz.) Czarn. *Navicula trivialis* Lange-Bert., *Gomphonema italicum* Kütz. and *G. gracile* Ehrenb. dominated the assemblages
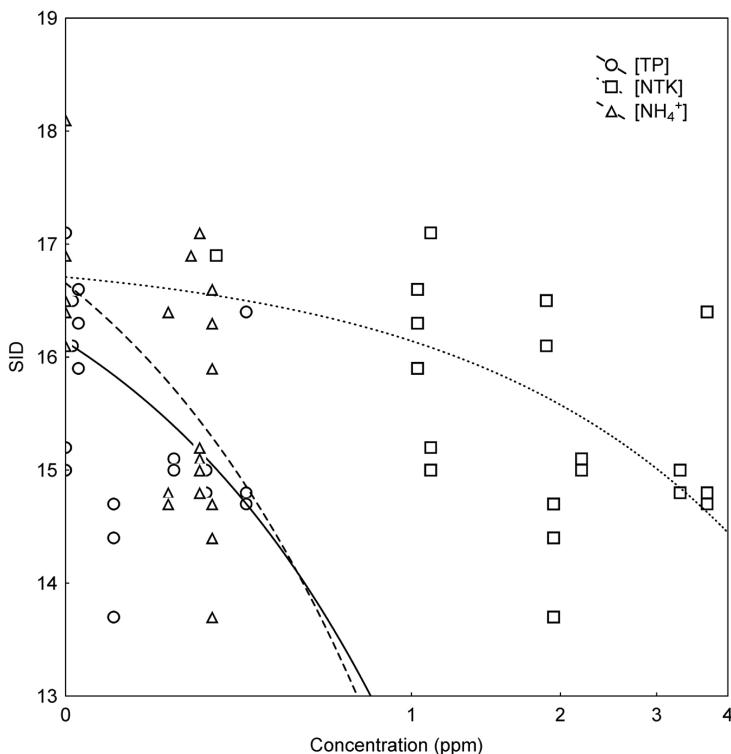
Fig. 1. – Plot of the SID index scores *versus* nutrient concentrations in the studied lakes. Data fitted to linear regression lines.

found at Lake Arkaute, while the most abundant taxa at Lake Betoño were *Epithemia adnata* (Kütz.) Bréb., *Gomphonema acidoclinatum* Lange-Bert. & E. Reichardt and *Achnanthidium 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 anagallisaquatica*. 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.
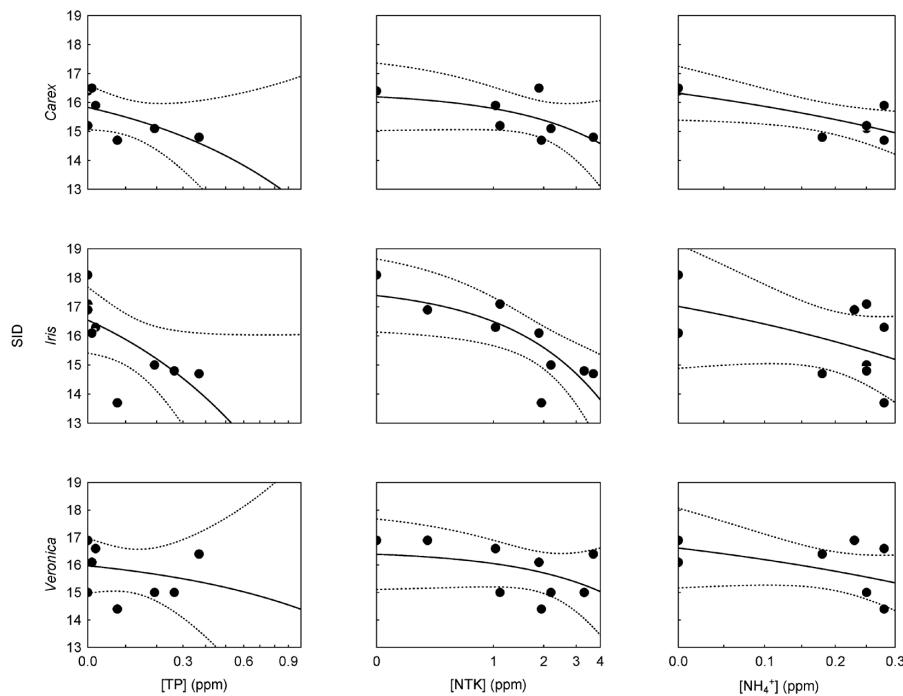
Fig. 2. – Regressions of the SID index scores calculated for the diatom assemblages growing on different plant substrata on nutrient correlations. Regression bands (95 % confidence limits) shown (dashed lines). Data fitted to linear regression lines.
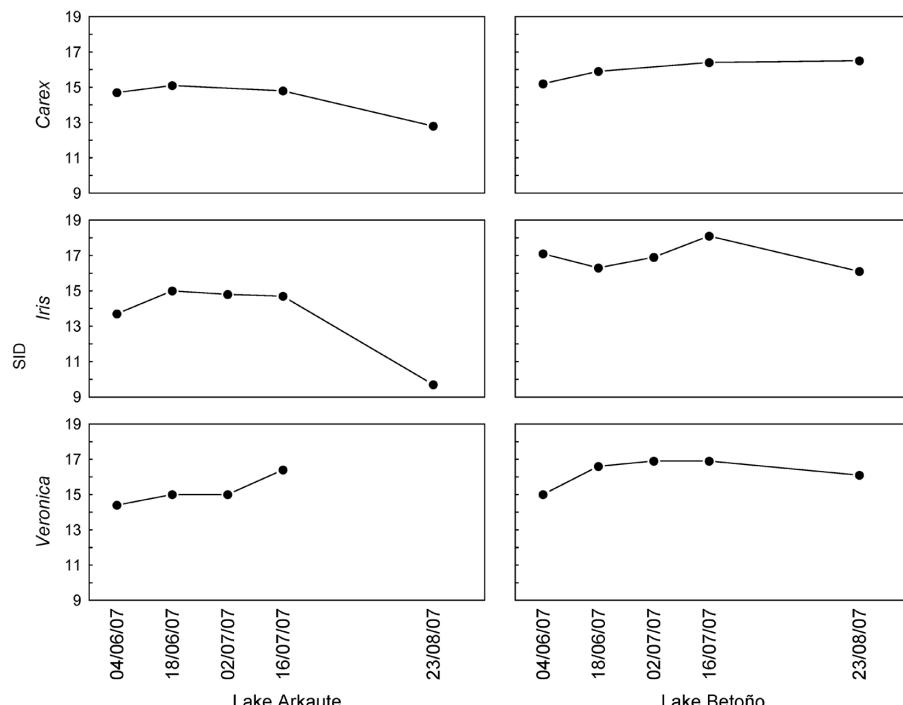


Fig. 3. – Temporal changes in the SID index scores calculated for the diatom assemblages growing on different plant substrata.

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 pseudocorus*, 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. Poulíčková *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. Kitner & Poulíčková (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, epipelic 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 Messyasz & 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.

## REFERENCES

Álvarez-Blanco I 2010. Spatio-temporal variations in diatom ecological profiles: indications for biomonitoring. *Limnology*. Accepted.

Ács É, Reskoné NM, Szabó K, Taba Gy, Kiss KT 2005. Application of epiphitic diatoms in water quality monitoring of lake Velence – recommendations and assignments. *Acta Bot Hung* 47: 211-223.

Ács É, Borsodi AK, Kiss É, Kiss KT, Szabó KÉ, Vladár P, Várbíró G, Záray Gy 2008. Comparative algological and bacteriological examinations on biofilms developed on different substrata in a shallow soda lake. *Aquat Ecol* 42: 521–531.

APHA 1998. Standard methods for the examination of water and wastewater. 20th ed. American Water Works Association, Washington, 1368 p.

Besse-Lototskaya A, Verdonschot PFM, Sinkeldam JA 2006. Uncertainty in diatom assessment, sampling, identification and counting variation. *Hydrobiologia* 566: 247-260.

Blanco S, Ector L, Bécares E 2004. Epiphytic diatoms as water quality indicators in Spanish shallow lakes. *Vie Milieu* 54: 71-79.

Blanco S, Bécares E 2006. Método de muestreo de diatomeas epífitas en lagunas para la aplicación de la Directiva Marco del Agua. *Tecnol Agua* 277: 42-47.

Blindow I 1987. The composition and density of epiphyton on several species of submerged macrophytes. The neutral hypothesis tested. *Aquat Bot* 29: 157-168.

Bolue Ingurumen Ikerketak 2008. Caracterización de las poblaciones de Galápagos autóctonos y control de las poblaciones de Galápagos exóticos del parque de Salburúa. Año 2008, Vitoria-Gasteiz, 49 p.

Cattaneo A, Amireault MC 1992. How artificial are artificial substrata for periphyton? *J N Am Benthol Soc* 11: 244-256.

Cattaneo A, Galanti G, Gentinetta S, Romo S 1998. Epiphytic algae and macroinvertebrates on submerged and floating-leaved macrophytes in an Italian lake. *Freshw Biol* 39: 725-740.

CEN 2003. Water quality – Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. EN 13946: 2003. Comité européen de Normalisation, Geneva.

Clarke KR 1993. Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* 18: 117-143.

Denys L 2004. Relation of abundance-weighted averages of diatom indicator values to measured environmental conditions in standing freshwater. *Ecol Indic* 4: 255-275.

Ector L, Rimet F 2005. Using bioindicators to assess rivers in Europe. An overview. *In* Modelling community structure in freshwater ecosystems, Lek S, Scardi M, Verdonschot PFM, Descy JP, Park YS eds, Springer-Verlag, Berlin, Heidelberg: 7-19.

Ekos 2006. Revisión del estado de conservación de las poblaciones de anfibios de los humedales de Salburúa, Vitoria-Gasteiz, 98 p.

Eminson D, Moss B 1980. The composition and ecology of periphyton communities in freshwaters. 1. The influence of host type and external environment on community composition. *Br Phycol J* 15: 429-446.

European Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *O J E C* L 327: 1-73.

Hilt S 2006. Allelopathic inhibition of epiphytes by submerged macrophytes. *Aquat Bot* 85: 252-256.

Hoffman G 1994. Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie. *Bibl Diatomol* 30: 1-241

King L, Clarke G, Bennion H, Kelly M, Yallop M 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *J Appl Phycol* 18: 15-25.

Kitner M, Poulíčková A 2003. Littoral diatoms as indicators for the eutrophication of shallow lakes. *Hydrobiologia* 506-509: 519-524.

Krammer K, Lange-Bertalot H 1986-1991. Süsswasserflora von Mitteleuropa Bacillariophyceae Teil 1-4. G. Fisher Verlag, Stuttgart, 2486 p.

Lange-Bertalot H ed 1995-2009. Iconographia Diatomologica. Annotated Diatom Micrographs. Vols I – XX. A. R. G. Gantner Verlag K. G.

Lange-Bertalot H ed 2000-2009. Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vols I-V. A. R. G. Gantner Verlag K. G.

Lecointe C, Coste M, Prygiel J 1993. OMNIDIA software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia* 269/270: 509-513.

Lecointe C, Coste M, Prygiel J, Ector L 1999. Le logiciel OMNIDIA version 2, une puissante base de données pour les inventaires de diatomées et pour le calcul des indices diatomiques européens. *Cryptogamie Algol* 20: 132-134.

Linares JE 2003. Las diatomeas bentónicas de las lagunas del Parque Nacional de Sierra Nevada. Estudio comparado con las colecciones del herbario de la Univ Granada (GDA). Ph D Diss, Univ Granada, Granada, 325 p.

Lobo Urrutia L, Sesma Ausejo J 2006. Ficha informativa de los humedales de Ramsar (FIR).

Messyasz B, Kuczyńska-Kippen N 2006. Periphytic algal communities, a comparison of *Typha angustifolia* L. and *Chara tomentosa* L. beds in three shallow lakes (West Poland). *Pol J Ecol* 54: 15-27.

Potapova M, Charles DF 2005. Choice of substrate in algae-based water-quality assessment. *J N Am Benthol Soc* 24: 415-427.

Poulíčková A, Duchoslav M, Dokulil M 2004. Littoral diatom assemblages as bioindicators of lake trophic status, a case study from perialpine lakes in Austria. *Eur J Phycol* 39: 143-152.

Rott E, Hofmann G, Pall K, Pfister P, Pipp E 1997. Indikationslisten für Aufwuchsalgen Teil 1, Saprobielle Indikation. Bundesministerium für Land- und Forstwirtschaft, Wien, 73 p.

Stenger-Kovács C, Buczkó K, Hajnal E, Padisák J 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL) developed in Hungary. *Hydrobiologia* 589: 141-154.

Trobajo Pujadas R 2007. Ecological analysis of periphytic diatoms in Mediterranean coastal wetlands (Empordà wetlands, NE Spain). *Diatom Monogr* 7: 1-210.

Winter JG, Duthie HC 2000. Stream epilithic, epipelic and epiphytic diatoms, habitat fidelity and use in biomonitoring. *Aquat Ecol* 34: 345-353.

Zimba PV, Hopson MS 1997. Quantification of epiphyte removal efficiency from submersed aquatic plants. *Aquat Bot* 58: 173-179.

# IV.

# Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited

*Cristina Cejudo-Figueiras*[A,C], *Irene Álvarez-Blanco*[A], *Eloy Bécares*[B] *and Saúl Blanco*[B]

[A]Institute of the Environment, La Serna 58, E-24007 León, Spain.
[B]Department of Biodiversity and Environmental Management,
  University of León, E-24071 León, Spain.
[C]Corresponding author. Email: ccejf@unileon.es

**Abstract.** For bioassessment of freshwaters, diatom indices have been mainly used in streams although their applicability in shallow lakes has been demonstrated in several studies. However, the influence of sampling substrata on periphytic diatom communities and on the ecological quality inferred from them has been paid little attention. In this paper, we test the 'neutral substrate hypothesis', which predicts no relevant influence of host plant type on their epiphytic community. Nineteen shallow permanent lakes from north-west Spain were studied and classified into three trophic levels. Epiphytic diatom communities growing on three different macrophytes for each trophic level were sampled and analysed. We assess: (1) which of the most common diatom indices provides a reliable water quality assessment, (2) how different plant substrata influence the diatom communities growing on them and (3) how these differences affect water quality assessment. Similarity tests showed significant differences in the composition of diatom assemblages among nutrient concentrations and host macrophytes. In contrast, ANOVA results for selected diatom-based metrics showed significant differences among trophic levels but not between different plant substrata. This supports the use of epiphytic diatoms as biological indicators for shallow lakes irrespective of the dominant macrophyte.

**Additional keywords:** bioassessment, biotic index, diatom assemblage, eutrophication, macrophytes, monitoring, shallow lake, Water Framework Directive.

## Introduction

Benthic diatoms have ecological and physiological features that make them suitable to monitor water quality in many aquatic environments. Several metrics (e.g. Blanco *et al.* 2007) based on ecology and relative abundances of species within epilithic diatom communities collected from rivers have been developed, yet similar metrics that have been developed specifically for lakes are scarce. Diatoms have potential value as ecological indicators of lacustrine environments in coastal wetlands (Trobajo 2003), mountain lakes (Linares 2003) and mediterranean ponds (Blanco *et al.* 2004). Sampling periphytic diatoms in shallow lakes, however, can present several methodological constraints owing, in many cases, to the absence of submerged hard substrates. Epiphyton may serve as a reliable, 'universal' community for biomonitoring, though physical and/or chemical constraints related to the architecture or physiology of submersed vegetation can influence epiphyte communities, owing to differences in colonisable surfaces or differential allelopathic effects (Cattaneo and Kalff 1980). Blanco *et al.* (2004) concluded that stalks of emergent macrophytes (helophytes) are the most suitable substrate in biomonitoring studies.

The 'neutral substrate hypothesis' (Blindow 1987) states that the structure of epiphytic communities is unaffected by host plant identity and reflects only the limnological characteristics of the water body. Although macrophytes are able to transfer a small amount of nutrients to their epiphytes, these interactions are of relatively minor importance (Cattaneo and Kalff 1979; Carignan and Kalff 1982). In contrast, Eminson and Moss (1980), investigating epiphytic communities in oligotrophic systems, reported that host-plant identity did influence the structure of epiphytic diatom assemblages. In this paper, we assess differences among diatom communities developed on different host plants in shallow lentic systems of contrasting trophic status, and compare diagnoses of environmental quality of these systems from several diatom-based indices. Our aim is to evaluate whether different substrata affect the reliability of diatom-based monitoring metrics in lentic habitats. According to previous works (Eminson and Moss 1980; Blindow 1987; Blanco *et al.* 2004), we hypothesise the absence of significant differences in the composition of the diatom communities related to host plants, together with a clear response of diatom indices to increasing nutrient concentrations.

## Materials and methods

### Study area

Duero River Basin (97 290 km$^2$, 40°17′N–43°1′N and 2°56′W–8°41′W) is on the northern Iberian plateau (700 m a.s.l.) and has

**Table 1.    Sampling design**

| Trophic level | *Scirpus* | | | *Typha* | | | *Myriophyllum* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lake | [TP] ($\mu g\,L^{-1}$) | | Lake | [TP] ($\mu g\,L^{-1}$) | | Lake | [TP] ($\mu g\,L^{-1}$) | |
| Low | Carucedo | 18 | | Diel | 10 | | Diel | 10 | |
| | Bercianos | 28 | | Santervás | 38 | | Santervás | 38 | |
| | Sentiz | 51 | | Sentiz | 51 | | Sentiz | 51 | |
| Medium | Antimio | 75 | | Bardal | 75 | | Antimio | 75 | |
| | Ribas | 95 | | Zarza | 88 | | Redos | 78 | |
| | Villaflores | 133 | | Berrueces | 111 | | Zarza | 88 | |
| High | Valdemorgate | 141 | | Villadangos | 308 | | Valdemorgate | 141 | |
| | Chozas | 176 | | Zuares | 424 | | Chozas | 176 | |
| | Tremedosa | 540 | | Muña | 1029 | | Cabeza | 425 | |

a Mediterranean bioclimate. Annual average precipitation in the basin is ~513.9 mm, 359.7 mm of which are lost by evapotranspiration. The lake systems included in this study are in the central endorheic depression of the basin, composed of Tertiary and Quaternary lacustrine and continental sediments, with several evaporitic inclusions.

### Field sampling and laboratory treatment

Nineteen shallow (depth <3 m), permanent lakes were studied and classified according to Moss *et al.* (2003) into three trophic levels based on average summer total phosphorus (TP) concentration (Low: <60, Medium: 60–140, High: >140 $\mu g\,TP\,L^{-1}$) (Table 1). Main limnological variables were measured following APHA (1998) protocols in all systems in summer 2003 except Lake Muña, sampled in 2004 (Table 2). Macrophyte abundance, expressed as the percentage of volume infested (PVI), was measured by estimating plant coverage and height at 10-m intervals along transects from the lakeshore to the lake centre. The estimate was done by inspecting the lake bottom at each point along the transect or, if visibility was low, by taking random samples with a rake at each point. Epiphythic communities growing on *Scirpus lacustris*, *Typha latifolia* and *Myriophyllum alterniflorum* were examined. Diatom sampling methods followed those described by Blanco and Bécares (2006) for epiphytic diatoms in shallow lakes. Submersed macrophyte stems were sampled, in triplicate (i.e. three stems), using a Kornijów sampler (Kornijów and Kairesalo 1994) to minimise the presence of phytoplankton, except in Sentiz (*T. latifolia*), Zarza (*M. alterniflorum*) and Zuares (*T. latifolia*) where, owing to technical problems, only two stands could be sampled, and Muña (*T. latifolia*) where only one stand was sampled. Thus, 76 samples were collected, comprising three replicates of each plant substrata in each trophic level (Table 1). Epiphyton was removed from all the fragments by shaking the samples for 2 min according to Zimba and Hopson (1997), which ensures the collection of more than 90% of the diatoms attached to the stems.

Aliquots from the original samples were cleaned by oxidation with hot hydrogen peroxide 8.5 M and then rinsed three times by decantation using distilled water. Air-dried aliquots were mounted on permanent glass slides using the refractive resin Naphrax (Brunel, Chippenham, Wiltshire, UK) at 140°C. Identification and enumeration of diatoms followed the method described in the European Standard UNE-EN 13946. Four

hundred individuals were identified in each sample to the lowest possible taxonomic level (species, subspecies, variety or form) based on the taxonomy and nomenclature proposed in Krammer and Lange-Bertalot (1986–1991), Lange-Bertalot (1995–2009) and Lange-Bertalot (2000–2002).

### Data analysis

Floristic inventories were processed with OMNIDIA version 4.1 software (Lecointe *et al.* 1993, 1999), which calculates eighteen diatom-based biotic indices (Table 3) related to the ecological status of water bodies. Most of these indices (APDI, BDI, DESCY, DI_CH, EPI-D, GDI, LMA, LOBO, SID, SLA, SPI, TDI and TID) are based on the Zelinka and Marvan (1961) formula:

$$ID = \sum (A_i \cdot S_i \cdot V_i) \Big/ \left( \sum A_i \cdot V_i \right)$$

where ID is the calculated diatom index, $A_i$ is the relative abundance of the *i*th taxon, $S_i$ is the pollution sensitivity of the *i*th taxon and $V_i$ is the indicative value or stenoecy degree of the *i*th taxon. Main differences among calculated indices lie in the taxonomic level considered, the number of taxa included for the calculation and their autoecological values. GDI requires determination to genus level and includes all freshwater genera. APDI includes 45 genera and 91 species. The other indices require determination to species level and each one includes a different number of taxa (BDI: 209; DESCY: 106; LMA: 210; SLA: 323; SPI: all known species). The CEC index is based on a 2-fold quality grid, with tolerance and indicator values for 208 species. The %PT index works as a supplement to TDI (which indicates the effect of inorganic nutrients) and indicates the proportion of the sample composed of organic pollution-tolerant taxa. The results of these indices are numeric values standardised from 1 to 20, representing the theoretical minimal and maximal water quality statuses respectively.

The diatom indices selected as the best-correlated with the studied variables and used for the subsequent statistical analyses were chosen by calculating the Correlation Index (CI) of each index against 18 limnological and physiographical variables (Table 2). CI was calculated applying the formula proposed by Blanco *et al.* (2007):

$$CI = \left[ \sum (\rho_S^2 \cdot S) \right] \cdot n^{-2}$$

**Table 2. Physical, chemical and physiographic parameters of the shallow lakes**

Average values from $n = 2$ measurements on summer 2003 and 2004, respectively. Coordinates are referred to the WGS84 datum. SRP, soluble reactive phosphorus; PVI, percentage of volume infested (Moss *et al.* 2003); *, bottom; –, no available data. Data from Conty (2007)

| Lake | Zone | UTM X | UTM Y | Mean depth (cm) | Area (ha) | Altitude (m a.s.l.) | Secchi depth (cm) | Turbidity (NTU) | Colour (abs400 nm) | Temp. (°C) | pH | Conductivity (μS cm⁻¹) | $O_2$ (% sat.) | $O_2$ (mg L⁻¹) | $N\text{-}NH_4^+$ (mg L⁻¹) | $N\text{-}NO_3$ (μg L⁻¹) | TP (μg L⁻¹) | SRP (μg L⁻¹) | TOC (mg L⁻¹) | Chl. *a* (μg L⁻¹) | PVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antimio | 30T | 284706 | 4708356 | 74 | 3.0 | 855 | 124* | 7.77 | 0.0255 | 20.9 | 7.8 | 239 | 65.0 | 3.71 | 25.0 | 13.7 | 75 | 5.70 | 20.2 | 10.0 | 91.3 |
| Bardal | 30T | 742446 | 4503933 | 99 | 2.8 | 865 | 83 | 7.85 | – | 24.6 | 8.5 | 226 | 100.0 | 7.05 | 6.6 | 52.0 | 75 | 3.65 | 84.3 | 20.4 | 21.5 |
| Bercianos | 30T | 323361 | 4695246 | 74 | 0.7 | 855 | – | 7.10 | 0.0243 | 18.0 | 7.6 | 118 | 102.4 | 8.83 | 8.8 | 131.1 | 28 | 8.86 | 2.5 | 0.0 | – |
| Berrueces | 30T | 325794 | 4647039 | 34 | 1.1 | 778 | 72* | 3.28 | – | 23.2 | 8.4 | 735 | 48.6 | 3.81 | 0.0 | 12.6 | 111 | 8.21 | 24.1 | 11.9 | 93.8 |
| Cabeza | 29T | 747779 | 4533469 | 49 | 1.0 | 770 | 62 | 31.85 | – | 20.0 | 7.4 | 168 | 45.5 | 3.59 | 14.0 | 52.4 | 425 | 15.00 | 28.4 | 66.6 | 100.0 |
| Carucedo | 29T | 682214 | 4707194 | 400 | 50.0 | 570 | 400 | 9.99 | 0.0000 | 25.3 | 7.5 | 340 | 84.4 | 6.65 | 59.3 | 77.5 | 18 | 5.33 | – | 1.0 | – |
| Chozas | 30T | 277008 | 4711412 | 84 | 2.0 | 940 | 30 | 29.23 | 0.0278 | 21.6 | 8.1 | 168 | 109.3 | 5.93 | 11.9 | 51.8 | 176 | 6.42 | 22.3 | 37.1 | 3.2 |
| Diel | 30T | 313652 | 4710771 | 67 | 1.5 | 910 | 155* | 1.20 | 0.0040 | 13.0 | 7.1 | 117 | 96.0 | 9.16 | 4.0 | 260.0 | 10 | 23.21 | – | 0.2 | 75.2 |
| Muña | 30T | 416224 | 4571575 | – | 6.1 | 910 | 57 | – | 0.0631 | – | 7.9 | 700 | 34.0 | – | 0.0 | 45.4 | 1030 | 47.39 | – | 171.4 | – |
| Redos | 30T | 317500 | 4705100 | 93 | 1.0 | 900 | 118* | 16.85 | – | 21.8 | 8.2 | 116 | 95.0 | 6.85 | 12.8 | 57.0 | 78 | 9.84 | 4.6 | 11.8 | 96.4 |
| Ribas | 30T | 374300 | 4669800 | 156 | 8.2 | 780 | 77 | 7.10 | – | 24.6 | 7.9 | 285 | 159.8 | 12.27 | 18.8 | 10.9 | 95 | 5.45 | 5.3 | 51.1 | 56.5 |
| Santervás | 30T | 351247 | 4707837 | 45 | 0.5 | 925 | 86* | 3.47 | 0.0359 | 18.2 | 7.8 | 246 | 67.5 | 91.58 | 36.6 | 23.2 | 38 | 16.00 | 18.4 | 3.9 | 75.8 |
| Sentiz | 30T | 318800 | 4714000 | 64 | 4.6 | 940 | 64* | 9.12 | 0.0502 | 19.5 | 7.6 | 340 | – | 4.53 | 5.3 | 12.4 | 51 | 4.55 | 43.3 | 13.3 | 87.7 |
| Tremedosa | 30T | 418709 | 4574617 | 52 | 0.3 | 850 | 33 | 23.71 | 0.0740 | 23.9 | 8.5 | 239 | 35.7 | 4.39 | 0.0 | 28.7 | 540 | 24.60 | 25.3 | 65.4 | 100.0 |
| Valdemorgate | 30T | 329574 | 4694455 | 62 | 0.6 | 850 | 117* | 10.10 | 0.0878 | 21.6 | 7.7 | 462 | 40.0 | 3.49 | 4.0 | 29.1 | 141 | 32.03 | 14.7 | 11.2 | 70.3 |
| Villadangos | 30T | 272100 | 4711400 | 54 | 9.4 | 940 | 48* | 23.63 | 0.0919 | 24.6 | 7.8 | 258 | 10.1 | 5.91 | 597.2 | 183.8 | 308 | 8.47 | 27.9 | 76.2 | – |
| Villaflores | 30T | 309498 | 4551076 | 69 | 0.3 | 730 | 130* | 5.48 | – | 27.4 | 7.8 | 2450 | 117.0 | 4.93 | 0.0 | 0.3 | 133 | 8.91 | 18.4 | 13.2 | 100.0 |
| Zarza | 29T | 725206 | 4525155 | 154 | 12.8 | 850 | 135* | 15.61 | – | 22.0 | 8.3 | 358 | 117.0 | 7.74 | 23.9 | 41.1 | 88 | 20.11 | 24.9 | 16.7 | 29.2 |
| Zuares | 30T | 279524 | 4690215 | 84 | 1.6 | 820 | 69 | 10.83 | 0.0593 | 25.0 | 7.8 | 373 | 34.3 | 5.31 | 1365.5 | 635.9 | 424 | 337.10 | 10.9 | 39.0 | – |

**Table 3.   Summary of calculated diatom indices**

| Abbreviation | Name | Reference |
|---|---|---|
| %PT | % of pollution Tolerant Taxa | Kelly and Whitton (1995) |
| APDI | Artois-Picardie Diatom Index | Prygiel *et al.* (1996) |
| BDI | Biological Diatom Index | Lenoir and Coste (1996) |
| CEC | European Index | Descy and Coste (1991) |
| DESCY | Descy's Index | Descy (1979) |
| DI_CH | Hurlimann's Index | Hürlimann and Niederhauser (2002) |
| EPI-D | Eutrophication Pollution Index | Dell'Uomo (2004) |
| Eq | Equitability | – |
| GDI | Generic Diatom Index | Rumeau and Coste (1988) |
| Ha | Shannon's Index | Shannon and Weaver (1949) |
| LMA | Leclerq and Maquet Index | Leclerq and Maquet (1987) |
| LOBO | Lobo's Index | Lobo *et al.* (2002) |
| PDI | Pampean Diatom Index | Gómez and Licursi (2001) |
| S | Species richness | – |
| SHE | Steinberg and Schiefele Index | Steinberg and Schiefele (1988) |
| SID | Rott's Saprobic Index | Rott *et al.* (1997) |
| SLA | Sládeček's Index | Sládeček (1986) |
| SPI | Specific Pollution Index | Coste in Cemagref (1982) |
| TDI | Trophic Diatom Index | Kelly and Whitton (1995) |
| TID | Rott's Trophic Index | Rott *et al.* (1997) |
| WAT | Diatom Assemblage Index | Watanabe *et al.* (1988) |

where $\rho_S^2$ is Spearman's correlation coefficient; S is the number of statistically significant correlations at $\alpha = 0.95$; and n is the number of studied variables.

CI ranges from 0 to 1, indicating the theoretical minimum and maximum relationships among variables.

A two-way ANOVA was performed to account for significant differences in the resulting diatom indices scores among the three established trophic levels and the three studied plant substrata. ANOVA test assumptions were verified by Kolmogorov's and Levene's tests. A similarity analysis (ANOSIM permutation test: Clarke 1993) and a Principal Coordinates analysis (PCO) were performed on relative abundances of the 309 taxa identified, to assess how the composition of epiphytic diatom communities varied with plant species and trophic status of studied lakes. Spearman's correlation and ANOVA tests were calculated using STATISTICA software (StatSoft, Inc. 2007), the ANOSIM test with Community Analysis Package version 3.11 (Pisces Conservation Ltd. 2004), and PCO with Past version 1.78 (Hammer *et al.* 2001).

**Results**

For this study, 309 diatom taxa were identified, including species, subspecies, varieties and forms. The most abundant species, with the highest occurrence, was *Achnanthidium minutissimum*, which was present in all systems except Zuares Lake. Its abundance was <10% in the lakes where TP concentration was over $300\,\mu g\,L^{-1}$.

*Selection of metrics*

Among the 18 physical or chemical attributes measured, dissolved oxygen, TP, macrophyte cover and colour correlated significantly with the highest number of diatom indices (Table 4). Seven variables (mean depth, area, Secchi depth,

turbidity, temperature, pH and nitrate concentration) did not show any significant relation to diatom metrics. Diatom indices with the highest number of significant statistical relations with analysed parameters were CEC, SPI and BDI. Four indices (S, DESCY, DI_CH and SID) showed no significant relationships with any limnological or physiographical variable. The indices that achieved high values for the Correlation Index were CEC, SPI and BDI (Fig. 1).

*Trophic status assessment*

With respect to the biological quality, ANOVA results (Fig. 2, Table 5) showed significant differences among trophic levels for eight indices (CEC, BDI, SPI, APDI, WAT, EPI-D, LOBO and GDI). CEC, BDI, APDI and LOBO showed significant differences for the pairs Low–High and Medium–High, whereas SPI, WAT, EPI-D and GDI only differed for the pair Low–High (Table 6).

*Neutral substrate hypothesis*

ANOVA results showed no significant differences for 17 of the 21 indices analysed when different plant substrata were compared (Table 5). BDI, APDI, EPI-D and SHE, indices detected differences in pair-wise comparisons among plant substrata for all species pairs except *M. alterniflorum–S. lacustris* (Table 6). No interactive effects were found between trophic levels and plant species (Table 5).

ANOSIM tests on the relative abundance of diatom species show significant differences between the diatom communities developed on the three different plants (*M. alterniflorum–S. lacustris*, $r = 0.019$; *M. alterniflorum–T. latifolia*, $r = 0.039$; *S. lacustris–T. latifolia*, $r = 0.01$; $P = 0.001$) and trophic levels (High–Low, $r = 0.001$; High–Medium, $r = 0.004$; Low–Medium, $r = 0.002$; $P = 0.001$). PCO shows higher overlap

**Table 4.** Spearman's correlation coefficients ($p_s^2$) between diatom indices and environmental variables. Significant correlations ($P < 0.05$) in bold

| Index | Mean depth | Area | Altitude | Secchi depth | Turbidity | Colour | T | pH | Conductivity | ($O_2$) (% sat.) | ($O_2$) | ($N$-$NH_4^+$) | ($N$-$NO_3^-$) | (TP) | (Orto-P) | (COT) | (Chl. $a$) | PVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ha | −0.21 | 0.01 | −0.29 | −0.20 | −0.10 | 0.30 | −0.23 | 0.02 | −0.08 | −0.16 | −0.20 | −0.36 | −0.57 | 0.04 | 0.00 | **0.58** | 0.17 | **0.49** |
| S | −0.20 | 0.04 | −0.29 | −0.17 | −0.06 | 0.24 | −0.26 | 0.05 | −0.10 | −0.25 | −0.24 | **−0.41** | −0.48 | 0.08 | 0.06 | **0.54** | 0.15 | **0.51** |
| Eq | −0.05 | 0.07 | −0.30 | −0.29 | −0.16 | 0.34 | −0.09 | 0.09 | 0.03 | 0.05 | −0.13 | −0.25 | −0.47 | −0.04 | −0.17 | 0.22 | 0.15 | 0.14 |
| SLA | 0.16 | 0.09 | 0.22 | 0.00 | −0.13 | −0.45 | −0.01 | −0.03 | −0.38 | **0.52** | **0.60** | **0.42** | 0.30 | **−0.59** | **−0.39** | −0.31 | −0.35 | **−0.57** |
| DESCY | −0.02 | 0.25 | 0.04 | −0.25 | −0.01 | 0.09 | −0.17 | 0.16 | −0.08 | 0.25 | 0.30 | 0.09 | 0.14 | −0.29 | −0.29 | −0.04 | 0.05 | −0.40 |
| LMA | 0.25 | 0.07 | 0.46 | 0.15 | −0.08 | −0.40 | −0.02 | 0.10 | −0.18 | 0.35 | **0.44** | 0.11 | 0.35 | −0.36 | −0.15 | −0.35 | −0.25 | −0.39 |
| SHE | −0.02 | −0.14 | 0.28 | 0.17 | −0.18 | −0.34 | −0.07 | 0.03 | −0.30 | 0.27 | **0.42** | 0.23 | 0.36 | **−0.45** | −0.19 | −0.35 | −0.33 | −0.36 |
| WAT | 0.23 | 0.01 | 0.24 | 0.32 | −0.23 | **−0.58** | 0.05 | 0.08 | −0.20 | **0.58** | **0.60** | 0.10 | 0.42 | **−0.54** | −0.21 | −0.23 | **−0.45** | **−0.50** |
| TDI | −0.15 | −0.06 | −0.26 | −0.22 | 0.31 | **0.58** | 0.02 | −0.02 | 0.38 | **−0.49** | **−0.53** | −0.23 | −0.16 | **0.62** | **0.39** | 0.41 | **0.46** | 0.26 |
| %PT | −0.12 | −0.15 | **−0.50** | 0.05 | 0.07 | 0.27 | 0.19 | −0.06 | 0.21 | −0.13 | **−0.45** | −0.23 | −0.24 | 0.37 | 0.12 | 0.22 | 0.11 | 0.29 |
| GDI | 0.09 | 0.12 | 0.28 | 0.02 | −0.14 | **−0.56** | −0.10 | −0.07 | −0.36 | **0.45** | **0.49** | 0.35 | 0.31 | **−0.55** | −0.32 | −0.37 | **−0.40** | −0.39 |
| CEC | 0.22 | 0.12 | 0.16 | 0.25 | −0.21 | **−0.68** | 0.03 | 0.00 | **−0.43** | **0.58** | **0.57** | 0.27 | 0.27 | **−0.63** | **−0.41** | −0.41 | **−0.44** | **−0.44** |
| SPI | 0.15 | 0.04 | 0.27 | 0.19 | −0.23 | **−0.64** | −0.04 | −0.04 | **−0.40** | **0.58** | **0.57** | 0.30 | 0.24 | **−0.62** | −0.35 | −0.40 | **−0.47** | **−0.47** |
| BDI | 0.18 | 0.04 | 0.10 | 0.21 | −0.17 | **−0.65** | 0.10 | −0.02 | **−0.44** | **0.62** | **0.59** | 0.39 | 0.31 | **−0.57** | **−0.42** | −0.38 | −0.38 | **−0.44** |
| APDI | 0.17 | −0.02 | 0.22 | 0.22 | −0.20 | **−0.57** | 0.15 | −0.06 | −0.36 | **0.61** | **0.57** | 0.38 | 0.39 | **−0.53** | **−0.39** | −0.35 | −0.36 | **−0.50** |
| EPI-D | 0.18 | 0.05 | 0.16 | 0.07 | −0.14 | **−0.47** | 0.15 | 0.02 | −0.27 | **0.60** | **0.53** | 0.38 | 0.30 | **−0.50** | **−0.41** | −0.35 | −0.32 | **−0.53** |
| DL_CH | 0.12 | 0.00 | 0.45 | 0.19 | −0.10 | 0.04 | −0.28 | 0.08 | −0.32 | −0.24 | 0.27 | 0.02 | 0.27 | −0.29 | 0.02 | −0.11 | −0.01 | −0.07 |
| PDI | −0.08 | 0.06 | 0.16 | 0.06 | −0.27 | 0.09 | −0.34 | −0.03 | −0.23 | −0.04 | 0.15 | −0.12 | −0.31 | **−0.40** | −0.18 | 0.07 | −0.15 | 0.08 |
| LOBO | 0.13 | −0.01 | 0.12 | 0.15 | −0.22 | **−0.57** | 0.23 | 0.00 | −0.24 | **0.66** | **0.53** | 0.38 | 0.28 | **−0.45** | −0.35 | −0.31 | −0.35 | **−0.46** |
| SID | 0.07 | −0.06 | 0.14 | 0.02 | −0.05 | 0.17 | −0.13 | 0.07 | −0.30 | −0.05 | 0.17 | 0.08 | −0.10 | −0.22 | −0.11 | −0.06 | 0.04 | −0.12 |
| TID | 0.20 | 0.05 | 0.26 | 0.13 | −0.15 | **−0.58** | 0.04 | −0.01 | **−0.42** | **0.61** | **0.64** | 0.39 | 0.42 | **−0.57** | −0.32 | −0.29 | −0.36 | **−0.53** |

when comparing plant species than comparing trophic levels (Fig. 3).

*Achnanthidium minutissimum* was the most abundant species in the diatom communities that developed on the three plant substrata, with its lowest abundance on *Typha latifolia* (Fig. 4). Species richness was 93, 96 and 72 for *S. lacustris*, *T. latifolia* and *M. alterniflorum*, respectively. Some differences in the mean abundances of certain diatom species were observed among epiphytic communities on different plant species (Fig. 4). *Amphora veneta*, common on *T. latifolia* stems, was nearly absent in the communities developed on *S. lacustris*, and completely absent in *M. alterniflorum*. In contrast, *Encyonopsis subminuta* was common in *M. alterniflorum* epiphyton, whereas the mean abundance of this taxon on the other plants did not exceed 0.5%. Some diatoms appeared in relatively high abundance on two of the three studied plant substrata, but in much lower densities on the last
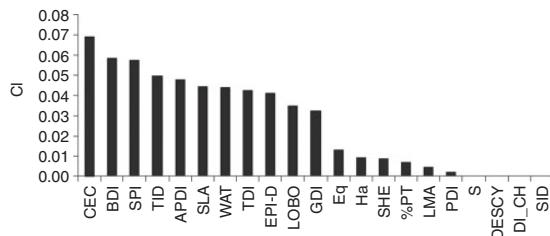


**Fig. 1.** Correlation index (CI) scores obtained from the different diatom metrics calculated.

substratum. This was the case with *Gomphonema gracile*, which grew mainly on *T. latifolia* and *M. alterniflorum*, or *Cocconeis euglypta*, which was common on *S. lacustris* and *T. latifolia*. Only two and three taxa with mean abundances above 5% were found on *S. lacustris* and *M. alterniflorum*, respectively. Five taxa with mean abundances higher than 5% were found on *T. latifolia*.

## Discussion

### Selection of metrics

Our results showed that the three indices that correlated best with the analysed parameters for the studied lakes were CEC, SPI and BDI. In a study on Mediterranean rivers (Blanco *et al.* 2007), these three indices, calculated from epilithic assemblages, were also determined to be the most suitable diatom indices for water quality diagnosis. These three indices are the most-used diatom-based metrics for freshwater biological quality assessment in Europe (Ector and Rimet 2005). In the Duero Basin, SPI has the best performance in bioassessment studies for rivers (Blanco *et al.* 2007), whereas we found similar results for SPI, CEC and BDI indices in the studied shallow lakes. Main differences between calculated diatom indices lie in the floristic spectrum (the species list, together with their autoecological values) considered by each method. Hence, contrasting results may be obtained from the same dataset when different indices are applied (Blanco *et al.* 2007; Feio *et al.* 2009). In general, correlation between diatom metrics and environmental variables depends on the percentage of the identified individuals considered by each index (Blanco *et al.* 2007; Cejudo-Figueiras 2007).
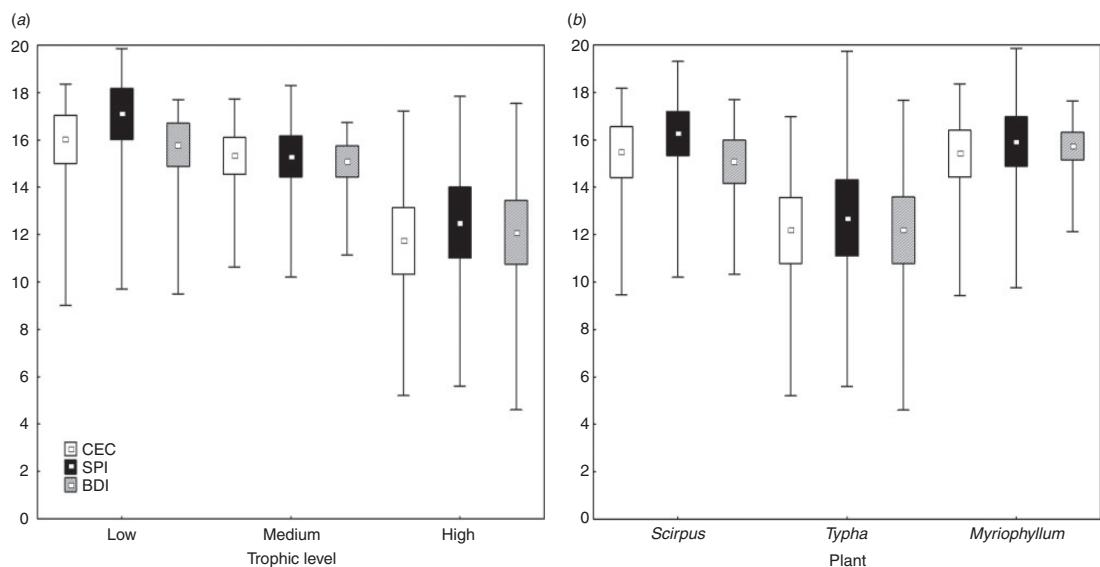


**Fig. 2.** CEC, SPI and BDI scores calculated from epiphytic diatom assemblages growing under different trophic levels (*a*) and on different plant substrata (*b*). Mean values ± s.e. ± non-outlier ranges.

## Trophic status assessment

None of the indices discriminated between Low and Medium levels of nutrient enrichment but they all discriminated between Low and High levels. The interpretation of data about trophic levels depends largely on the classification model used. For example, a deep lake with TP $\sim 30\,\mu g\,L^{-1}$ may be considered as mesotrophic, whereas a shallow lake with comparable TP concentration would be classified as ultra-oligotrophic. Our classification is based on the one for shallow lakes by Moss *et al.* (2003). The slightly better performance exhibited by CEC may be thus rather attributed to more accurate autoecological (S and V) values assigned to the same taxa in the CEC metric than in the SPI metric.

## Neutral substrate hypothesis

Several studies have focussed on the influence of sampling substrata on the ecological quality inferred from the analysis of periphytic assemblages (Cattaneo and Amireault 1992; Poulíčková *et al.* 2004); for example, in a bioassessment study of the Plaine River, France, Besse-Lototskaya *et al.* (2006) observed that 8 of the 17 metrics calculated (including CEC) varied depending on the type of the substratum sampled (macrophytes, stones or sediments), and found that the most accurate substratum for CEC was macrophytes, whereas SPI and BDI were not affected by the choice of substrate. Poulíčková *et al.* (2004) compared diatom communities from stones, mud and young reed stalks, and illustrated that differences in species composition among these substrata affected the assessment of trophic status. Using samples collected from streams across the United States, Potapova and Charles (2005) found significantly lower species richness, diversity and diatom biovolumes in assemblages on hard substrata (rocks and wood) than on soft substrata (sand and silt), whereby the relationship among these attributes and environmental factors was similar regardless of substratum type. In our study, the diagnostic capacities of CEC and SPI were unaffected by macrophyte species identity, whereas BDI values did differ among species. Results obtained by Eminson and Moss (1980) suggest that epiphytic algal assemblages may be more influenced by environmental factors than by host macrophytes. Likewise, Caput and Plenković-Moraj (2000) concluded that the high similarity between diatom communities growing on live and dead stalks of *Cladium mariscus* was likely related to the uniformity of physico–chemical conditions. Nevertheless,

**Table 5.   ANOVA test results of the effects of the studied factors on the results of the diatom and diversity indices**
Significant *P* values highlighted in bold

| Factor | Statistic | Trophic level (d.f. = 2) | Plant (d.f. = 2) | Trophic level × plant (d.f. = 4) |
|---|---|---|---|---|
| CEC | *F* | 5.10 | 3.43 | 0.73 |
|  | *P* | **0.018** | 0.580 | 0.580 |
| BDI | *F* | 5.08 | 4.69 | 1.16 |
|  | *P* | **0.018** | **0.023** | 0.362 |
| SPI | *F* | 4.68 | 3.39 | 1.07 |
|  | *P* | **0.023** | 0.056 | 0.401 |
| APDI | *F* | 4.14 | 3.98 | 0.64 |
|  | *P* | **0.033** | **0.037** | 0.640 |
| WAT | *F* | 4.21 | 1.63 | 0.62 |
|  | *P* | **0.032** | 0.223 | 0.655 |
| EPI-D | *F* | 3.90 | 4.46 | 1.21 |
|  | *P* | **0.039** | **0.027** | 0.343 |
| LOBO | *F* | 5.71 | 3.01 | 1.21 |
|  | *P* | **0.012** | 0.074 | 0.342 |
| GDI | *F* | 3.75 | 1.79 | 0.57 |
|  | *P* | **0.043** | 0.195 | 0.688 |
| SHE | *F* | 1.68 | 3.59 | 1.00 |
|  | *P* | 0.215 | **0.049** | 0.433 |

**Table 6.   Post-hoc pairwise comparison test between studied factors on the scores provided by different diatom indices**
*P* values shown. Significant differences ($P < 0.01$) highlighted in bold. Degrees of freedom for all tests = 18

| Comparison | Category | CEC | BDI | SPI | APDI | WAT | EPI-D | LOBO | GDI | SHE |
|---|---|---|---|---|---|---|---|---|---|---|
| Low *v.* Medium | Plants | 0.63 | 0.58 | 0.25 | 0.60 | 0.26 | 0.53 | 0.89 | 0.09 |  |
| Medium *v.* High |  | **0.02** | **0.03** | 0.08 | **0.04** | 0.10 | 0.06 | **0.01** | 0.37 |  |
| Low *v.* High |  | **0.01** | **0.01** | **0.01** | **0.01** | **0.01** | **0.02** | **0.01** | **0.01** |  |
| *Scirpus v. Typha* | Nutrients |  | **0.03** |  | **0.03** |  | **0.01** |  |  | **0.03** |
| *Typha v. Myriophyllum* |  |  | **0.01** |  | **0.03** |  | **0.03** |  |  | **0.04** |
| *Scirpus v. Myriophyllum* |  |  | 0.60 |  | 1.00 |  | 0.78 |  |  | 0.81 |

we have observed (Cejudo-Figueiras *et al.* in press) that the relationship between epiphytic diatom assemblages and limnological variables may be weaker in certain plant substrata (e.g. *Veronica anagallis-aquatica*), but not when dominant host macrophytes are compared.

Analysis of similarity relating to species abundance and composition of diatom assemblages throughout trophic levels confirm that epiphytic communities show environment-related structural changes, thereby providing a basis for their implementation as bioindicators for shallow lakes. Analogous differences have been found comparing different plant substrata. Kitner and Poulícková (2003) observed significant differences

in the diatom species richness found growing on different substrata (rocks, plants and sediment). In contrast, Winter and Duthie (2000) found no substantive structural differences among river epilithic, epipelic and epiphytic diatom assemblages, though some taxa were more abundant in certain habitats. Yallop *et al.* (2009) found no significant differences in assessment of trophic status (TDI), species richness, species diversity or the relative abundance of motile taxa within epilithic diatom assemblages, whereas differences in trophic status among systems was evident from variation in epiphyton.

Several studies have shown differences at the community level comparing different host macrophytes. For instance,
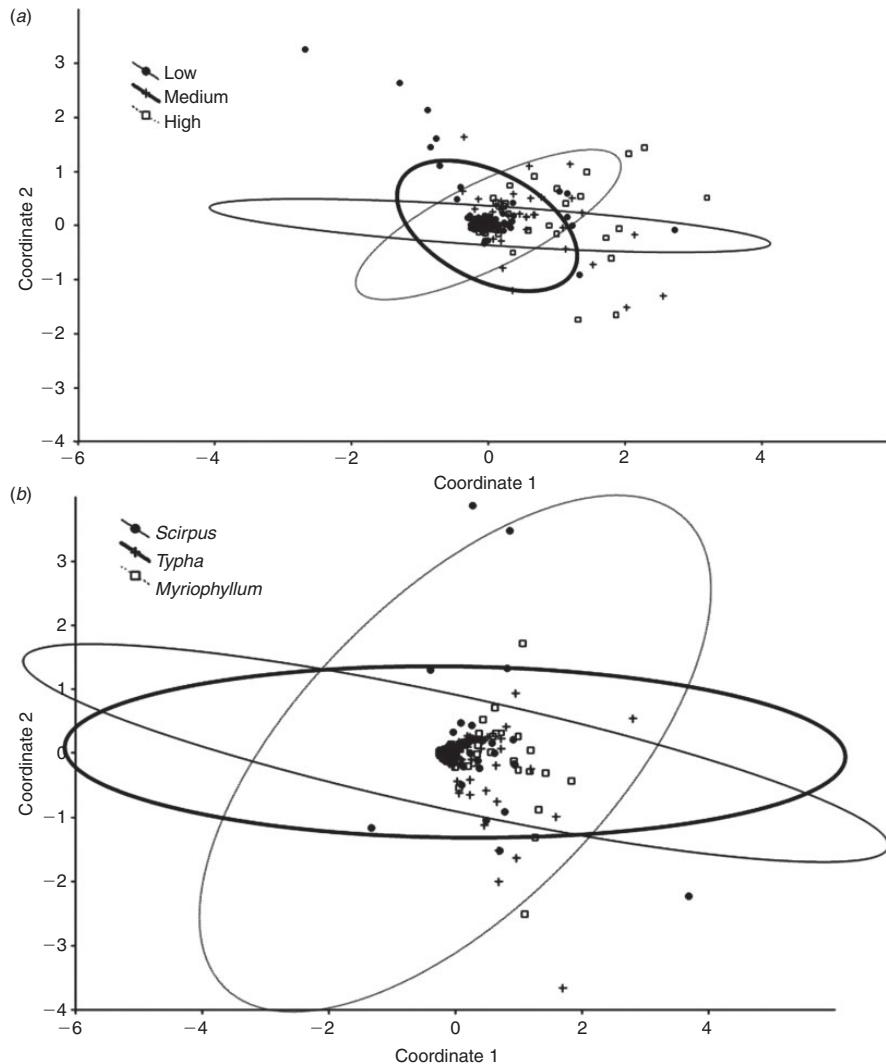


**Fig. 3.** Principal coordinates diagram of diatom abundances: (*a*) different trophic levels, (*b*) different host macrophytes. Confidence ellipses (95%) shown.
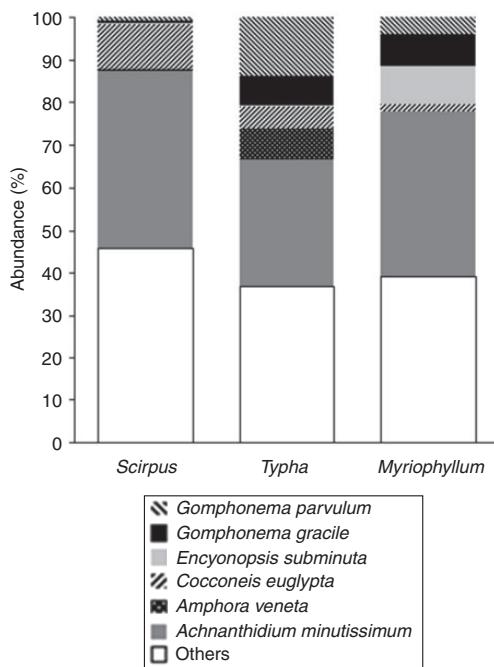
**Fig. 4.** Average individual abundances (%) of diatom species at abundances of over 5% in at least one plant substrata.

Townsend and Gell (2005) found that diatom assemblages growing on *Juncus* sp. and *Chara* sp. were similar, but those developing on *Vallisneria* sp. leaves were species-impoverished. Interspecific differences in macrophytic epiphyton may also affect functional parameters such as algal biomass or chlorophyll *a* content (Laugaste and Reunanen 2005). Such differences might be unrelated to the morphological similarity among plants; Blindow (1987) found larger differences in epiphyton composition between *Chara globularis* and *Chara tormentosa* than between the former and *Nitellopsis obtusa*. Additionally, he observed differences in the density of the epiphyton developed on different macrophytes, with some diatom species more abundant in certain plants (e.g. *Cocconeis placentula*, which showed clear affinity for *Potamogeton pectinatus*). This preference for particular substrata has been reported in many papers (e.g. Caput and Plenković-Moraj 2000; Townsend and Gell 2005; Messyasz and Kuczyńska-Kippen 2006). In the present study, we have observed these kinds of preferences (e.g. *Gomphonema parvulum* and *Amphora veneta* for *T. latifolia*, *Encyonopsis subminuta* for *M. alterniflorum*, or *Cocconeis euglypta* for *S. lacustris*), together with a higher species richness in diatom assemblages developed on *M. alterniflorum*. These differences might be caused, for example, by stochastic factors associated with diatom colonisation (Townsend and Gell 2005) or the release of allelopathic substances by macrophytes (Gross 2003), despite the fact that epiphyton is less sensitive to these substances than phytoplankton (Hilt 2006).

The relative abundance of *Achnanthidium minutissimum*, a species associated with low nutrient concentrations (Ponader and Potapova 2007), was less than 10% in the lakes where TP concentration was greater than 300 $\mu$g L$^{-1}$, such as Zuares. This relatively common species may be totally absent from several lakes within this eco-region (see Blanco *et al.* 2004), perhaps owing to biogeographical constraints, though this hypothesis has not been thoroughly explored up to date.

Data shown in the present study indicate that the ecological quality inference using CEC is more accurate when compared with other diatom-based metrics. However, the CEC index did not adequately discriminate between low and medium trophic levels. Because CEC was developed for lotic systems, it presumably does not include some typical species from lacustrine habitats or epiphytic taxa. *Aulacoseira subarctica*, *Fragilaria bicapitata* and *Navicula cryptocephala* were present in the studied assemblages but not considered in the CEC metric, suggesting that this metric needs to be adapted for lentic environments.

To conclude, our data show that CEC was the diatom index with best results for water quality assessment in the shallow lakes studied because it obtained the highest CI, the trophic status assessment was not affected by the plant substrata, and it differentiated trophic levels, with the exception of Low–Medium. The composition of epiphytic diatom assemblages differed depending on the host macrophyte and the trophic status of the shallow lake. Nevertheless, water quality assessment was not affected by the host macrophyte sampled. The results corroborate the validity of the neutral substrate hypothesis in epiphyton-based trophic classification of shallow lakes. Therefore, epiphytic diatoms can be used as bioindicators of trophic status in a wide variety of shallow lacustrine systems, regardless of the species composition of the resident macrophyte community. However, it is necessary to recalibrate diatom indices or, eventually, to develop new metrics with a more accurate fit to the floristic and auto-ecological particularities of lake epiphytic diatom communities.

## References

APHA (1998). 'Standard Methods for the Examination of Water and Wastewater.' (American Water Works Association: Washington.)

Besse-Lototskaya, A., Verdonschot, P. F. M., and Sinkeldam, J. A. (2006). Uncertainty in diatom assessment, sampling, identification and counting variation. *Hydrobiologia* **566**, 247–260. doi:10.1007/S10750-006-0092-5

Blanco, S., and Bécares, E. (2006). Método de muestreo de diatomeas epífitas en lagunas para la aplicación de la Directiva Marco del Agua. *Tecnología del Agua* **277**, 42–47.

Blanco, S., Ector, L., and Bécares, E. (2004). Epiphytic diatoms as water quality indicators in Spanish shallow lakes. *Vie et Milieu* **54**, 71–79.

Blanco, S., Bécares, E., Cauchie, H. M., Hoffmann, L., and Ector, L. (2007). Comparison of biotic indices for water quality diagnosis in the Duero Basin (Spain). *Archiv für Hydrobiologie – Supplement Large Rivers* **17**, 267–286.

Blindow, I. (1987). The composition and density of epiphyton on several species of submerged macrophytes. The neutral hypothesis tested. *Aquatic Botany* **29**, 157–168. doi:10.1016/0304-3770(87)90093-3

Caput, K., and Plenković-Moraj, A. (2000). Epiphytic diatoms on sawgrass (*Cladium mariscus*) in the karstic Plitvice Lakes, Croatia. *Biologia* **55**, 343–350.

Carignan, R., and Kalff, J. (1982). Phosphorus release by submerged macrophytes: significance to epiphyton and phytoplankton. *Limnology and Oceanography* **27**, 419–427. doi:10.4319/LO.1982.27.3.0419

Cattaneo, A., and Amireault, M. C. (1992). How artificial is artificial substrata for periphyton? *Journal of the North American Benthological Society* **11**, 244–256. doi:10.2307/1467389

Cattaneo, A., and Kalff, J. (1979). Primary production of algae growing on natural and artificial aquatic plants: a study of interactions between epiphytes and their substrate. *Limnology and Oceanography* **24**, 1031–1037. doi:10.4319/LO.1979.24.6.1031

Cattaneo, A., and Kalff, J. (1980). The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. *Limnology and Oceanography* **25**, 280–289. doi:10.4319/LO.1980.25.2.0280

Cejudo-Figueiras, C. (2007). Diatomeas epífitas en lagos europeos, diversidad florística y relación con factores ambientales. MSc Thesis, University of León.

Cejudo-Figueiras, C., Blanco, S., Álvarez-Blanco, I., Ector, L., and Bécares, E. (2010). Nutrient monitoring in Spanish wetlands using epiphytic diatoms. *Vie et Milieu* **60**, in press.

Cemagref (1982). Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Q. E. Lyon-A. F. Bassin Rhône-Méditerranée-Corse.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**, 117–143. doi:10.1111/J.1442-9993.1993.TB00438.X

Conty, A. (2007). El bucle microbiano en las lagunas someras esteparias de Castilla y León, importancia ecológica e influencia en la eutrofización. PhD Thesis, University of León.

Dell'Uomo, A. (2004). 'L'Indice Diatomico di Eutrofizzacione/Polluzione (EPI-D) nel Monitoraggio delle Acque Correnti. Linee Guida.' (APAT: Roma.)

Descy, J. P. (1979). A new approach to water quality estimation using diatoms. *Nova Hedwigia* **64**, 305–323.

Descy, J. P., and Coste, M. (1991). A test of methods for assessing water quality based on diatoms. *Verhandlungen – Internationale Vereinigung für Theoretische und Angewandte Limnologie* **24**, 2112–2116.

Ector, L., and Rimet, F. (2005). Using bioindicators to assess rivers in Europe: an overview. In 'Modelling Community Structure in Freshwater Ecosystems'. (Eds S. Lek, M. Scardi, P. F. M. Verdonschot, J. P. Descy and Y. S. Park.) pp. 7–19. (Springer-Verlag: Berlin.)

Eminson, D., and Moss, B. (1980). The composition and ecology of periphyton communities in freshwaters. 1. The influence of host type and external environment on community composition. *British Phycological Journal* **15**, 429–446. doi:10.1080/00071618000650431

Feio, M. J., Almeida, S. F. P., Craveiro, S. C., and Calado, A. J. (2009). A comparison between biotic indices and predictive models in stream water quality assessment based on benthic diatom communities. *Ecological Indicators* **9**, 497–507. doi:10.1016/J.ECOLIND.2008.07.001

Gómez, N., and Licursi, M. (2001). The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina. *Aquatic Ecology* **35**, 173–181. doi:10.1023/A:1011415209445

Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences* **22**, 313–339. doi:10.1080/713610859

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 1–9.

Hilt, S. (2006). Allelopathic inhibition of epiphytes by submerged macrophytes. *Aquatic Botany* **85**, 252–256. doi:10.1016/J.AQUABOT.2006.05.004

Hürlimann, J., and Niederhauser, P. (2002). 'Méthodes d'étude et d'appréciation de l'état de Santé des Cours d'eau: Diatomées–Niveau R (Région).' (Office Fédéral de l'Environnement, des Forêts et du Paysage: Berne.)

Kelly, M. G., and Whitton, B. A. (1995). The Trophic Diatom Index, a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology* **7**, 433–444. doi:10.1007/BF00003802

Kitner, M., and Poulíčková, A. (2003). Littoral diatoms as indicators for the eutrophication of shallow lakes. *Hydrobiologia* **506–509**, 519–524. doi:10.1023/B:HYDR.0000008567.99066.92

Kornijów, R., and Kairesalo, T. (1994). A simple apparatus for sampling epiphytic communities associated with emergent macrophytes. *Hydrobiologia* **294**, 141–143. doi:10.1007/BF00016854

Krammer, K., and Lange-Bertalot, H. (1986–1991). Bacillariophyceae. In 'Susswasserflora von Mitteleuropa'. (Eds H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer.) pp. 1–2458. (Spektrum Akademischer Verlag: Heidelberg.)

Lange-Bertalot, H. (Ed.) (1995–2009). 'Iconographia Diatomologica. Annotated Diatom Micrographs.' Vols. I–XX. (A. R. G. Gantner Verlag K. G: Ruggell.)

Lange-Bertalot, H. (Ed.) (2000–2002). 'Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats.' Vols. I–IV. (A. R. G. Gantner Verlag K. G: Ruggell.)

Laugaste, R., and Reunanen, M. (2005). The composition and density of epiphyton on some macrophyte species in the partly meromictic Lake Verevi. *Hydrobiologia* **547**, 137–150. doi:10.1007/S10750-005-4155-9

Leclerq, L., and Maquet, B. (1987). Deux nouveaux indices chimique et diatomique de qualité d'eau courante. Application au Samson et à ses affluents (Bassin de la Meuse Belge). Comparaison avec d'autres indices chimiques biocénotiques et diatomiques. *Institut Royal des Sciences Naturelles de Belgique Documents de Travail* **38**, 113.

Lecointe, C., Coste, M., and Prygiel, J. (1993). OMNIDIA software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia* **269–270**, 509–513. doi:10.1007/BF00028048

Lecointe, C., Coste, M., Prygiel, J., and Ector, L. (1999). Le logiciel OMNIDIA versión 2, une puissante base de données pour les inventaires de diatomées et pour le calcul des indices diatomiques européens. *Cryptogamie. Algologie* **20**, 132–134.

Lenoir, A., and Coste, M. (1996). Development of a practical diatom index of overall water quality applicable to the French national water board network. In 'Use of Algae for Monitoring Rivers II'. (Eds B. A. Whitton and E. Rott.) pp. 29–43. (Botany Institute, University of Innsbruck: Innsbruck.)

Linares, J. E. (2003). Las diatomeas bentónicas de las lagunas del Parque Nacional de Sierra Nevada. Estudio comparado con las colecciones del herbario de la Universidad de Granada (GDA). PhD Thesis, The University of Granada.

Lobo, E. A., Callegaro, V. L. M., and Bender, E. P. (2002). 'Utilizaçao de Algas Diatomáceas Epilíticas como Indicadores da Qualidade da Água em Rios e Arroios da Regiao Hidrográfica do Guaíba, RS, Brasil.' (EDUNISC: Santa Cruz do Sul.)

Messyasz, B., and Kuczyńska-Kippen, N. (2006). Periphytic algal communities, a comparison of *Typha angustifolia* L. and *Chara tomentosa* L. beds in three shallow lakes (West Poland). *Polish Journal of Ecology* **54**, 15–27.

Moss, B., Stephen, D., Álvarez, C., Bécares, E., Van der Bund, W., *et al.* (2003). The determination of ecological quality in shallow lakes – a

tested expert system (ECOFRAME) for implementation of the European Water Framework Directive. *Aquatic Conservation-Marine and Freshwater Ecosystems* **13**, 507–549. doi:10.1002/AQC.592

Pisces Conservation Ltd. (2004). Community Analysis Package version 3.11. Pisces Conservation Ltd., Lymington, Hampshire, UK.

Ponader, K. C., and Potapova, M. G. (2007). Diatoms from the genus *Achnanthidium* in flowing waters of the Appalachian Mountains (North America): ecology, distribution and taxonomic notes. *Limnologica* **37**, 227–241. doi:10.1016/J.LIMNO.2007.01.004

Potapova, M. G., and Charles, D. F. (2005). Choice of substrate in algae-based water-quality assessment. *Journal of the North American Benthological Society* **24**, 415–427. doi:10.1899/03-111.1

Poulíčková, A., Duchoslav, M., and Dokulil, M. (2004). Litoral diatom assemblages as bioindicators of lake trophic status. A case study from perialpine lakes in Austria. *European Journal of Phycology* **39**, 143–152. doi:10.1080/0967026042000201876

Prygiel, J., Leveque, L., and Iseretant, R. (1996). Un nouvel indice diatomique pratique pour l'evaluation de la qualité des eaux en réseau de surveillance. *Revue des Sciences de l'Eau* **9**, 97–113.

Rott, E., Hofmann, G., Pall, K., Pfister, P., and Pipp, E. (1997). 'Indikationslisten für Aufwuchsalgen Teil 1, Saprobielle Indikation.' (Bundesministerium für Land- und Forstwirtschaft: Wien.)

Rumeau, A., and Coste, M. (1988). Initiation à la systématique des diatomées d'eau douce. *Bulletin Francais de la Peche et de la Pisciculture* **309**, 1–69. doi:10.1051/KMAE:1988009

Shannon, C. E., and Weaver, W. (1949). 'The mathematical theory of communication.' (The University of Illinois Press: Urbana, IL, USA.)

Sládeček, V. (1986). Diatoms as indicators of organic pollution. *Acta Hydrochimica et Hydrobiologica* **14**, 555–566. doi:10.1002/AHEH.19860140519

StatSoft, Inc. (2007). STATISTICA (data analysis software system), version 8.0. Available at http://www.statsoft.com [Verified 30 September 2010].

Steinberg, C., and Schiefele, S. (1988). Biological indication of trophy and pollution of running waters. *Zeitschrift für Wasser und Abwasser Forschung* **21**, 227–234.

Townsend, S. A., and Gell, P. A. (2005). The role of substrate type on benthic diatom assemblages in the Daly and Roper Rivers of the Australian wet/dry tropics. *Hydrobiologia* **548**, 101–115. doi:10.1007/S10750-005-0828-7

Trobajo, R. (2003). Ecological analysis of periphytic diatoms in Mediterranean coastal wetlands (Empordà wetlands, NE Spain). PhD Thesis, University of Girona.

Watanabe, T., Asai, K., and Houki, A. (1988). Numerical water quality monitoring of organic pollution using diatom assemblages. In 'Proceedings of the 9th International Diatom Symposium 1986'. (Ed. F. E. Round.) pp. 123–141. (Koeltz Science Books: Königstein.)

Winter, J. G., and Duthie, H. C. (2000). Stream epilithic, epipelic and epiphytic diatoms, habitat fidelity and use in biomonitoring. *Aquatic Ecology* **34**, 345–353. doi:10.1023/A:1011461727835

Yallop, M., Hirst, H., Kelly, M., Juggins, S., Jamieson, J., *et al.* (2009). Validation of ecological status concepts in UK rivers using historic diatom samples. *Aquatic Botany* **90**, 289–295. doi:10.1016/J.AQUABOT.2008.11.005

Zelinka, M., and Marvan, P. (1961). Zur präzisierung der biologischen klassifikation der reinheit fliessender gewässer. *Archiv fuer Hydrobiologie* **57**, 389–407.

Zimba, P. V., and Hopson, M. S. (1997). Quantification of epiphyte removal efficiency from submersed aquatic plants. *Aquatic Botany* **58**, 173–179. doi:10.1016/S0304-3770(97)00002-8

# Epiphytic diatoms along environmental gradients in European shallow lakes

Cristina Cejudo-Figueiras[1,3], Irene Álvarez-Blanco[1,4], Eloy Bécares[2,5], AND Saúl Blanco[2,6]

[1] *Institute of the Environment, La Serna 58, E-24007 León, Spain*
[2] *Department of Biodiversity and Environmental Management, University of León, E-24071 León, Spain*

**Abstract.** Diatom-based methods have been successfully used worldwide to assess the trophic status in lenthic habitats. Several studies have demonstrated the efficacy of epiphytic diatoms as indicators of nutrient load in shallow lakes and wetlands. The purpose of this study was to examine the relative importance of environmental factors in explaining the structure of epiphytic diatom communities in European shallow lakes. The effects of lake chemical, morphometric and geographical predictors on diatom assemblages was tested using distance-based, canonical correspondence and regression analyses. Results show that epiphytic diatom communities respond mainly to chemical variables, overriding the effects of lake depth, size and location. Moreover, the clustering of studied systems based on TP levels is found consistent with their classification according to the indication value of inhabiting diatom taxa. Epiphytic diatoms offer then a workable tool for developing shallow lake typologies and classification systems in European countries. Further testing and concretion of the most relevant limnological variables would improve the applicability of diatoms as environmental proxies for shallow lakes.

**Key words:** epiphython, Bacillariophyta, bioindication, eutrophication, total phosphorus.

Shallow lakes are numerically the most abundant lentic ecosystems in the world (Wetzel 2001). Their heterogeneous nature and anthropogenic impacts pose challenges for developing standard bioassessment methodologies. These lakes are characterized by a high ratio of littoral and pelagic component, so that the area of aquatic plant growth is proportionately larger, potentially occupying its entirety. In Europe, most of these ecosystems have been severely affected by anthropogenic perturbations, among which one of the most important processes is eutrophication. In this context, the European Union Water Framework Directive (WFD, European Union 2000), requires state members to develop standardized methods to assess the ecological status of their waters using bioindicators. Among them, diatoms are suitable indicators of a variety of disturbances, particularly those affecting the chemical condition of waters. Diatoms fulfill the requirements of good indicator organisms in aquatic ecosystems, having additional advantages with respect to other communities such as macroinvertebrates (Blanco et al. 2008). They occur in a wide variety of environments, showing a broad range of tolerance along several gradients of abiotic factors, while individual species have specific water chemistry requirements (Round 1991). Finally, sampling and processing are relatively simple and cost-effective.

While many studies support the efficacy of diatom-based biological indices for monitoring the ecological status of running waters (e.g., Ector and Rimet 2005), there is still a lack of a common assessment methodology for shallow lakes. The study of diatoms in lentic systems has usually focused on their use as paleoenvironmental proxies (see an extensive review in Smol and Stoermer 2009), although several recent papers show also the applicability of biotic indices based on diatoms in the establishment of the ecological status of lakes (Hoffman 1994, Mayer and Galatowitsch 1999, Kitner and Poulíčková 2003, Trobajo 2003).

---

[3] E-mail addresses: ccejf@unileon.es
[4] ialvb@unileon.es
[5] ebecm@unileon.es
[6] sblal@unileon.es

In lenthic facies, the use of diatoms as ecological predictors achieves a more realistic approach for water quality assessment than other taxonomic groups and, particularly, epiphytic diatoms play a major structural and functional role in the aquatic biota of shallow freshwater ecosystems. Blanco et al. (2004) argue the advantages of sampling epiphytic diatom assemblages on littoral helophytes for biomonitoring purposes. Cejudo-Figueiras (2010a, b) explored the effect of different plant substrata on the resulting ecological classification of shallow lakes inferred from epiphytic diatom assemblages, evidencing that diatom-based metrics provide an accurate diagnosis of their trophic status irrespective of the sampled host macrophyte. The present study aims to explore the effect of further abiotic predictors on the configuration of epiphytic diatom communities in shallow lakes, namely the response to lake geographical, morphological and chemical features. The literature (Pan et al. 2000, Della Bella et al. 2007, Denys 2007) suggest a great dependence of the structure of this community to water chemistry, and we predict that this effect may override the influence of additional variables, hence providing a reliable support for the implementation of methods based on epiphytic diatoms for the ecological assessment of European shallow lakes.

TABLE 1. Descriptive statistics of the variables measured in the studied lakes (n = 44).

| Group | Variable | Mean | s.e. | Range |
|---|---|---|---|---|
| Geographic | Latitude | 53.71 | 0.92 | 42.23-68.44 |
| | Altitude (m a.s.l.) | 259.66 | 55.50 | (-2)-1,448 |
| Morpho-metric | Area (ha) | 788.59 | 614.90 | 1-27,000 |
| | Mean depth (m) | 1.77 | 0.13 | 0.4-3.7 |
| Chemical | Secchi depth (m) | 1.19 | 0.18 | 0.01-6.30 |
| | pH | 8.01 | 0.15 | 5.1-9.8 |
| | Conductivity ($\mu S \cdot cm$-1) | 284.58 | 32.94 | 9-1010 |
| | [TP] (ppb) | 109.28 | 18.59 | 6-521 |
| | [TN] (ppb) | 1422.96 | 270.00 | 26-11,544 |
| | [Chl $a$] (ppb) | 44.32 | 10.08 | 0.9-378.0 |

## Materials and methods

Littoral helophyte stems (mainly adult reeds Phragmites australis (Cav.) Trin. ex Steud.) were collected from 44 shallow lakes in 9 European countries in summer 2000 or 2001 (Fig. 1) in the context of a pan-European study (ECOFRAME) aiming at developing an assessment system for the ecological quality of shallow lakes. Main limnological characteristics and further data on the studied systems are presented on Table 1. Lake selection pretended to cover a wide range of geographical regions and limnological conditions. All lakes were natural in origin or naturalized if ultimately manmade, and none were 'heavily modified waters' as outlined in the WFD (Peeters et al. 2009). Water chemistry and other parameters were measured in situ or in the laboratory following standard protocols. Further data on the selected lakes and methodological details are available in Moss et al. (2003) and Nöges et al. (2003).
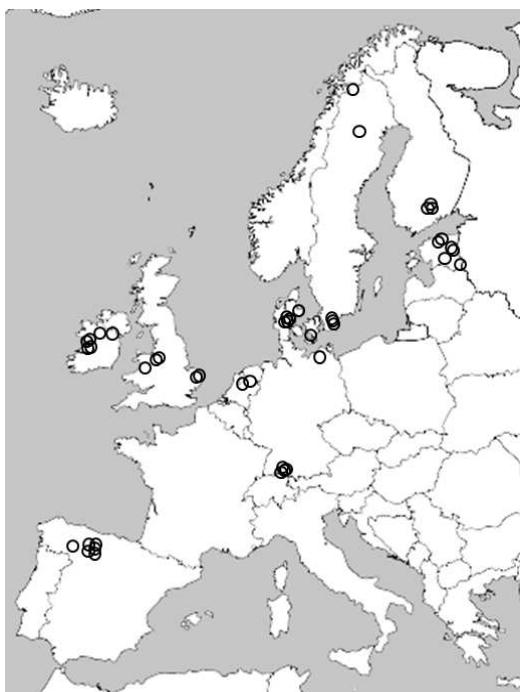


FIG. 1. Geographic location of the studied shallow lakes.

Helophyte 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 stan-

dard European standard protocol EN14407 (2004). At least 400 valves were counted and identified on each slide. Diatom taxonomy followed Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) and other reference works. Diatom taxa were classified according to their sensitivity to general pollution (S) in the SPI index (Coste 1982) which considers five classes (S = 1 to S = 5) for species indicating highly impaired and near-pristine conditions, respectively. This metric is significantly correlated with nutrient levels in shallow lakes (Blanco et al. 2004).
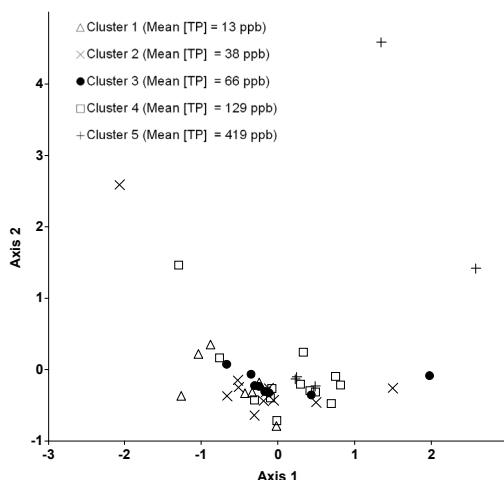


FIG. 2. CCA ordination plot of the 44 studied lakes grouped into 5 clusters according to their TP concentration.

Total phosphorus concentrations (TP) was considered as the main operational criterion for the classification of studied lakes according to their trophic status. Following the WFD recommendations (Stenger-Kovacs et al. 2007), a 5-level classification was obtained by means of a k-means clustering (Hartigan and Wong 1979) for this variable. Abiotic factors were sorted into three groups: chemical (pH, conductivity, Secchi depth, TP, total nitrogen and pelagic chlorophyll a), morphometric (lake area and mean depth) and geographic (latitude and altitude) variables. The differential response of diatom assemblages to these groups was assessed by means of distance-based multivariate analysis for a linear model using forward selection (DISTLM, Anderson 2003). This analysis performs a multivariate multiple regression on the basis of a given distance measure, resulting in a forward selection of the predictor va-

riables sets with tests by random permutation (McArdle and Anderson 2001). The distance measure used for DISTLM was the Manhattan distance, which was chosen as it is a function of absolute values of distances, and is therefore robust to outliers (Lear and Lewis 2009). Additionally, a canonical correspondence (CCA) and a stepwise regression (SRA) analysis were performed in order to explore the dependence of diatom relative abundances on water chemical characteristics. A similarity test (ANOSIM) was used to check for significant differences between lake clusters in the resulting CCA plot. Statistical analyses were conducted using Statistica v. 8.0 (StatSoft 2007) and PAST v. 2.04 (Hammer et al. 2001) softwares.

TABLE 2. Results of k-means clustering of studied lakes based on TP concentrations.

| Cluster | No. of lakes included | [TP] mean (s.e.) (ppb) |
|---------|-----------------------|------------------------|
| 1 | 7 | 13.43 (2.28) |
| 2 | 11 | 37.64 (2.44) |
| 3 | 8 | 65.63 (4.96) |
| 4 | 13 | 129.19 (7.15) |
| 5 | 5 | 419.20 (33.25) |

## Results

Lake clusters according to their TP concentrations are presented on Table 2. These groups are also displayed in Fig. 2 showing the distribution of studied systems along main CCA axes. ANOSIM test proved these groups to be statistically different (R = 0.098, p = 0.038). An examination of original datasets revealed floristic discrepancies between lake classes, e.g., systems with the lowest TP levels were dominated by oligotrophilous species such as *Achnanthidium minutissimum* (Kütz.) Czarn. (mean abundance: 27%) or *Achnanthes linearioides* Lange-Bert. (mean abundance: 10%) in the epiphytic assemblages, while taxa indicating eutrophic conditions (*Gomphonema parvulum* Kütz, mean abundance: 3 %) were specially frequent in cluster 5 (mean [TP] = 419 ppb). Taxa with mean relative abundances above 1% were plotted against CCA axes (Fig. 3) and the ANOSIM test results (R = 0.056, p = 0.044) on each species' coordinates in the plot show statistically significant differences between the a-priori defined taxa sets based on their sensitivity value (S) to pollution in the SPI index.

TABLE 3. Results of DISTML analysis (conditional tests) on the basis of the Manhattan distance. p values were obtained using 99999 permutations of residuals.

| Group | SS (trace) | Pseudo-F | Proportion | Cumulative | p |
|---|---|---|---|---|---|
| Chemical | 1712·10⁶ | 13.8 | 0.21 | 0.21 | 0.0686 |
| Morphometric | 471·10⁶ | 11.6 | 0.06 | 0.32 | 0.3023 |
| Geographic | 452·10⁶ | 11.0 | 0.06 | 0.26 | 0.3446 |

CCA plot of abiotic vectors (Fig. 4) shows graphically the differential importance of studied environmental variables in the corresponding distribution of lakes and diatom taxa. The eigenvalues of the first two axes are 0.59 and 0.54, explaining 19 % and 17 % of the cumulative variance of the species dataset, respectively (sum of all canonic eigenvalues: 3.14). The statistical significance of the defined sets of variables was assessed through DISTLM analysis (Table 3), revealing that only chemical predictors achieve near-significant relationships with the abundance and distribution of diatoms. The relationships with singular chemical descriptors was more thoroughly addressed by means of SRA (Table 4) which pointed out significant effects of TP, conductivity and pelagic chlorophyll a in the configuration of epiphytic diatom assemblages in the studied lakes.
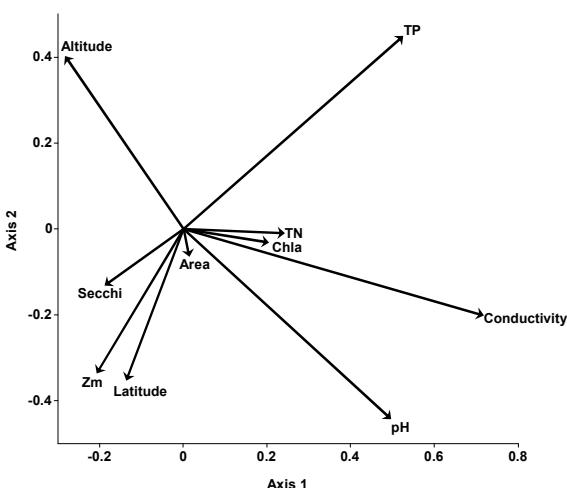


FIG. 3. CCA plot of environmental variables for the analyzed systems.

## Discussion

Littoral helophytes (Poulíčková et al. 2004, Stenger-Kovacs et al. 2007) and, particularly, reeds (Ács et al. 2008) are regarded as the most suitable substrata for the assessment of lake trophic status using diatom-based metrics. Epiphytic diatoms exhibit comparable performances to mid-lake sediment diatoms for monitoring TP levels (Denys 2007) and provide robust environmental inferences for water quality assessment. In our study, associations between epiphytic diatom assemblages and environmental variables were detected through several complementary multivariate methods. The predictive power of different sets of abiotic factors was tested using DISTLM analysis, which evidenced that this community responds mainly to water chemical predictors, being other parameters of minor relevance.

TABLE 4. Results of SRA analysis with forward stepwise selection of chemical variables.

| | Wilk's test | F | p |
|---|---|---|---|
| Chl $a$ | 0.000032 | 941.08 | 0.0258 |
| Conductivity | 0.000104 | 291.02 | 0.0463 |
| TP | 0.000110 | 274.30 | 0.0477 |
| Secchi depth | 0.000243 | 124.64 | 0.0708 |
| TN | 0.002443 | 12.37 | 0.2220 |
| pH | 0.003800 | 7.94 | 0.2749 |

### Chemical variables

Identification of the primary chemical stressors structuring the studied diatom communities resulted in the detection of chlorophyll a, conductivity and TP concentration. Despite limnological data were collected from one date only whereas ideally time-weighted averages may have proved more suitable for environmental inferences, epiphytic diatoms were proved to provide a statistically robust prediction of nutrient status in the studied lakes. Additionally, CCA demonstrated a concordance between a biological lake classification based on epiphytic diatoms and a classification based on TP levels. This analysis was also used to test how well the studied variables discriminated between diatom ecological groups, showing congruence between this clustering and diatom specific indication values. However, it has been suggested that epiphytic assemblages could be less efficient in reflecting ambient conditions

than other communities (Denys 2007). The composition and the architecture of the epiphyton are subject not only to physicochemical attributes of the surrounding environment (Ács et al. 2008), and this may indicate that many taxa may not show a direct easily interpretable response to eutrophication pressure (Free et al. 2009). For instance, Denys (2007) found that epiphytic assemblages from acid lakes are more weakly related to TP concentrations. Despite this, broadscale studies show that nutrient concentrations were the main variables determining the composition and structure of epiphytic diatom communities (Pan et al. 2000, Della Bella et al. 2007). Our results suggest also that epiphytic diatom assemblages may be useful bioindicators of shallow lake conditions, especially eutrophication.
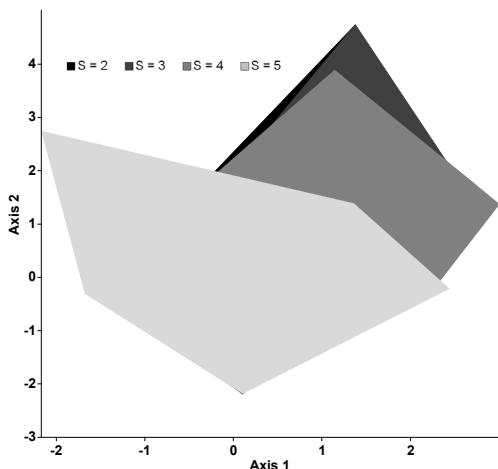


Fig. 4. Ordination plots of main (> 1% in mean relative abundance) epiphytic diatom taxa with respect to environmental variables based on CCA. Species were classified according to their pollution sensitivity value (S) in the SPI index. Points (omitted) fitted to convex hulls.

### Morphometric variables

Both depth and size are considered as core mandatory descriptors for the ecological classification of lakes in Europe (Nykänen et al. 2005, McElarney and Rippey 2009). However, there is so far less knowledge on large scale responses to these factors in lakes (Lyche Solheim et al. 2008). Within several biological communities, the abundance of different taxonomic groups is greatly dependent on the lake's morphometry (Nykänen

et al. 2005). Particularly, macroinvertebrate richness, especially of small lakes, increases with lake size (Zenker and Baier 2009). Thus, morphometric variables could play an important role across diverse lake types and this variability may introduce a bias in the dependence of biotic communities to water quality. Anyway, our data suggest that the sensitivity of epiphytic diatoms to lake morphometry is low, this pattern may be common also for macroinvertebrates (Free et al. 2009).

### Geographical variables

The responses of epiphytic algae to trophic state could be modified by the influence of other factors such as lake altitude or latitude that determine lake characteristics and, therefore, the biological community structure and its composition. Some authors (e.g., Catalan et al. 2009) suggest a regional element in the response of the biota to environmental predictors in European lakes. Adding to this, the relative influence of nutrients on water quality of shallow lakes appears to increase southward in the area covered by this study (Beklioglu et al. 2007) so that nutrient levels may be of less importance in northern shallow lakes than in similar lakes at lower latitudes. Therefore, environmental inferences from epiphytes are also presumed to be subject to spatial variation (Denys 2007). Altitude is also frequently found as an important surrogate for relevant descriptors, usually correlated to anthropogenic eutrophication (McElarney and Rippey 2009). The data collected within the present study showed, however, general trends in response mainly to increasing nutrient levels along a wide latitudinal gradient. This is supported by several studies (e.g., Donohue et al. 2009, McElarney and Rippey 2009) that consistently find a poor amount of variation in biological communities in freshwater habitats, local effects being stronger than large-scale characteristics. This suggests strongly that epiphytic diatoms can provide a robust indication of trophic conditions in shallow lakes despite floristic regional particularities.

### Concluding remarks

This paper reports a concordance between a classification based on epiphytic diatoms and lake types derived using TP levels for European shallow lakes. This indicates that, despite the complexity of epiphytic diatom communities, it is

possible to successfully develop bioindication methods based on these assemblages to be applied under a wide range of morphometric conditions and geographic locations. Our data suggest that such metrics could be advantageous compared to macroinvertebrate indices, whose dependence on habitat features prevents their use in ecological assessment (Donohue et al. 2009, Free et al. 2009). Future investigations may explore inclusion in similar analyses of more lake typologies and ecological conditions in order to develop reliable biomonitoring methods for these ecosystems.

## Literature cited

Ács, É., A. K. Borsodi, É. Kiss, K. T. Kiss, K. É. Szabó, P. Vladár, G. Várbíró, and Gy. Záray. 2008. Comparative algological and bacteriological examinations on biofilms developed on different substrata in a shallow soda lake. Aquatic Ecology 42:521-531.

Anderson, M. J. 2003. DISTLM forward: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model using forward selection. Department of Statistics, University of Auckland, New Zealand.

Beklioglu, M., S. Romo, I. Kagalou, X. Quintana and E. Bécares. 2007. State of the art in the functioning of shallow Mediterranean lakes: Workshop conclusions. Hydrobiologia 584:317-326.

Blanco, S., L. Ector, and E. Bécares. 2004. Epiphytic diatoms as water quality indicators in Spanish shallow lakes. Vie et Milieu / Life and Environment 53 (2/3):71-79.

Blanco, S., L. Ector, V. Huck, O. Monnier, H. M. Cauchie, L. Hoffmann, and E. Bécares. 2008. Diatom assemblages and water quality assessment in the Duero Basin (NW Spain). Belgian Journal of Botany 141:39-50.

Catalan, J., C. J. Curtis, and M. Kernan. 2009. Remote European mountain lake ecosystems: regionalisation and ecological status. Freshwater Biology 54:2419-2432.

Cejudo-Figueiras, C., I. Álvarez-Blanco, E. Bécares, and S. Blanco. 2010. Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited. Marine and Freshwater Research. In press.

Cejudo-Figueiras, C., S. Blanco, I. Álvarez-Blanco, L. Ector, and E. Bécares. Nutrient monitoring in Spanish wetlands using epiphytic diatoms. Vie et Milieu / Life and Environment 60. In press.

Coste, M. 1982. Étude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport d'étude CEMAGREF Lyon, Agence de I'Eau, Rhône – Méditérannée – Corse, France.

Della Bella, V., C. Puccinelli, S. Marcheggiani, and L. Mancini. 2007. Benthic diatom communities and their relationship to water chemistry in wetlands of central Italy. Annales de Limnologie-International Journal of Limnology 43 (2):88-89.

Denys, L. 2007. Water-chemistry transfer functions for epiphytic diatoms in standing freshwaters and a comparison with models based on littoral sediment assemblages (Flanders, Belgium). Journal of Paleolimnology 38:97–116.

Donohue, I., L. A. Donohue, B. Ní Ainín, and K. Irvine. 2009. Assessment of eutrophication pressure on lakes using littoral invertebrates. Hydrobiologia 633:105–122.

Ector, L., and F. Rimet. 2005. Using bioindicators to assess rivers in Europe: An overview. Pages 7-19 in S. Lek, M. Scardi, P. F. M. Verdonschot, J. P. Descy, Y.-S. Park (editors). Modelling Community Structure in Freshwater Ecosystems. Springer-Verlag, Berlin, Heidelberg.

European Standard EN 14407. 2004. Water quality - Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. European Committee for Standardization, Brussels.

European Union. 2000. Directive 2000/60/EC of the European Parliament and the Council of 23 October establishing a framework for Community action in the field of water policy. Official Journal of the European Communities L327:1–73.

Free, G., A. Solimini, B. Rossaro, L. Marziali, R. Giacchini, B. Paracchini, M. Ghiani, S. Vaccaro, B. M. Gawlik, R. Fresner, G. Santner, M. Schönhuber, and A. C. Cardoso. 2009. Modelling lake macroinvertebrate species in the shallow sublittoral: relative roles of habitat, lake morphology, aquatic chemistry and sediment composition. Hydrobiologia 633:123–136.

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4 (1):1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hartigan, J. A., and M. A. Wong. 1979. Algorithm AS 136: A k-means Clustering Algorithm. Applied Statistics 28:100-108.

Hoffman, G. 1994. Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie. Bibliotheca Diatomologica 30:1-241.

Kitner, M., and A. Poulíčková. 2003. Littoral diatoms as indicators for the eutrophication of shallow lakes. Hydrobiologia 506-509:519-524.

Krammer, K., and H. Lange-Bertalot. 1986. Bacillariophyceae, Teil 1. Naviculaceae. VEB Gustav Fisher Verlag, Stuttgart.

Krammer, K., and H. Lange-Bertalot. 1988. Bacillariophyceae, Teil 2. Epithemiaceae, Bacillariaceae, Surirellaceae. VEB Gustav Fisher Verlag, Stuttgart.

Krammer, K., and H. Lange-Bertalot. 1991a. Bacillariophyceae, Teil 3. Centrales, Fragilariaceae, Eunotieaceae, Achnanthaceae. VEB Gustav Fisher Verlag, Stuttgart.

Krammer, K., and H. Lange-Bertalot. 1991b. Bacillariophyceae, Teil 4. Achnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae) und Gomphonema. VEB Gustav Fisher Verlag, Stuttgart.

Lear, G., and G. D. Lewis. 2009. Impact of catchment land use on bacterial communities within stream biofilms. Ecological Indicators 9:848-855.

Lyche Solheim, A., S. Rekolainen, S. J. Moe, L. Carvalho, G. Phillips, R. Ptacnik, W. E. Penning, L. G. Toth, C. O'Toole, A. L. Schartau, and T. Hesthagen. 2008. Ecological threshold responses in European lakes and their applicability for the Water Framework Directive (WFD) implementation: synthesis of lakes results from the REBECCA project. Aquatic Ecology 42:317-334.

Mayer, P. M., and S. M. Galatowitsch. 1999. Diatom communities as ecological indicators of recovery in restored prairie wetlands. Wetlands 19:765-774.

McArdle, B. H., and M. J. Anderson. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82(1):290-297.

McElarney, Y. R., and B. Rippey. 2009. A comparison of lake classifications based on aquatic macrophytes and physical and chemical water body descriptors. Hydrobiologia 625: 195-206.

Moss, B., D. Stephen, C. Álvarez, E. Bécares, W. van de Bund, S. E. Collings, E. van Donk, E. de Eyto, T. Feldmann, C. Fernández-Aláez, M. Fernández-Aláez, R. J. M. Franken, F. García-Criado, E. M. Gross, M. Gyllström, L.-A. Hansson, K. Irvine, A. Järvalt, J.-P. Jensen, E. Jeppesen, T. Kairesalo, R. Kornijów, T. Krause, H. Künnap, A. Laas, E. Lill, B. Lorens, H. Luup, M. R. Miracle, P. Nõges, T. Nõges, M. Nykänen, I. Ott, W. Peczula, E. T. H. M. Peeters, G. Phillips, S. Romo, V. Russell, J. Salujõe, M. Scheffer, K. Siewertsen, H. Smal, C. Tesch, H. Timm, L. Tuvikene, I. Tonno, T. Virro, E. Vicente, and D. Wilson. 2003. The determination of ecological quality in shallow lakes - a tested system (ECOFRAME) for implementation of the European Water Framework Directive. Aquatic Conservation: Marine and Freshwater Ecosystems 13:507-549.

Nõges, P., T. Nõges, L. Tuvikene, H. Smal, S. Ligeza, R. Kornijow, W. Peczula, E. Bécares, F. García-Criado, C. Alvarez-Carrera, M. Fernández-Aláez, C. Ferriol, M. R. Miracle, E. Vicente, S. Romo, E. van Donk, W. Van de Bund, J. P. Jensen, E. Gross, L. A. Hansson, M. Gyllström, M. Nykänen, E. de Eyto, K. Irvine, D. Stephen, S. Collings, and B. Moss. 2003. Factors controlling hydrochemical and trophic state variables in 86 shallow lakes in Europe. Hydrobiologia 506-509:51-58.

Nykänen, M., T. Kairesalo, S. Mäkela, E. Huitu, P. Ala-Opas, and J. Mannio. 2005. A typology and ecological classification system for Finnish lakes: applicability of the ECOFRAME scheme. Boreal Environment research 10:159-179.

Pan, Y., R. J. Stevenson, P. Vaithiyanathan, J. Slate, and C. J. Richardson. 2000. Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, USA. Freshwater Biology 44:339-354.

Peeters, E. T. H. M., R. J. M. Franken, E. Jeppesen, B. Moss, E. Bécares, L-A. Hansson, S. Romo, T. Kairesalo, E. M. Gross, E. van Donk, T. Nõges, K. Irvine, R. Kornijów, and M. Scheffer. 2009. Assessing ecological quality of shallow lakes: Does knowledge of transparency suffice? Basic and Applied Ecology 10:89-96.

Poulíčková, A., M. Duchoslav, and M. Dokulil. 2004. Littoral diatom assemblages as indicators of lake trophic status: A case study from perialpine lakes in Austria. European Journal of Phycology 39:143-152.

Round, F. E. 1991. Diatoms in river water-monitoring studies. Journal of Applied Phycology 3:129-145.

Smol, J. P., and E. F. Stoermer. 2009 The diatoms: applications for the environmental and earth sciences. Tweede herziene druk. Cambridge University Press, Cambridge.

StatSoft, Inc. 2007. STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Stenger-Kovacs, C., K. Buczko, E. Hajnal, and J. Padisak. 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL) developed in Hungary. Hydrobiologia 589:141-154.

Trobajo, R. 2003. Ecological analysis of periphytic diatoms in Mediterranean coastal wetlands (Empordà wetlands, NE Spain). Tesis Doctoral, Universitat de Girona, Girona, 200 pp.

Wetzel, R. G. 2001. Limnology. Lake and river ecosystems. Academic Press, San Diego, USA.

Zenker, A., and B. Baier. 2009 Relevance of abiotic criteria used in German lake typology for macroinvertebrate fauna. Hydrobiologia 636:379-392.

I.  Cejudo-Figueiras C., Morales E.A., Wetzel C.E., Blanco S., Hoffmann L. & Ector L. 2011. Analysis of the type of *Fragilaria construens* var. *subsalina* (Bacillariophyceae) and description of two morphologically related taxa from Europe and the United States. *Phycologia*, 50 (1): 67-77.

II.  Blanco S., Cejudo-Figueiras C., Tudesque L., Bécares E., Hoffmann L. & Ector L. Are diatom diversity indices reliable monitoring metrics? *Hydrobiologia*. Aceptado.

III.  Cejudo-Figueiras C., Blanco S., Álvarez-Blanco I., Ector L. & Bécares E. 2010. Nutrient monitoring in Spanish wetland using epiphytic diatoms. *Vie et milieu - life and enviroment*, 60 (2): 89-94.

IV.  Cejudo-Figueiras C., Álvarez-Blanco I., Bécares E. & Blanco S. 2010. Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited. *Marine and Freshwater Research*, 61: 1457–1467.

V.  Cejudo-Figueiras C., Álvarez-Blanco I., Bécares E. & Blanco S. 2010. Epiphytic diatoms along environmental gradients in European shallow lakes. *The Journal of the North American Benthological Society*. Enviado.