Water-chemistry transfer functions for epiphytic diatoms in standing freshwaters and a comparison with models based on littoral sediment assemblages (Flanders, Belgium)

Luc Denys

Abstract Quantitative inference models for water-chemistry variables are derived from epiphytic diatom assemblages in 186 lentic and mostly shallow freshwaters in lower Belgium (Flanders). When the complete pH range is considered (pH 3.4–9.3), robust transfer functions are obtained for median pH (jack-knifed $r^2 = 0.88$, RMSEP = 0.38 pH units or 6.4% of the observed range) and dissolved inorganic carbon concentration (jack-knifed $r^2 = 0.86$, RMSEP = 0.194 $\log_{10}$ mg DIC l^{-1} or 10.2% of the observed range) by means of weighted-averaging partial least squares regression (WA-PLS). For these variables, the calibration models are as reliable as those based on sedimentary diatom assemblages. Inferences of pH may be improved by combining estimates from epiphytic and sediment assemblages. In circumneutral and alkaline conditions, WA-PLS calibration of maximum or median total phosphorus is possible (log-transformed; jack-knifed $r^2 = 0.64$ or 0.66 and RMSEP = 14% or 12.3% of the observed range, respectively). It makes little difference if taxa showing no response to TP are taken into consideration or not. These models considerably expand the prospects of using historical herbarium materials to hindcast environmental conditions and also allow more accurate interpretation of current compositional changes in epiphytic communities. Compared to littoral sediment assemblages, fewer water-column variables can be inferred reliably from epiphyton. This probably results from differences between the effective gradients in both habitats, together with lower in situ species diversity and less effective spatial integration (i.e. lower recruitment of phytoplankton) in the epiphyton. A comparison of the HOF response-model types and WA-optima of diatom taxa for epiphytic and sediment assemblages shows that the relationship to individual variables, and in particular to those related to trophic status, may differ with habitat. Thus, the combination of samples from both habitat types in the same calibration model is not recommended.

Keywords Bacillariophyceae Æ Phytobenthos Æ Shallow waters Æ Water quality Æ Microhabitat Æ Herbarium macrophytes

L. Denys (&)
Research Institute for Nature and Forest (INBO), Kliniekstraat 25, Brussels B-1070, Belgium
e-mail: luc.denys@inbo.be

L. Denys
Biology Department, PLP, University of Antwerpen, Universiteitsplein 1, Wilrijk B-2610, Belgium

Introduction

Reference to former status to validate ecological standards and goals for freshwater conservation and restoration (Battarbee 1999; CEC 2000) is faced with constraints for shallow lakes and ponds. Disturbance of sediment records, dating problems and poor preservation of microfossils, such as diatom frustules, complicate the use of sediment cores, while shifts in habitat availability due to varying macrophyte abundance and temporary sequestering of nutrients in sediments and biomass further challenge the reconstruction of environmental conditions in such systems (Sayer 2001; Denys 2003, 2006). Moreover, substantial between-site variation may need to be dealt with. Denys (2006) presented calibration models relating diatom assemblages from littoral surface sediments to water-chemistry variables measuring base status, salinity, silica concentration, organic matter and phytoplankton productivity in standing waters from lower Belgium. These models allow quantitative inferences of past water chemistry from historical samples, such as sediment adhering to the roots of herbarium macrophytes, and can provide environmental information substituting or complementing core-based paleolimnological observations. Alternatively, epiphytic diatoms on herbarium specimens are a possible source of information. The composition of epiphytic diatom assemblages on herbarium plants is not affected significantly by the drying process (Vogel et al. 2005) and is already used to hindcast general environmental conditions in standing waters (Arzet and van Dam 1986; van Dam and Mertens 1993; Sayer et al. 1999; Cocquyt and De Wever 2002; Denys 2003), often by applying indices using indicator values (e.g. Hofmann 1994; van Dam et al. 1994). Robust transfer functions would improve the reliability of such inferences (DeNicola et al. 2004; Denys 2004) and their application to herbarium materials offers unique possibilities for regional water-quality assessments and ecological base-line setting with a pertinent temporal perspective, without hinging on the availability of in situ sediment records with sufficient resolution and integrity. Moreover, such models may have useful applications to biomonitoring, particularly because epiphyton integrates environmental signals within a narrower time frame and on a more distinct habitat scale than algal assemblages found in sediments (King et al. 2000; DeNicola et al. 2004). So far, no diatom calibration models based exclusively on epiphytic samples appear to have been developed for standing waters. In this respect, it should be noted that phytobenthos-monitoring efforts developed for implementing the European Water Framework Directive (CEC 2000) are more likely to focus on this community.

Potential problems associated with the development of calibration models based on epiphytic diatoms include the complex interactions between macrophytes and the epiphytic communities they support (e.g. Eminson and Moss 1980; Burkholder and Wetzel 1990) which may confound quantitative inferences of water-column chemistry (Bennion 1995; Hall and Smol 1999). Previous studies suggest that nutrient supply from the substrate can intervene with total-phosphorus (TP) estimation from benthic diatom assemblages (Anderson et al. 1993; Bennion 1994; Siver 1999; Poulíčová et al. 2004). Epiphytic assemblages could also be less efficient in reflecting ambient conditions than sedimentary assemblages by storing less environmental information due to their generally lower species richness and evenness (Birks 1994; Hofmann 1994; Siver 1999), with less spatial integration of the flora from other habitats (Battarbee et al. 2001). On the other hand, less control by physical habitat conditions, such as light availability, might be expected for epiphyton, which is reported as a problem in nutrient reconstructions from sediment assemblages originating from deeper water where variable turbidity can interfere (Bennion 1995; Bennion et al. 2001; Sayer 2001).

This contribution examines the calibration of epiphytic diatom assemblages and the possibilities for further exploiting the environmental archive represented by herbarium specimens of aquatic plants more completely. In order to be generally applicable to this type of material, inference tools need to span a wide range of limnological conditions (Denys 2006). Next to developing transfer models in addition to those based on littoral sediment assemblages from the same region, this paper aims to compare the range of

variables that can be calibrated from epiphytic and sediment assemblages, as well as the precision attained by shared transfer functions. The use of the same sites and methodology as in the study presented by Denys (2006) allows to compare models for both assemblages without the usual constraints imposed by reference to training sets of different data quality and covering unequal environmental gradients. Furthermore, apparent species optima for epiphytic and littoral sedimentary assemblages are compared in order to investigate whether development of "hybrid" transfer functions (Schönfelder 1997; Schönfelder et al. 2002) and assessment methods (Schaumburg et al. 2004) from simultaneous sampling of communities from both hard and soft substrates is warranted, despite the potentially significant habitat specificity in environmental relationships (e.g. Poulíčová et al. 2004).

The general conditions and types of surface waters in the study region are similar to those in the remaining densely populated near-coastal region of Western Europe, so it is likely that observations presented in this paper will be relevant to diatom-epiphyton studies in this considerably larger area.

Study area

Geographical characteristics of the study area, Flanders, the northern part of Belgium (51°00¢N 4°15¢E, 13.522 km², mainly below 200 m a.s.l.), are summarized by Verbruggen et al. (1996) and Franklin et al. (2003). Surface geology largely consists of Quaternary marine, fluvial and niveo-eolian deposits with well-developed clayey to sandy, locally peaty, top soils. The climate is mild and of a temperate maritime type (average yearly temperature 9.8°C, precipitation 780 mm yr^{-1}). Population density is very high ($\Diamond$443 persons per km²) and land use is intense. Industrial, agricultural and recreational activities result in high anthropogenic pressure on most surface waters (eutrophication, fish stocking, acidification, physical perturbation; Denys 2001). Nearly all standing waters are man-made and most are small and shallow. Stones are scarce or often completely absent in the littoral areas, where submerged or floating-leaved aquatic plants, helophytes and woody debris are the more usual natural substrates for periphytic communities.

Materials and methods

Data collection

The entire training set consists of samples from 186 standing waters distributed throughout the region and selected following a pilot survey (Fig. 1). These cover the range of permanent freshwater conditions, with the exception of canals, ditches, watercourses, and distinctly brackish waters. Most of the water bodies are small (median surface area = 1.33 ha), shallow, and well mixed (Table 1). Surrounding land use is highly varied (mainly semi-natural heaths, shrubs and woodlands, tree plantations, lawns, pastures and agricultural fields), and so is the degree of
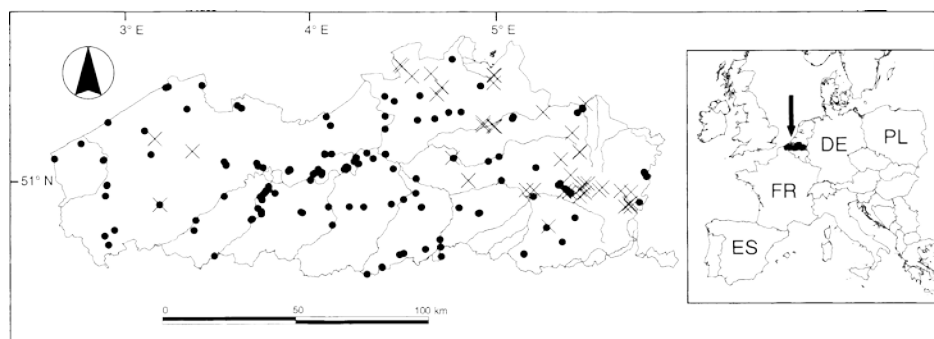

Fig. 1 Map of Flanders with main rivers and location of sampling sites (sites included in the non-acid waters training set are represented by •, acid sited by · ; distinction based on species composition of epiphyton, see "Data analysis")

Table 1 General features of the sampled waters and the species data sets

	Unit	Entire data set					Non-acid sites				
		Minimum	Median	Mean	SD	Maximum	Minimum	Median	Mean	SD	Maximum
Surface area	ha	0.02	1.33	4.60	9.58	73.97	0.02	1.47	5.32	10.86	73.97
Maximum depth	m	<1	1.5–3	–	–	ca. 18	< 1	1.5–3	–	–	ca. 18
Shoreline with trees and shrubs	%	0	60	55	36	100	0	60	54	36	100
Submerged vegetation	%	0	1	21	35	100	0	1	16	31	100
Emergent vegetation	%	0	1	10	21	100	0	1	10	21	100
pH		3.4	7.7	7.4	1.1	9.3	6.5	7.9	7.9	0.5	9.3
EC	$lS\,cm^{-1}$	24	460	520	393	3,520	202	559	642	381	3,520
Alkalinity	$meq\,l^{-1}$	−0.33	1.25	1.29	1.05	5.20	0.11	1.60	1.73	0.90	5.20
DIC	$mg\,l^{-1}$	<1.6	15.3	16.2	12.6	62.6	0.8	20.5	21.3	12.5	62.6
Sodium	$mg\,l^{-1}$	1.9	21.0	32.6	52.5	571.0	7.3	24.4	39.5	58.3	571.0
Calcium	$mg\,l^{-1}$	1.4	61.3	63.9	44.0	307.0	22.4	81.7	81.9	36.7	307.0
Magnesium	$mg\,l^{-1}$	0.6	6.8	9.1	7.9	65.8	3.4	8.8	11.3	8.1	65.8
Iron	$mg\,l^{-1}$	0.01	0.24	0.49	0.77	5.62	0.01	0.22	0.34	0.39	2.52
Sulphate	$mg\,l^{-1}$	<4.0	38.0	52.9	61.2	390.0	<4.0	48.0	59.9	62.1	374.0
Chloride	$mg\,l^{-1}$	3.0	37.0	51.6	79.8	921.0	9.0	42.0	63.7	89.7	921.0
TIN	$mg\,l^{-1}$	0.07	0.24	0.78	1.38	7.95	0.07	0.28	0.90	1.53	7.95
TP	$mg\,l^{-1}$	<0.07	0.13	0.29	0.45	2.89	<0.07	0.19	0.37	0.50	2.89
Silica	$mg\,l^{-1}$	0.06	3.96	6.55	6.85	30.30	0.10	5.53	7.96	7.30	30.30
A440	m^{-1}	0.28	4.05	5.37	4.43	24.40	0.45	4.00	5.31	4.57	24.40
A440$_f$	m^{-1}	<0.1	1.18	1.81	2.31	19.85	0.10	1.10	1.52	2.11	19.85
Oxygen saturation	%	12	92	91	29	206	15	92	93	31	206
Chlorophyll a	$lg\,l^{-1}$	<3	21	42	56	310	<3	25	49	62	310
pGOP	$mg\,l^{-1}$	−1.0	3.3	6.6	8.4	41.8	−1.0	4.6	8.0	9.3	41.8
COD	$mg\,l^{-1}$	<1.5	37.4	40.7	27.5	309.0	9.3	38.4	42.7	29.8	309.0
TON	$mg\,l^{-1}$	0.34	1.27	1.47	0.84	7.40	0.34	1.36	1.61	0.90	7.40
Occurrences/taxon		1	23	34	34	160	1	26	35	31	132
Hill's $N2$ (taxon)		1.0	4.3	7.1	8.2	67.2	1.0	4.4	7.4	7.2	38.5
Dominance (taxon)	%	1.0	5.7	15.0	20.0	100.0	1.0	5.4	13.0	18.0	94.4
Taxa/sample		2	46	44	19	109	13	50	50	15	103
Hill's $N2$ (sample)		1.0	4.0	5.2	3.9	20.1	1.1	4.5	6.0	4.3	19.9
Dominance (sample)	%	10.0	41.5	45.9	21.8	100.0	10.0	38.1	41.9	20.5	94.4

Water-chemistry characteristics are based on median values of samples collected during a single vegetation season in either 1998 or 1999

impairment as judged from turbidity, vegetation and shoreline modification.

Surface area is determined from recent air-survey photographs, whereas maximum depth and underwater slope at the shore are ordinal field estimates (Denys 2006). Major soil-texture and land-use types are assessed proportionally within a periphery of 50 m from soil maps and the most recent "biological evaluation" survey maps available (De Blust et al. 1994).

Water-column variables are for the vegetation period (May–November) of a single year, either 1998 or 1999. Water samples are taken at a depth of 0.5 m, or at half of the maximum depth in basins less than 1 m deep, close to the site of diatom sampling, but not within dense vegetation. Samples are preferably from near the outflow, if this is present, or from a well-mixed site, as far from the bank as can be reached without a boat. Oxygen saturation (O_2%), pH and electric conductivity (EC) are determined from 5 to 6 field measurements. Usually, three analyses are available for major ions, inorganic nitrogen, total phosphorus (TP) and soluble reactive phosphorus, Kjeldahl nitrogen, dissolved inorganic carbon (DIC) and calculated alkalinity (ALK). Variables

reflecting biochemical and metabolic status or phytoplankton abundance have 4–5 measurements. These include chemical oxygen demand (COD), biochemical oxygen demand, potential gross oxygen production (pGOP) and potential net oxygen production (all for a 24 h period), chlorophyll *a* and phaeopigments, as well as absorbance of organic matter at 254 nm and of yellow substances (gilvin) at 440 nm, before and after filtering (A440, A440$_f$). Because of the limited number of measurements, site median values are preferred to averages; maxima are also considered for N- and P-fractions and chlorophyll *a*. Levels below detection limits are set at 50% of the threshold for all calculations. Denys (2006) provides more details on water sampling and analysis.

Epiphytic diatom assemblages are sampled from representative substrates, with preference for true aquatics. Helophytes (mostly reed, *Phragmites australis*) or, if necessary, parts of riparian vegetation hanging in the water are sampled where true aquatic plants are lacking. Bryophytes and senescent plant parts are excluded from sampling. A compound sample is taken in the case that multiple aquatics are present at the sampling site. All samples are from permanently submerged substrates occurring at a depth of approximately 20–30 cm. Plant parts are fixated immediately with dilute formaldehyde. From soft-leaved plants, �2 cm^3 (with roughly equal volumetric representation of collected plant species) is processed entirely for diatom analysis, but epiphyton on reeds, etc., is scraped off with a razor before digestion. Sample preparation, identification and counting (exactly 500 valves; additional taxa occurring outside the count are attributed 0.02%) follows Denys (2006). Only taxa occurring at least once with an abundance of 1% are considered in the analyses, from which the reworked or transported marine taxon *Delphineis minutissima* is excluded.

Data sets are characterized by the total number of taxa, the number of taxa per sample, the dominance (% abundance of the most abundant taxon), the number of occurrences per taxon and the estimated number of effective occurrences (*N*2; Hill 1973).

Data analysis

Data analysis is the same as reported in more detail by Denys (2006) for sediment assemblages. Transformations include square-root (sqrt) for species abundances and logarithms (base 10, adding a constant if necessary) or sqrt for environmental data.

Variables for calibration are identified by a series of ordination analyses (with rare taxa down-weighted; CANOCO for Windows version 4.5; ter Braak and Šmilauer 2002). Compositional gradient length is estimated by means of detrended correspondence analyses (DCA, detrending by segments, rare taxa down-weighted, non-linear rescaling) to determine the most appropriate ordination method. A minimal set of environmental variables significantly influencing diatom variation between sites is determined by canonical correspondence analysis (CCA) with forward selection (1,000 Monte–Carlo permutations; $P \pounds 0.05$, Bonferroni corrected). Colinearity is avoided by inspecting correlations and variance inflation factors (VIFs). The variance in species composition explained by a forward-selected variable (marginal effect) is estimated by a constrained CCA (CCCA) and its significance tested using a Monte–Carlo permutation test (1,000 permutations). The ratio of the eigenvalues for the two principal axes (k_1/k_2) in this CCCA measures the relative importance of the constrained variable in explaining the species data. Interaction with secondary gradients is examined by partial CCCAs with all other forward-selected variables as covariables. Significance of the first axis, representing the unique contribution of the variable, is again tested by permutation. The ratio of the principal eigenvalues ($k_1\pounds/k_2\pounds$) and the amount of variance explained in this analysis are compared with the overall CCCA to examine independent behaviour of the target variable. Finally, the species-gradient length associated with selected variables is determined by detrended constrained canonical correspondence analysis (DCCA).

To account for varying combinations of influential variables along the prominent environmental gradient affecting species composition, results of a minimum variance cluster analysis of the

diatom data are considered (Euclidean distance; Statistica 6.1, StatSoft 2003). This suggests that the training set can be split into 137 samples from circumneutral to alkaline waters (pH ‡ 6.5) and 49 samples representing more or less acid waters. The latter are mainly from the north-eastern part of the study area which is characterized by carbonate-poor sandy soils (Fig. 1). Because species composition is used to allocate the samples, the number of samples from non-acid waters is slightly smaller than in the analogous analysis of sediment assemblages (141 samples; Denys 2006). No separate inference models are developed for the acid–water samples because of their limited number.

The response of taxa occurring at least 10 times in a data set to selected variables is assessed by fitting their distribution (sqrt-transformed abundance) to a hierarchical set of models (Huisman et al. 1993) using the HOF version 2.3 program (Poisson-approximated error structure; http://www.cc.oulu.fi/~jarioksa/; Oksanen and Minchin 2002). These include a skewed unimodal distribution (model V), a symmetric unimodal distribution (model IV), a monotonic relationship with a plateau (model III), a monotonic increase or decrease (model II) and, finally, a no-relationship null model (model I).

The C^2 1.3 program (Juggins 2003) for weighted averaging (WA), weighted averaging with tolerance down weighting (WAT), weighted averaging with partial least squares regression (WA-PLS) and partial least squares regression (PLS) is used to develop transfer functions. The best option is selected based on the highest r^2 and the lowest root mean squared error of prediction estimated by jack-knifing ($r^2_{jack.}$, RMSEP), as well as the mean and maximum bias in the jack-knifed residuals. Model performance is also documented by the apparent r^2 and root mean squared error (RMSE). With WA-PLS, a minimal improvement of RMSEP by 5% determines the number of components. Samples with an extreme v^2 distance in a constrained CCA and an estimated value differing more than 1 standard deviation from the observed value for the reconstructed variable are considered outliers and removed. No attempt is made to discount samples dominated by taxa characterizing an early colonization stage. Finally, model improvement is attempted by stepwise removal of taxa with extreme tolerances, and deletion of all taxa having no significant relationship (type I response) to the variable according to the HOF analysis. This last option also potentially reduces the influence of confounding environmental variation (Racca et al. 2004).

All taxa in the data sets are listed in the Appendix (available online as Electronic Supplementary Material) with their authorities, abundance characteristics, as well as back-transformed WA optima and tolerances for variables yielding useful calibration models.

Correlations between environmental variables are Spearman rank coefficients (Statistica 6.1). Apparent species optima obtained from different training sets are compared by Wilcoxon matched pairs tests (Statistica 6.1).

Results

Entire data set

Details on the sampled waters are given in Table 1. Their surface area ranges from 0.02 to 74 ha and maximum depth from about 0.5 to 18 m, with most waters remaining mixed continuously. While many have a partially wooded (including shrubs) shoreline, development of submerged or emergent vegetation is often poor. The pH ranges from 3.4 to 9.3, with a median at 7.7, and most waters are well buffered. EC varies by two orders of magnitude (24–3,520 lS cm^{-1}). TP, TIN and chl *a* concentrations are often considerable. Many water-chemistry variables show positively skewed distributions (compare median with average values in Table 1).

A total of 241 taxa occurs with an abundance of 1% in at least one of the 186 samples, with *Gomphonema parvulum*, *Achnanthidium minutissimum*, *Encyonema silesiaca*, *Amphora copulata*, *Navicula cryptotenella*, *Eolimna minima*, *Nitzschia paleacea*, *Navicula cryptocephala*, *N. veneta*, *Cocconeis placentula* var. *euglypta*, *Amphora pediculus*, *Sellaphora pupula* aggr. and *Rhoicosphenia abbreviata* as the 5% with highest *N*2 values (Appendix, available as Electronic

Supplementary Material). The species matrix is very sparse (81.6% zeros). Individual samples have 2–109 taxa, with an average of 44 taxa. Dominance varies from 10 to 100% (Table 1). In a DCA, the two principal axes explain 11.7% of the variance in species composition and represent species-gradient lengths of 5.6 and 2.9 standard deviations (SD), respectively (Table 2). Consequently, CCA is chosen for further analyses.

The forward selection procedure of CCA retains DIC, pH, oxygen saturation, alkalinity, maximum TP, pGOP, silica, COD, surface area, percentage heathland and the proportion of shoreline with woody vegetation as the most parsimonious set of variables explaining species variation. However, alkalinity is colinear with DIC and not retained for further analyses. With the 10 remaining variables, the first two CCA axes ($k_1 = 0.6$, $k_2 = 0.23$) account for 9.3 and 3.5% of the species variation, or 46.2 and 17.6% of the species-environment relation, respectively (Table 2, Fig. 2). Several moderate correlations are noted between the selected variables

($r \lozenge 0.60$) but final VIFs are low (<4), confirming that all variables contribute independent information explaining species variation (Table 3).

CCCAs constrained to individual water-chemistry variables indicate the strongest marginal effect for pH (7.7%), closely followed by DIC (6.7%); all other variables explain less than half this amount of variation (Table 2). The ratio of eigenvalues for the constrained and principal unconstrained axis, k_1/k_2, is relatively high for pH (0.93) and for DIC (0.68), suggesting that their inference will be possible. For the remaining variables, k_1/k_2 is low ($\lozenge 0.2$ or less). With the effects of all other significant variables partialled-out, every variable still explains a limited amount of species variation (1–3.8%). The eigenvalue ratios ($k_1\text{¢}/k_2\text{¢}$) decline to 0.50 for pH and to 0.31 for DIC, evidencing interactions with other gradients. In view of these ordination results, only pH and DIC are considered candidates for calibration. Gradient lengths in DCCA are 5.1 SD for pH and 3 SD for DIC, pointing to WA or WA-PLS as the most appropriate

Table 2 Results of constrained CCA analyses with forward-selected variables for the complete training set and for non-acid sites, only

Training set	Variable (transformation)	Constrained CCA—marginal effect					Partial constrained CCA— unique effect (forward selected variables as covariables)					DCCA gradient length (SD)
		k_1	k_1/k_2	% Species variance	F	P	$k_1\text{¢}$	$k_1\text{¢}/k_2\text{¢}$	% Species variance	F	P	
All sites	pH	0.500	0.93	7.7	15.3	£0.001	0.200	0.50	3.8	6.9	£0.001	5.10
	DIC (log$x$)	0.430	0.68	6.7	13.3	£0.001	0.130	0.31	2.4	4.2	£0.001	3.00
	O$_2$%	0.070	0.09	1.1	2.0	0.005	0.070	0.18	1.4	2.5	0.002	1.60
	TP$_{max.}$ (log$x$)	0.166	0.22	2.6	4.9	£0.001	0.060	0.15	1.2	2.1	£0.001	2.10
	pGOP (log(x+2))	0.112	0.15	1.7	3.3	£0.001	0.070	0.17	1.3	2.4	£0.001	1.50
	Silica (log$x$)	0.128	0.18	2.0	3.7	£0.001	0.050	0.13	1.0	1.7	0.003	1.20
	COD (log$x$)	0.123	0.17	1.9	3.6	£0.001	0.050	0.13	1.0	1.8	0.002	3.00
Non-acid sites	ALK (log$x$)	0.088	0.34	2.3	3.2	£0.001	0.072	0.37	2.1	2.8	£0.001	1.60
	Sodium (log$x$)	0.076	0.28	2.0	2.7	£0.001	0.046	0.24	1.4	1.8	£0.001	1.64
	TP$_{max.}$ (log$x$)	0.125	0.56	3.3	4.6	£0.001	0.075	0.38	2.2	2.9	£0.001	1.84
	TP (log$x$)*	0.115	0.51	3.0	4.2	£0.001	0.065	0.34	1.9	2.5	£0.001	1.71
	TIN (log$x$)	0.044	0.16	1.2	1.6	0.005	0.045	0.23	1.3	1.7	£0.001	0.84
	O$_2$%	0.079	0.34	2.1	2.9	£0.001	0.050	0.26	1.5	1.9	£0.001	1.62
	pGOP (log(x+2))	0.082	0.32	2.1	3.0	£0.001	0.084	0.43	2.5	3.3	£0.001	1.29

* Median TP used as an alternative to TP$_{max}$.

Fig. 2 Canonical correspondence analysis (CCA) biplot of samples and selected forward-selected environmental variables for the entire training set (non-acid sites represented by •, acid sites by ⋅)
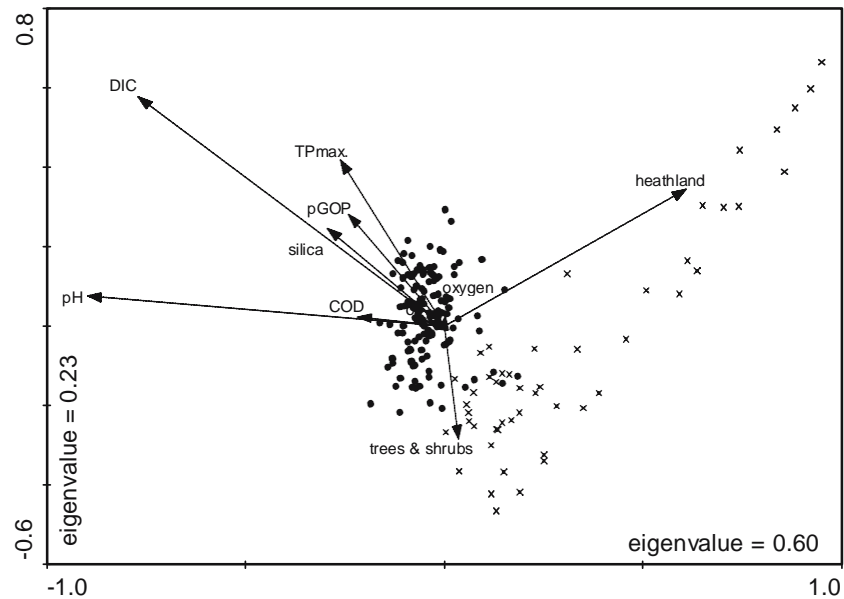
Table 3 Spearman rank correlations and variance inflation factors (VIF) for variables included in the minimal CCA model with the complete training set

	DIC	pH	O$_2$%	TP$_{max.}$	pGOP	Silica	COD	Surface area	Heathland	VIF
DIC	1	–	–	–	–	–	–	–	–	3.9
pH	*0.58*	1	–	–	–	–	–	–	–	3.4
O$_2$%	–0.11	*0.46*	1	–	–	–	–	–	–	1.7
TP$_{max.}$	*0.60*	*0.28*	*–0.28*	1	–	–	–	–	–	2.0
pGOP	*0.26*	*0.16*	0.08	*0.32*	1	–	–	–	–	1.4
Silica	*0.59*	0.11	*–0.32*	*0.49*	0.12	1	–	–	–	1.7
COD	*0.29*	0.09	*–0.18*	*0.61*	*0.39*	*0.14*	1	–	–	1.9
Surface area	*–0.17*	*0.24*	*0.38*	*–0.18*	–0.03	*–0.21*	*–0.19*	1	–	1.3
Heathland	*–0.42*	*–0.35*	*0.16*	*–0.28*	*–0.16*	*–0.31*	*–0.18*	0.10	1	1.3
Wooded shore	–0.12	*–0.17*	*–0.16*	–0.07	–0.08	0.07	*–0.15*	0.04	–0.13	1.1

Correlations with $P£ 0.05$ are in italic

regression options for developing inference models.

Of the 174 taxa occurring 10 times or more, less than 10% have relative abundance patterns that are unrelated (type I) to pH (e.g. *Craticula buderi, Eunotia soleirolii, Fragilaria capucina* var. *rumpens, F. famelica, Staurosira construens, S. venter, Mayamaea atomus* var. *permitis, Nitzschia acicularis* and *Rhopalodia gibba*) or DIC (e.g. *Ctenophora pulchella, Eunotia soleirolii, Gomphonema parvulum, Mayamaea atomus* var. *permitis, Stauroneis kriegerii* and *Staurosira subsalina* (Table 4 and Appendix, available online as Electronic Supplementary Material). Almost 60% presents a symmetric unimodal relationship (type IV) to these variables.

A fairly large proportion has a skewed unimodal relationship to pH, whereas a monotonic pattern is more frequent with DIC.

Table 4 Proportion of taxa according to HOF response-model type for the complete training set and the non-acid sites (transformed variables, pH excepted)

	All sites (174 taxa)		Non-acid sites (149 taxa)	
	pH	DIC	TP$_{max.}$	TP
I—flat	8.6	6.3	20.1	24.2
II—monotonic	12.6	26.4	19.5	22.8
III—monotonic, plateau	0.6	1.7	1.3	2.7
IV—symmetric unimodal	59.2	57.5	37.6	32.9
V—skewed unimodal	19.0	8.0	21.5	17.4

Both for pH and DIC, five samples are identified as outliers and removed to optimize the transfer functions. For pH, the best result is given by WA-PLS with two components (Table 5). The jack-knifed error is 0.38 pH units, or 6.4% of the observed range, and the $r^2_{jack.}$ is 0.88 when all taxa are included in the model. Excluding the limited number of taxa with no apparent relationship to pH hardly affects RMSEP and mean bias, but lowers the maximum bias from 0.73 to 0.49 units. This improvement does not appear to be substantial enough to promote this type of taxa selection. Figure 3a shows that residual values are similar throughout the model range. Three components are preferred for WA-PLS inference of DIC, yielding a model nearly as strong as for pH ($r^2_{jack.}$ = 0.86 and RMSEP ◆10% of the observed range). Here also, removal of taxa based on their general response characteristics appears unnecessary. As suggested by Fig. 3b, the model may lose sensitivity at concentrations above 25 mg l^{-1}, potentially due to the low number of data points in this region.

Non-acid sites

The pH range is less than three units for this data set, spanning only the circumneutral to alkaline part of the gradient (pH 6.5–9.3; Table 1), while lower values of EC, calcium and COD are truncated.

Only 196 taxa are included in the species data, which is 19% less than for the entire data set. The matrix remains sparse (77.2% zero values) and, except for *Sellaphora pupula* aggr., the same taxa have the highest number of effective occurrences. Samples never include less than 13 taxa, but average sample diversity is not different from the complete data set (Table 1).

Species-gradient lengths in DCA are 3.0 and 2.8 SD for the main axes and therefore, unimodal CCA remains appropriate. The two principal CCA axes with forward-selected variables account for only 4.0 and 2.6% of the species variation, but for 28.0 and 18.1% of the species-environment relation. Maximum TP, potential gross oxygen production, alkalinity, surface area, percentage of shoreline with woody vegetation, oxygen saturation, sodium and total inorganic

Table 5 Performance statistics for calibration models (most appropriate options in italics)

|	Variable	Transformed range	Method	Taxa excluded	Number of taxa	% Outliers	r^2	RMSE	$r^2_{jack.}$	RMSEP	Mean bias jack-knifed	Max. bias jack-knifed	RMSEP % trans. range	Mean bias % trans. range	Max. bias % trans. range
All sites	DIC (log(10x))	0.903–2.797	WAPLS-3	None	241	2.7	0.93	0.132	0.86	0.194	0.001	0.177	10.2	0.1	9.3
		0.903–2.797	WAPLS-3	Type I	230	2.7	0.94	0.130	0.86	0.192	0.000	0.147	10.1	0.0	7.8
	pH	3.4–9.3	WAPLS-2	None	241	2.7	0.93	0.290	0.88	0.376	-0.017	0.734	6.4	-0.3	12.4
		3.4–9.3	WAPLS-2	Type I	225	2.7	0.93	0.290	0.88	0.370	-0.016	0.490	6.3	-0.3	8.3
Non-acid sites	TP$_{max.}$ (logx)	1.544–3.810	WAPLS-2	None	224	1.5	0.81	0.234	0.62	0.330	0.028	0.694	14.6	1.2	30.6
		1.544–3.810	WAPLS-2	Type I	194	1.5	0.81	0.230	0.64	0.318	0.031	0.701	14.0	1.4	30.9
	TP (logx)	1.544–3.461	WAPLS-2	Type I	194	1.5	0.82	0.198	0.66	0.278	0.029	0.718	12.3	1.3	31.7
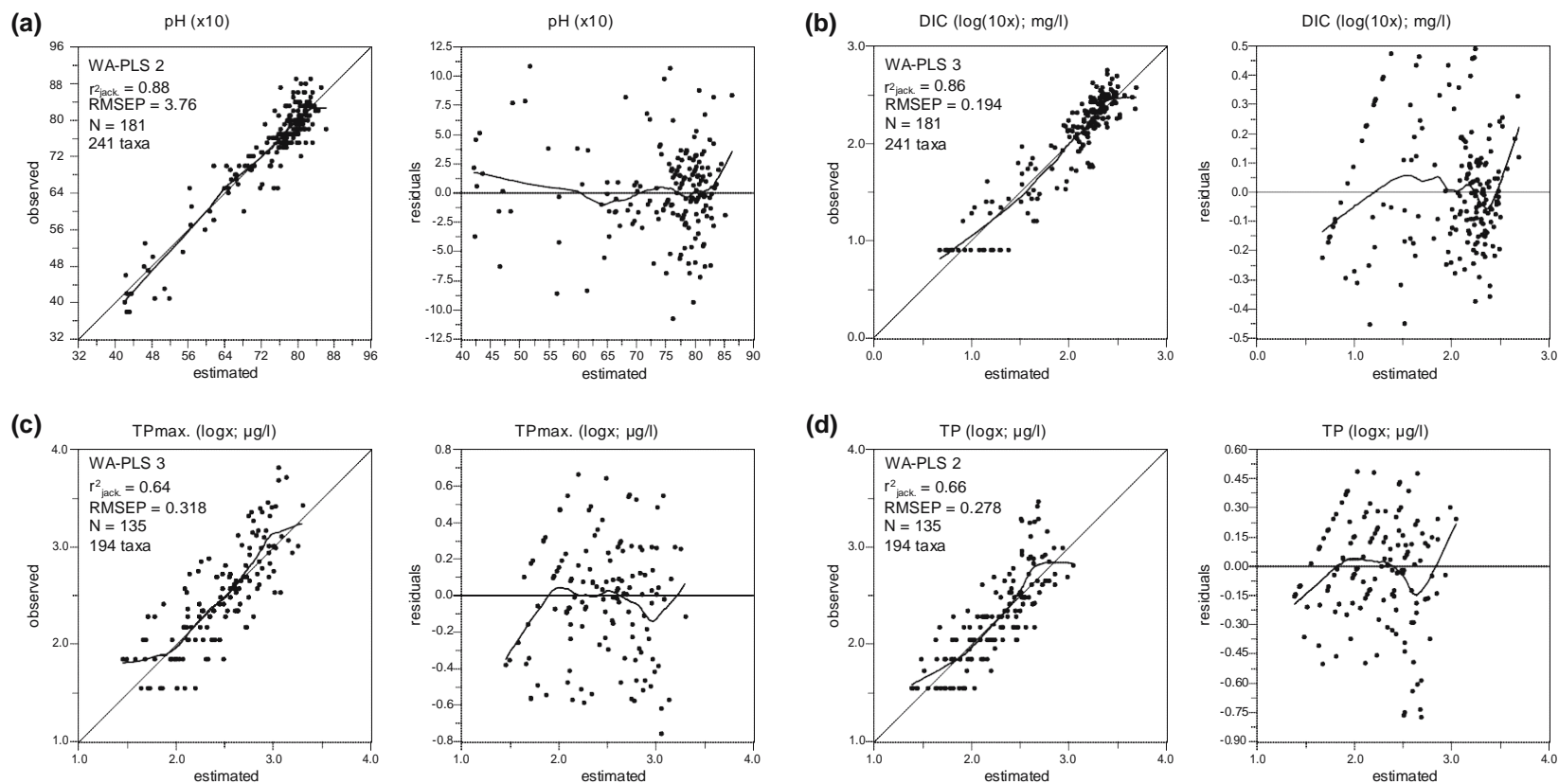
Fig. 3 Scatter plots of observed values and residuals vs. jack-knifed estimates for calibration models based on the entire data set for (a) pH (type I taxa included), (b) DIC (type I taxa included), and on the non-acid sites for (c) maximum TP (type I taxa excluded) and (d) median TP (type I taxa excluded). Thick trend lines are LOWESS smooth fits (stiffness 0.4)
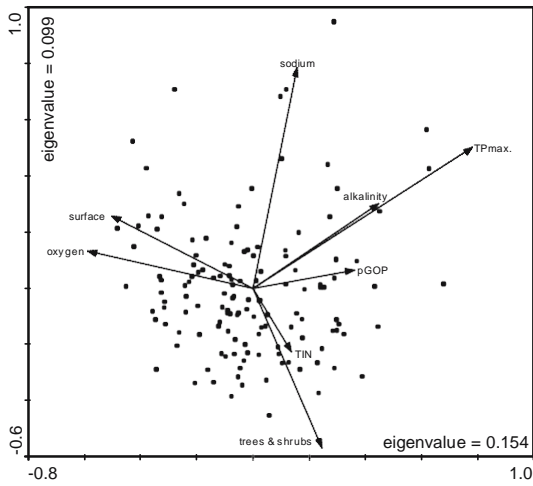
Fig. 4 Canonical correspondence analysis (CCA) biplot of samples and selected forward-selected environmental variables for the non-acid sites

nitrogen, in order of explained variation, are identified as the most important variables, all having very low VIFs (Fig. 4, Table 6). The strongest correlation ($r = 0.48$) is noted for $TP_{max.}$ and ALK (Table 6).

Although the marginal effect of all forward-selected water-chemistry variables is rather similar (ranging from 3.3% for $TP_{max.}$ to 1.2% for TIN) and all are highly significant in the CCCAs, k_1/k_2 only exceeds 0.5 for $TP_{max.}$ (Table 2), making this the best candidate for modelling. Although its unique effect is slightly smaller than that of pGOP, the associated DCCA species-gradient length for $TP_{max.}$ (1.84 SD) is larger (Table 2). The ratio of the principal eigenvalues in the partial analysis constrained to $TP_{max.}$ is

reduced by only ◆30% in comparison to the analysis including interactions with other variables, suggesting that the $TP_{max.}$ signal is relatively robust. Moreover, almost 60% of the 149 taxa with at least 10 occurrences has a unimodal relationship (HOF types IV and V) to $TP_{max.}$, whereas only ◆20% shows a monotonic decrease or increase. Only 1 in 5 taxa does not respond to this variable according to the HOF analysis (Table 4). These include several common taxa that may be quite abundant in the epiphytic community, e.g. *Achnanthidium eutrophilum*, *Amphora pediculus*, *Cocconeis placentula* var. *placentula*, *Gomphonema acuminatum*, *G. exilissimum*, *G. gracile*, *Fragilaria capucina* var. *vaucheriae*, *Nitzschia dissipata*, *N. fonticola*, *Rhoicosphenia abbreviata*, *Staurosira brevistriata* and *S. venter*.

The best inference model for $TP_{max.}$ is obtained by WA-PLS with two components (Table 5). Calibration statistics are fairly similar when all taxa are considered or when the 30 taxa with a type I HOF relationship to the response variable are excluded. The jack-knifed r^2 is 2% higher for the latter (0.64) and RMSEP slightly lower (0.318 $\log_{10}$ lg $TP l^{-1}$), supporting a slight preference for the model with less taxa. Plots of observed values and residuals against estimates point to a generally uniform response along the gradient (Fig. 3c). Median TP is colinear with the maximum concentration ($r = 0.94$, $P < 0.001$) and, consequently not retained in the CCA variable-selection procedure. However, it may be a more attractive measure for water managers as it is less prone to peak values, while constrained ordination analyses indicate that its

Table 6 Spearman rank correlations and variance inflation factors (VIF) for water-chemistry variables included in the minimal CCA model with the non-acid waters training set

	ALK	Sodium	$TP_{max.}$	TIN	O_2%	pGOP	Surface area	VIF
ALK	1	–	–	–	–	–	–	1.6
Sodium	*0.30*	1	–	–	–	–	–	1.5
$TP_{max.}$	*0.48*	*0.28*	1	–	–	–	–	1.7
TIN	0.14	0.15	0.10	1	–	–	–	1.0
O_2%	*−0.22*	−0.03	*−0.38*	−0.10	1	–	–	1.3
pGOP	0.14	0.12	0.20	0.06	0.12	1	–	1.1
Surface area	*−0.38*	0.13	*−0.28*	0.02	0.35	−0.03	1	1.3
Wooded shore	*−0.18*	*−0.24*	0.00	0.07	−0.13	−0.10	0.05	1.1

Correlations with $P \pounds 0.05$ are in italic

explanatory power is not much lower than that of $TP_{max.}$ (Table 2). With exactly the same data options, an alternative model using two WA-PLS components is obtained for median TP with an $r^2_{jack.}$ of 0.66 and an RMSEP which are slightly lower than those obtained for the $TP_{max.}$ model (Table 5). Here, some particularly negative residuals are noted in the range from 2,000 to 3,000 $lg\,l^{-1}$ (Fig. 3d). Inspection of the species composition and chemical characteristics of these sites reveals only their extremely high TP as a distinctive feature. Exclusion of the 11 water bodies with a maximum depth of at least 6 m does not improve precision of the TP models, nor does the use of untransformed species data.

Comparison and combination of estimates from sediment assemblages and epiphyton

Figure 5 compares the inferred values obtained by the most effective pH and DIC models obtained from littoral sediment assemblages (Denys 2006) and epiphytes for the complete data set. Inferred values are strongly correlated in both cases (pH: $r = 0.97$, DIC: $r = 0.95$, both $P < 0.001$). The largest pH discrepancies occur in acid conditions, and conversely to the epiphyte model, sediment assemblages hardly trace any pH variation at values > 7.5. Inferences for (log-transformed) DIC are also most variable at lower values, whereas the epiphyte model tends towards some underestimation relative to the sediment-based model in the lowermost part of this gradient.

The usefulness of inferring pH and DIC simultaneously from sediment assemblages and epiphyton is assessed by regressing observed values to the median, minimum and maximum estimate obtained by both models (Table 7; Fig. 6). This indicates that combined estimation can slightly improve the pH approximation, whereas precision also increases marginally over the sediment-based estimate for DIC (RMSEP—6.5%). However, in spite of its better overall accuracy, the median pH inherits the insensitivity of the sediment-derived model at high values (Fig. 6a). Therefore, the epiphyte model remains more appropriate at pH > 7.5.

Apparent species response in sediment and epiphyton assemblages

To compare the apparent response of taxa in sediment and epiphyton assemblages to measured environmental conditions, only the variables yielding transfer functions pertaining most directly to base or trophic status are considered, i.e. pH and DIC (entire training set; both sediment and epiphyton), $TP_{max.}$ (non-acid sites; epiphy-
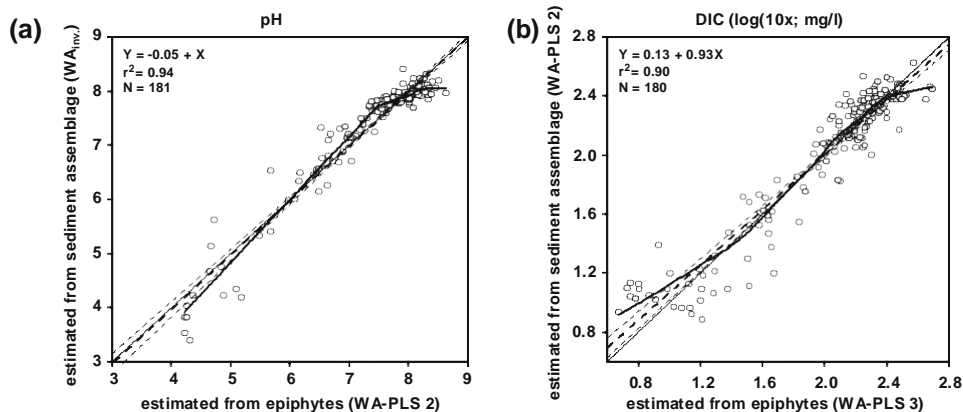

Fig. 5 Scatter plots of jack-knifed estimates for (a) pH based on epiphyton (WA-PLS 2; type I taxa included) and littoral sediment assemblages ($WA_{inv.}$; type I taxa included), (b) DIC based on epiphyton (WA-PLS 3; type I taxa included) and littoral sediment assemblages (WA-PLS 2.; type I taxa included). Thick solid trend lines are LOWESS smooth fits (stiffness 0.4); thick dashed lines are linear regressions with thin dashed lines indicating 95% confidence limits; thin solid lines represent 1:1 correspondence

Table 7 Error statistics for individual transfer functions and combined estimates

	pH ($N = 181$)		DIC ($N = 180$)	
	$r^2_{jack.}$	RMSEP	$r^2_{jack.}$	RMSEP
Sediment only*	0.85	0.43	0.88	0.183
Epiphyton only	0.88	0.38	0.86	0.194
Median of both	0.90	0.35	0.89	0.171
Minimum of both	0.90	0.36	0.88	0.186
Maximum of both	0.89	0.38	0.89	0.186

* Most appropriate models for littoral sediment assemblages according to Denys 2006; pH: $WA_{inv.}$ with type I taxa included, DIC: WA-PLS 2 with type I taxa included

ton) and pGOP (non-acid sites; sediment assemblages) (see also Denys 2006).

Of the 156 taxa assessed by means of HOF analysis that the epiphyton and sediment assemblages have in common, only 3 (1.9%; *Aulacoseira ambigua*, *Rhopalodia gibba* and *Staurosira venter*) are unrelated to pH and DIC. Another 1.9% of the taxa displays a significant relationship to pH in the epiphyton but not in the sediment assemblages, whereas 7.0% appears to be insensitive to this variable in the epiphyton but not in the sediment. The proportion of taxa unrelated to DIC in one of the assemblage types is more similar (4.5% in sediment but not in epiphyton, versus 3.2% in epiphyton but not in sediment). Sediment and epiphyton of non-acid sites have

only 9 out of 134 taxa (6.7%) in common with a type I response to $TP_{max.}$ and 15 (11.2%) for pGOP. The proportion of taxa showing no response to $TP_{max.}$ or to pGOP is markedly larger for sediments than for epiphyton (29.1 and 32.8% vs. 9.0 and 11.9%, respectively).

Apparent species optima estimated by WA from epiphyton and sediment assemblages are compared in Fig. 7. Overall, optima do not differ significantly for pH and DIC ($P > 0.05$, Wilcoxon matched pairs test). However, a pattern emerges in the range of deviation between the estimated values. For taxa with pH optima above 6.8, the difference is usually small, not exceeding about 0.5 units, whereas obvious disparities are more common in the more acid part of the gradient (up to 0.7 pH units, e.g. for *Pinnularia subcapitata* and *Tabellaria quadriseptata*; Fig. 7a). The reverse is observed for DIC, where optima are more similar at low concentrations and deviations become larger above 8 mg l^{-1} (Fig. 7b). Differences between optima for maximum TP and pGOP, derived from the non-acid sites only, are significant ($P = 0.006$ and $P < 0.001$, respectively). Although a tendency may be noted for higher $TP_{max.}$ and pGOP optima in the epiphyton, this is not a general rule and some marked discrepancies occur in the opposite direction as well, e.g. for *Asterionella formosa* and $TP_{max.}$ or *Nitzschia acicularis* and pGOP (Fig. 7c, d).

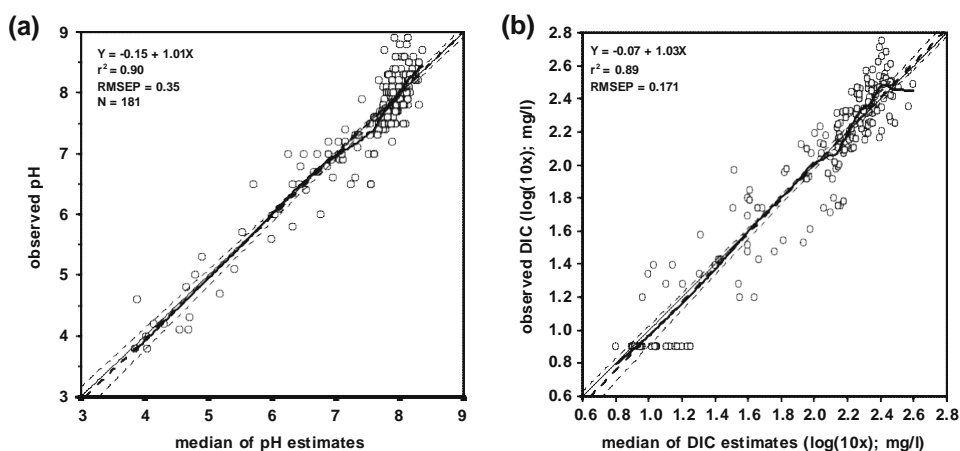Fig. 6 Linear regression of (a) median pH and (b) median DIC, estimated as the median value of inferences based on the littoral sediment and the epiphyton assemblage at each site (models as in Fig. 5) against observed values. Thick solid lines are LOWESS smooth fits, stiffness 0.4; thick dashed lines are linear regressions with thin dashed lines indicating 95% confidence bands; thin solid lines represent 1:1 correspondence

(a)

139 taxa

Staurosira elliptica

Amphora veneta, Nitzschia dissipata var. media, N. palea var. tenuirostris

Planothidium engelbrechtii

Gomphonema micropus
Discostella pseudostelligera
Nitzschia palea var. debilis, Pseudost. parasitica var. subcon.
Fragilaria tenera

Diatoma tenuis
Gyrosigma acuminatum
Nitzschia filiformis

Navicula seminulum

Nitzschia gracilis

Eunotia glacialis
Synedra rump.s.s. Hust.

Encyonopsis microcephala

Eunotia pectinalis var. undulata

Eunotia bilunaris

Fragilaria radians

Eunotia incisa

Encyonema neogracile

Nitzschia palaeformis

Achnanthidium jackii
Cymbella lanceolata
Gomphonema parv. f. sapr.

Frustulia saxonica
Frustulia crassinervia
Eunotia paludosa

Pinnularia subcapitata

G. subclavatum
G. utae

Tabellaria quadriseptata

Eunotia exigua

x-axis: pH optimum epiphyton
y-axis: pH optimum sediment assemblages

(b)

141 taxa

Craticula buderi

Cymbella neoleptoceros
Achnanthidium eutrophilum
Amphora veneta
Nitzschia vermicularis

Eolimna subminuscula
Nitzschia filiformis
Fragilaria famelica

Navicula menisculus, Nitzschia acicularis
Cocconeis pediculus, Fragilaria danica

Fragilaria fasciculata
Gomphonema dichotomum

Gomphonema subclavatum

A
Achnanthidium saprophilum
Navicula antonii

Fragilaria capucina var. rumpens
Craticula molestiformis

Nitzschia archibaldii
Nitzschia dissipata var. media

Fragilaria perminuta

Achnanthidium exiguum
Epithemia adnata, Gomphonema utae
Achnanthidium jackii
Gomphonema truncatum

Eolimna minima
Nitzschia lacuum

Fragilaria radians
Nitzschia palaeacea

Encyonopsis microcephala

Synedra rumpens sensu Hust.

A Navicula lundii
Rhopalodia gibba
Planothidium frequentissimum

x-axis: DIC optimum epiphyton (mg l^{-1})
y-axis: DIC optimum sediment assemblages (mg l^{-1})

(c)

105 taxa

Asterionella formosa
Gomphonema subclavatum
Epithemia adnata
Pseudostaurosira par. var. subc.

Craticula buderi

Navicula slesvicensis

Amphora veneta

Stauroneis kriegerii

Achnanthidium exiguum
Planothidium lanceolatum
Navicula seminulum
Eunotia glacialis, Fragilaria bidens, Planothidium frequentissimum
Nitzschia palea

Fragilaria famelica

Nitzschia frustulum
Nitzschia dissipata var. media

Nitzschia pumila

Nitzschia supralitorea
Mayamea atomus var. permitis, Staurosira subsalina
Planothidium engelbrechtii
Achnanthidium saprophilum
Fragilaria capucina var. distans
Navicula antonii

x-axis: optimum TPmax. epiphyton (μg l^{-1})
y-axis: optimum TPmax. sediment assemblages (μg l^{-1})

(d)

103 taxa

Nitzschia filiformis
Nitzschia acicularis

Cyclotella atomus

Ulnaria ulna var. angustissima

Cyclotella meneghiniana
Cyclostephanos dubius
Staurosira construens

Planothidium engelbrechtii

Platessa conspicua
Nitzschia supralitorea
Nitzschia lacuum
Amphora pediculus, Nitzschia dissipata
Navicula rhynchocephala
Gomphonema parvulum f. saprophilum
N. antonii
Gomphonema utae
Achnanthidium saprophilum

Achnanthidium jackii
Gomphonema dichotomum
Gomphonema truncatum
Rhopalodia gibba

Nitzschia amphibia
Nitzschia frustulum
Mayamaea atomus var. permitis, Nitzschia palaeacea

x-axis: optimum pGOP epiphyton (mg l^{-1})
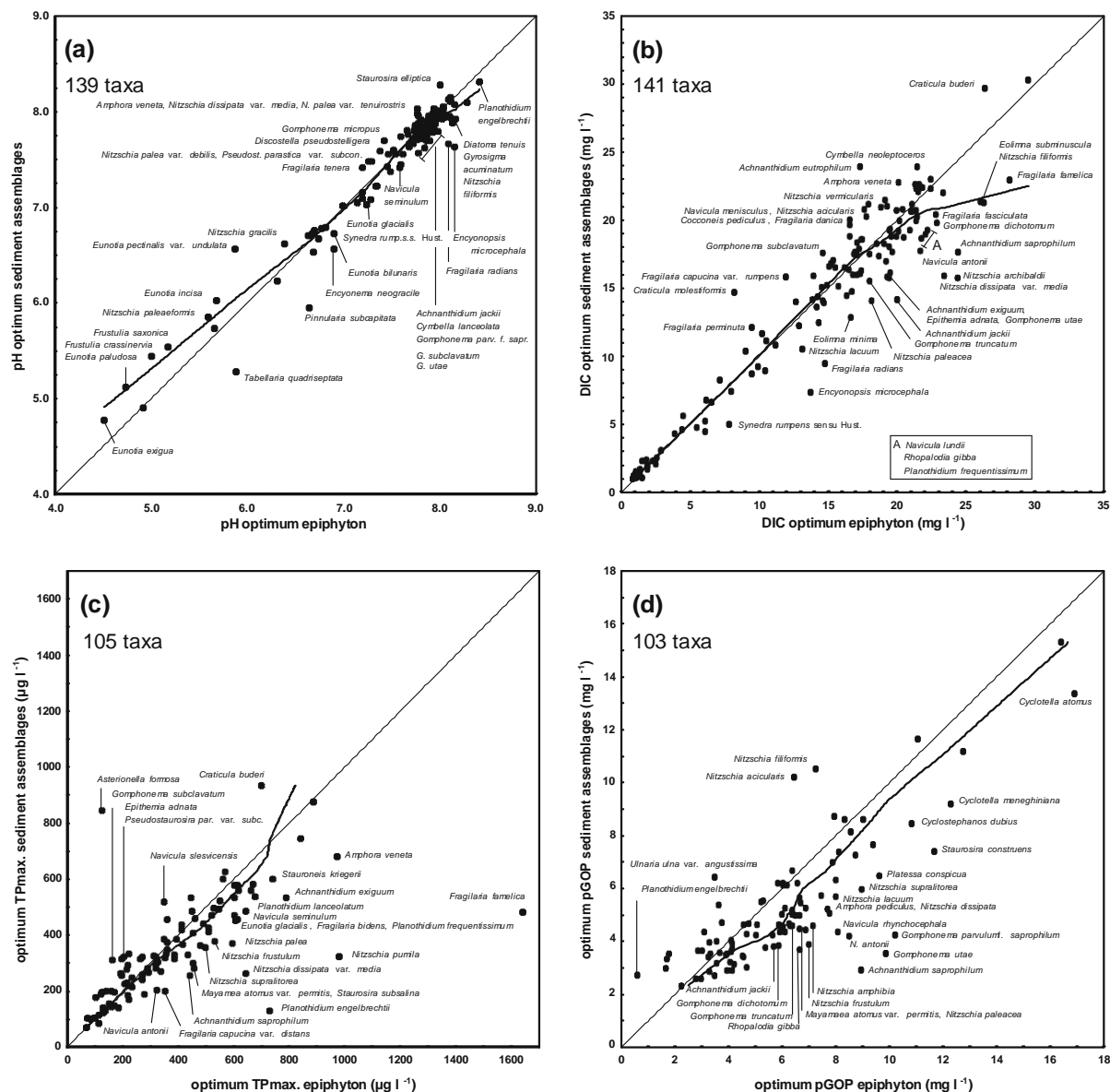y-axis: optimum pGOP sediment assemblages (mg l^{-1})

Fig. 7 Comparison of species optima estimated by weighted averaging in epiphyton and sediment assemblages for (a) median pH and (b) median DIC based on the entire training set, and for (c) maximum TP and median pGOP based on non-acid sites. Only taxa with at least 10 occurrences and a significant response to the variable in both assemblages are included; names are only shown for taxa where the difference between both optima is in the upper 25th percentile. Dashed line represents 1:1 correspondence, solid line is a LOWESS smooth fit (stiffness 0.4)

Discrepancies between estimated optima appear to be influenced by taxon occurrences. If only those are considered where the difference between optima is most severe (upper 25th percentile), the proportion of taxa having an $N2 \ddagger 10$ in both of the training sets is 11.4% in case of pH (*Eunotia bilunaris, E. exigua, Gomphonema subclavatum, Navicula seminulum*) and 22.2% for DIC (*Eolimna minima, Fragilaria capucina* var. *rumpens, F. fasciculata, Navicula antonii, N. lundii, N. menisculus, Nitzschia paleacea, Planothidium frequentissimum*). Higher percentages are noted for TP$_{max.}$ (30.8%; *Gomphonema subclavatum, Mayamaea atomus* var. *permitis,*

Navicula antonii, N. seminulum, Nitzschia frustulum, N. supralitorea, Planothidium frequentissimum, P. lanceolatum) and pGOP (28%; *Amphora pediculus, Cyclostephanos dubius, Cyclotella meneghiniana, Navicula antonii, Nitzschia amphibia, N. paleacea, N. supralitorea*). Most often, these common taxa have higher optima when they are found in the epiphyton, but there appears to be no clear relation with their principal source community or motility, as they include sessile epiphytes, motile epipelic species, as well as planktonic forms.

Discussion

Epiphytic diatom assemblages from standing waters in lower Belgium yield useful transfer functions for estimating median pH and DIC and maximum (alternatively median) TP, thereby enhancing opportunities for obtaining information on the former base and nutrient status of surface waters in the absence of sedimentary diatom material.

The number of useful transfer functions is lower for epiphyton than for sediment assemblages (2 vs. 3 with the entire training set, 1 against 6 for the non-acid sites). As shown by the ordination analyses, certain variables that are important to the distribution of sediment-associated diatoms, e.g. organic matter and certain cations (Denys 2006), are less influential for epiphytes. Possibly, this is because their effective gradients are less strong in the epiphytic microhabitat. As indicated by the results of Philibert and Prairie (2002), the lower number of possible models is most likely also related to the lower complexity of the species matrix, restraining the information content on subordinate environmental gradients. Compared to sediment assemblages, the total number of taxa in the epiphyton (–35.6 and –43.6% for all sites and non-acid sites, respectively), as well as the number of taxa per sample (all sites 44 ± 19 vs. 79 ± 27, non-acid sites 50 ± 15 vs. 87 ± 22; $P < 0.001$) are lower, whereas dominance values are higher (all sites 45.9 ± 21.8 vs. 31.2 ± 18.9, non-acid sites 41.9 ± 20.5 vs. 27.6 ± 15.1; $P < 0.001$). For variables to which even a large proportion of all taxa

occurring in the sediment assemblages appears to be unresponsive (e.g. silica, pGOP and COD; Denys 2006), the lower diversity of epiphyton may be a constraint. Additionally, the lower representation of planktonic taxa in epiphyton is likely to limit the reflection of phytoplankton productivity.

Although lower eigenvalue ratios are obtained in CCAs constrained to pH or DIC with the complete epiphyte training set than with littoral sediment samples, and marginal as well as unique effects are smaller, cross-validated statistics for inference models using epiphytes are marginally better for pH and nearly as good for DIC compared to those of models based on sediment assemblages (Denys 2006). Overall, the signal from both assemblage types is quite similar, which seems remarkable as metabolic processes can change pH and DIC concentrations within periphyton layers considerably from those in the surrounding water (Jones et al. 1996; Dodds 2003). However, slightly more outlier samples are encountered with epiphytes (2.7% against 1.1% with sediment assemblages) and there also appear to be minor differences in the sensitivity of the models along these predominant gradients. The plateau in estimated median pH values occurring with sediment diatoms at pH > 7.5 is almost absent with epiphytes, while DIC estimation is slightly less precise for epiphyton at very low concentrations (Denys 2006). Similar to the joint application of transfer functions using different algorithms (Racca et al. 2001), pH inferences in the region below pH 7.5 may be improved further by combining estimates from epiphyton and sedimentary diatom assemblages to a median value if the opportunity exists to sample both, as is often the case with herbarium specimens. However, epiphytes alone allow the best approximation at higher pH. For DIC, the gain of such an approach will be rather limited compared to the estimation from sedimentary diatoms only.

The epiphyte-based models for maximum or, alternatively, median TP of non-acid sites have a cross-validated r^2 of ◊0.65 and RMSEPs corresponding to about 14% of the gradient. This makes their performance as good as, or better than, many other published TP models using

mid-lake sediment diatoms (e.g. Köster et al. 2004; Reid 2005; overview by Werner and Smol 2005), river diatoms on *Cladophora* (O'Connel et al. 1997) or multiple algal groups in littoral lake periphyton (King et al. 2000; DeNicola et al. 2004). This also holds when only apparent statistics are considered (see Anderson 1997; King et al. 2000). Most of these models cover a much narrower TP range than that sampled in Flanders. Bennion et al. (2001) were able to obtain a substantially higher accuracy ($r^2_{jack.}$ = 0.82, relative RMSEP 8.8%) using mid-lake sediment assemblages from 152 northwest European lakes with annual mean TP between 5 and 1,190 $\lg l^{-1}$ (Bennion et al. 1996). Whilst containing predominantly shallow lakes as well, higher accuracy of this model may be related to its focus on somewhat lower TP concentrations (median TP value = 108 $\lg l^{-1}$) compared to the Belgian data set (median TP = 190 $\lg l^{-1}$), on average more frequent nutrient assays, and inclusion of data from studies where sites were strategically selected to cover regional TP gradients. With a jack-knifed RMSEP corresponding to 12.6% of the gradient, the model of Schönfelder et al. (2002) based on different assemblages from lakes, flushed lakes and rivers from northeastern Germany ranging in annual average TP from 9 to 1,687 $\lg l^{-1}$ also appears to allow a slightly better estimation. It is suspected that measurement of the TP concentration with a substantially lower detection limit than achieved in the present study would also result in a more effective transfer function in Flanders. Notably, the relationship of epiphytic assemblages to TP remains similar throughout the gradient and does not slacken conspicuously in any part, although there may be an exception at extremely high values if median concentrations are considered. Although macrophytes can supply nutrients to the epiphyton (Allen 1971; Carignan and Kalff 1982; Burkholder 1996) and the results of Kahlert and Petterson (2002) suggest that the importance of such sources does not decrease in more nutrient-rich waters, this does not appear to be a major impediment for epiphytic diatoms to track relative changes in TP concentration in Belgian standing waters. Even though rather few taxa have distinct optima at extremely high TP

concentrations, the transfer model does not appear to suffer from insufficient species turnover (Anderson and Odgaard 1994) in this region.

Epiphytic assemblages from the non-acid sites are more strongly related to TP concentration than littoral sediment assemblages. For the latter, TP is not among the most influential variables and calibration was not performed (Denys 2006). Variables covarying with TP, such as pGOP, COD, and also cations, are more influential and probably account for much of the species variation in sediment assemblages along the TP gradient. The stronger link of epiphytes to TP may be explained by a less representative assessment of nutrient conditions at the sediment–water interface by water-column sampling (Hansson 1989; Wetzel 1996), better signal quality by the coincidence of TP measurements with the period of epiphyton development (DeNicola et al. 2004) and/or broader nutrient tolerances of sediment-dwelling diatoms. For instance, a poor relationship to TP is often reported for a group of small benthic taxa formerly placed within *Fragilaria* and attributed to *Staurosira* in this paper (e.g. Bennion 1995; Bennion et al. 2001; Sayer 2001; Reid 2005). *Staurosira brevistriata*, *S. elliptica* and *S. venter* are the only species from this group that occur more frequently in the epiphyton and are unrelated to TP_{max}. While *S. elliptica* has a very similar representation in sediment and epiphyton samples of the non-acid sites ($N2$ = 5.9 and 5.6, respectively), *S. brevistriata* and *S. venter* are considerably less abundant in the epiphyton ($N2$ = 7.4 and 8.9, against 32.1 and 49.2 in sediments, respectively). Additionally, the shorter accumulation period of epiphyton allows a more rapid response to abrupt annual changes in nutrient levels, for instance, associated to weather conditions or the transition phase between a more stable clear or turbid water state (Scheffer et al. 1993).

Because the highest TP values in the sampled waters often occur during the summer, the forward selection of maximum and not median TP values in CCA may suggest a closer link between epiphyton composition and TP concentration immediately preceding diatom sampling. Alternatively, maximum TP may simply discriminate more effectively between water bodies,

comparable to spring TP in stratifying lakes. Yet, the slightly better overall performance of the inference model for median TP shows that its calibration is a valid option, in particular as nutrient-sampling frequency is suboptimal for this data set (Gibson et al. 1996; Bradshaw et al. 2002). Variability of TP concentration is especially high in more eutrophic shallow waters, compromising accurate assessment of $TP_{max.}$ from a few measurements (Bennion and Smith 2000). This results in a more noisy relation to diatom species composition and lower model accuracy.

The HOF analyses presented by Denys (2006) and in this study demonstrate that many diatom taxa can display a significant relationship to a variable or not depending on the habitat they are sampled from. This already points out that habitat provenance needs to be accounted for in environmental inferences. Proportionally more taxa respond to $TP_{max.}$ and pGOP in the epiphyton than in sediment assemblages. Differences are also observed in the apparent WA optima of taxa determined from epiphyton and sediment assemblages of the same water bodies. Distribution patterns of diatom taxa along a common gradient may differ with habitat type for a variety of reasons, including physical–chemical environment, biotic interactions, productivity, species recruitment, species traits and taphonomic processes, such as sediment mixing and differential valve preservation (see in the context of paleo-environmental reconstruction, e.g. Jones and Flower 1986; Flower 1993; Anderson 1994; Cameron 1995; Cameron et al. 1999; Siver 1999; Bradshaw et al. 2002; Philibert and Prairie 2002). The effect of habitat on the position and resolution of optima appears to be taxon-specific, but is less important for the predominant variables, pH and DIC, than for $TP_{max.}$ or pGOP, representing more secondary gradients. Species optima for pH are fairly independent of habitat in well-buffered conditions, but this is less so at lower pH. The reverse is observed for DIC. This suggests that differences between (ambient) microhabitat and water-column pH or DIC due to biological and chemical processes are involved in the positioning of apparent optima. However, only certain species are affected and in a dissimilar way, which could be due to physiological

characteristics in relation to pH and DIC or associated conditions. More prominent and consequent differences in species optima emerge for total phosphorus. Especially at elevated TP concentrations, these tend to be higher in the epiphyton relative to the sediment, which may be explained by higher ambient TP concentrations at the sediment–water interface relative to the water column and/or more intense interspecific interactions in the denser biofilms developing at higher nutrient levels (Van Der Grinten et al. 2004). Indirect effects of eutrophication may also play a role. In particular, this could be the case for increased turbidity, which would affect epiphytes growing at shallower water depth less severely than bottom-dwelling diatoms. The very similar observations for pGOP optima support such a connection to light conditions. Whatever the reason for these differences, they point out that one should remain cautious with estimating species characteristics or abundances from a combination of samples representing different littoral habitats within a water body, and this in particular with regard to variables related to trophic status. In spite of internal consistency, inference models derived from such data may be misleading if applied to assemblages that are not of a similar composite nature.

The relation between model performance and heterogeneity in epiphyton composition due to host-plant specificity (e.g. Mols 1976; Eminson and Moss 1980; Pip and Robinson 1984; Jones et al. 2000) is beyond the scope of the present paper and comparisons are limited to the general habitat level. As discussed by Potapova and Charles (2005), substrate-related differences in the structure of algal assemblages are usually reported from small-scale studies (often within single water bodies). In regional studies, such as the present one, this variation is masked by patterns ensuing from stronger environmental gradients that are structured at larger spatial scales, e.g. in water chemistry. At present, the more subtle differences between epiphytic diatom communities that may follow from host-plant specificity must be coped with by the extent of the training set and the error statistics for transfer functions reported here encompass this potential source of variation. It remains to be determined

whether models designed for single, or groups of morphologically similar, macrophyte species will be more accurate. However, these may be laborious to develop and their field of application limited, considering that herbarium materials include a wide range of different species, while most macrophytic plants also present restricted distributions along hydrochemical gradients. The calibration models developed in this paper are for summer samples only. Especially in spring, marked seasonal changes in diatom-epiphyton composition are possible (Hofmann 1994) that may compromise their application. For studies using herbarium specimens this is usually less of a problem, as most are collected in summer. It should not be forgotten, however, that inferences from epiphytes may also be subject to spatial variation within a basin and, if possible, analysis of multiple contemporaneous samples is advised to improve their reliability.

Conclusions

Although less diverse than sedimentary diatom assemblages, epiphytic diatom assemblages can provide robust environmental inferences using calibration methods. The present study develops epiphyton-based transfer functions for median pH, median DIC and maximum (alternatively medium) TP and demonstrates that these match the accuracy of transfer functions based on either mid-lake or littoral sediment assemblages. This substantially enlarges the scope for using herbarium materials as an information source on former freshwater conditions in the study area and comparable neighbouring regions. Also, it provides a reliable basis for interpreting compositional changes observed in epiphyton in the course of monitoring. As with sediment assemblages, a posteriori reduction of the predominating pH gradient proves successful to expand calibration possibilities. Exclusion of non-responsive taxa hardly influences model accuracy. The analysis of epiphyton and sediment assemblages from the same or simultaneously collected herbarium specimens can slightly improve the estimation of pH or DIC. More importantly, however, the combined use of epiphyton and sediment assemblages allows

for the joint assessment of phytoplankton productivity, TP, the availability of organic matter for heterotrophic pathways, as well as inorganic carbon and conservative ions for non-acid sites. These are important preconditions for the ecological status and biodiversity of lakes and ponds.

References

Allen HL (1971) Primary productivity, chemo-organotrophy and nutritional interactions of epiphytic algae and bacteria on macrophytes in a lake. Ecol Monogr 41:97–127

Anderson NJ (1994) Inferring diatom palaeoproduction and lake trophic status from fossil diatom assemblages. Mem Calif Acad Sci 17:539–547

Anderson NJ (1997) Reconstructing historical phosphorus concentrations in rural lakes using diatom models. In: Tunney H, Carton OT, Brookes PC, Johnston AE (eds) Phosphorus loss from soil to water. CAB International, Oxford, pp 95–118

Anderson NJ, Odgaard BV (1994) Recent palaeolimnology of three shallow Danish lakes. Hydrobiologia 275/276:411–422

Anderson NJ, Rippey B, Gibson CE (1993) A comparison of sedimentary and diatom-inferred phosorus profiles: implications for defining pre-disturbance nutrient conditions. Hydrobiologia 253:357–366

Arzet K, van Dam H (1986) Assessment of changes in pH by the study of diatoms in cores and old samples. In: Ricard M (ed) Proceedings eight international diatom symposium. Koeltz, Koenigstein, pp 748–749

Battarbee RW (1999) The importance of palaeolimnology to lake restoration. Hydrobiologia 395/396:149–159

Battarbee RW, Jones VJ, Flower RJ, Cameron NG, Bennion H, Carvalho L, Juggins S (2001) Diatoms. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments. Volume 3: terrestrial, algal, and siliceous indicators. Kluwer Academic Publishers, Dordrecht, pp 155–200

Bennion H (1994) A diatom-phosphorus transfer function for shallow, eutrophic ponds in southeast England. Hydrobiologia 275/276:391–410

Bennion H (1995) Surface-sediment diatom assemblages in shallow, artificial, enriched ponds, and implications for reconstructing trophic status. Diatom Res 10:1–19

Bennion HB, Smith MA (2000) Variability in water chemistry of shallow ponds in southeast England, with special reference to the seasonality of nutrients and implications for modelling trophic status. Hydrobiologia 436:145–158

Bennion H, Juggins S, Anderson NJ (1996) Predicting epilimnetic phosphorous concentrations using an improved diatom-based transfer function and its application to lake eutrophication management. Env Sci Technol 30:2004–2007

Bennion H, Appleby PG, Phillips G (2001) Reconstructing nutrient histories in the Norfolk Broads, UK: implications for the role of diatom-total phosphorus transfer functions in shallow lake management. J Paleolimnol 26:181–204

Birks HJB (1994) The importance of pollen and diatom taxonomic precision in quantitative palaeoenvironmental reconstructions. Rev Palaeobot Palynol 83:107–117

Bradshaw EG, Anderson NJ, Jensen JP, Jeppesen E (2002) Phosphorus dynamics in Danish lakes and the implications for diatom ecology and palaeoecology. Freshw Biol 47:1963–1975

Burkholder JM (1996) Interactions of benthic algae with their substrata. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic Press, New York, pp 253–297

Burkholder JM, Wetzel RG (1990) Epiphytic alkaline phosphatase on natural and artificial plants in an oligotrophic lake: re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. Limnol Oceanogr 35:736–747

Cameron NG (1995) The representation of diatom communities by fossil assemblages in a small acid lake. J Paleolimnol 14:185–223

Cameron NG, Birks HJB, Jones VJ, Berge F, Catalan J, Flower RJ, Garcia J, Kawecka B, Koinig KA, Marchetto A, Sánchez-Castillo P, Schmidt R, Šiško M, Solovieva N, Štefková E, Toro M (1999) Surface-sediment and epilithic diatom pH calibration sets for remote European mountain lakes (AL:PE Project) and their comparison with the surface waters acidification programme (SWAP) calibration set. J Paleolimnol 22:291–317

Carignan R, Kalff J (1982) Phosphorus release by submerged macrophytes: significance to epiphyton and phytoplankton. Limnol Oceanogr 27:419–427

CEC (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 J L 327:1–73

Cocquyt C, De Wever A (2002) Epiphytic diatom communities on herbarium material from Lake Naivasha and Lake Sonachi, Eastern Rift Valley, Kenya. Belg J Bot 135:38–49

De Blust G, Paelinckx D, Kuijken E (1994) Up-to-date information on nature quality for environmental management in Flanders. In: Klijn F (ed) Ecosystem classification for environmental management. Kluwer Academic Publishers, Dordrecht, Boston, London, pp 223–249

DeNicola DM, de Eyto E, Wemaere A, Irvine K (2004) Using epilithic algal communities to assess trophic status in Irish lakes. J Phycol 40:481–495

Denys L (2001) Stilstaande zoete wateren. In: Kuijken E, Boeye D, De Bruyn L, De Roo K, Dumortier M, Peymen J, Schneiders A, Van Straaten D, Weyembergh G (red.) Natuurraport 2001. Toestand van de Natuur in Vlaanderen: Cijfers voor het Beleid. Meded Inst Natuurbehoud 18:79–87

Denys L (2003) Environmental changes in man-made coastal dune pools since 1850 as indicated by sedimentary and epiphytic diatom assemblages (Belgium). Aquat Conserv 13:191–211

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

Denys L (2006) Calibration of littoral diatoms to water chemistry in standing fresh waters (Flanders, lower Belgium): inference models for historical sediment assemblages. J Paleolimnol 35:763–787

Dodds WK (2003) The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J Phycol 39:840–849

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

Flower RJ (1993) Diatom preservation: experiments and observations on dissolution and breakage in modern and fossil material. Hydrobiologia 269/270:473–484

Franklin A, Peeters M, Leentjes V (2003) A country profile. In: Peeters M, Franklin A, Van Goethem JL (eds) Biodiversity in Belgium. Royal Belgian Institute of Natural Sciences, Brussels, pp 21–48

Gibson CE, Foy RH, Bailey-Watts AE (1996) An analysis of the total phosphorus cycle in some temperate lakes: the response to enrichment. Freshw Biol 35:525–532

Hall R, Smol JP (1999) Diatoms as indicators of lake eutrophication. In: Stoermer EF, Smol JP (eds) The diatoms. Applications for the environmental and earth sciences. Cambridge University Press, Cambridge, pp 128–168

Hansson L-A (1989) The influence of a periphytic biolayer on phosphorus exchange between substrate and water. Arch Hydrobiol 115:21–26

Hill MO (1973) Diversity and evenness: a unifying notation and is consequences. Ecology 54:427–432

Hofmann G (1994) Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie. Bibl Diatomol 30:1–241

Huisman J, Olff H, Fresco LFM (1993) A hierarchical set of models for species response analysis. J Veg Sci 4:37–46

Jones VJ, Flower RJ (1986) Spatial and temporal variability in periphytic diatom communities: palaeoecological significance in an acidified lake. In: Smol JP, Battarbee RW, Davis RB, Meriläinen J (eds) Diatoms and lake acidity. Junk, Dordrecht, pp 73–85

Jones JI, Hardwick K, Eaton JW (1996) Diurnal carbon restrictions on the photosynthesis of dense stands of

Elodea nuttallii (Planch.) St. John Hydrobiologia 340:11–16

Jones JI, Moss B, Eaton JW, Johnstone OY (2000) Do submerged aquatic plants influence periphyton community composition for the benefit of invertebrate mutualists? Freshw Biol 43(4):591–604

Juggins S (2003) C^2. Software for ecological and palaeo-ecological data analysis and visualisation. User Guide Version 1.3. University of Newcastle, Newcastle upon Tyne

Kahlert M, Pettersson K (2002) The impact of substrate and lake trophy on the biomass and nutrient status of benthic algae. Hydrobiologia 489:161–169

King L, Barker P, Jones RI (2000) Epilithic algal communities and their relationship to environmental variables in lakes of the English Lake District. Freshw Biol 45:425–442

Köster D, Racca MJ, Pienitz R (2004) Diatom-based inference models and reconstructions revisited: methods and transformations. J Paleolimnol 32:233–245

Mols EJPJ (1976) Periphyton diatoms in a ditch near Tienhoven. Hydrobiol Bull 10:40–47

O'Connell JM, Reavie ED, Smol JP (1997) Assessment of water quality using epiphytic diatom assemblages on *Cladophora* from the St. Lawrence River (Canada). Diatom Res 12:55–70

Oksanen J, Minchin PR (2002) Continuum theory revisited: what shape are species responses along ecological gradients? Ecol Model 157:119–129

Philibert A, Prairie YT (2002) Is the introduction of benthic species necessary for open-water chemical reconstruction in diatom-based transfer functions? Can J Fish Aquat Sci 59:938–951

Pip E, Robinson GGC (1984) A comparison of algal periphyton composition on eleven species of submerged macrophytes. Hydrobiol Bull 18:109–118

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

Poulíčová 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

Racca JMJ, Philibert A, Racca R, Prairie YT (2001) A comparison between diatom-based pH inference models using artificial neural networks (ANN), weighted averaging (WA) and weighted averaging partial least squares (WA-PLS) regressions. J Paleolimnol 26:411–422

Racca JMJ, Gregory-Eaves I, Pienitz R, Yves T, Prairie YT (2004) Tailoring palaeolimnological diatom-based transfer functions. Can J Fish Aquat Sci 61:2440–2454

Reid M (2005) Diatom-based models for reconstructing past water quality and productivity in New Zealand lakes. J Paleolimnol 33:13–38

Sayer CD (2001) Problems with the application of diatom-total phosphorus transfer functions: examples from a shallow lake. Freshw Biol 46:743–775

Sayer CD, Roberts N, Sadler J, David C, Wade PM (1999) Biodiversity changes in a shallow lake ecosystem:

a multi-proxy palaeolimnological analysis. J Biogeogr 26:97–114

Schaumburg J, Schranz C, Hofmann G, Stelzer D, Schneider S, Schmedtje U (2004) Macrophytes and phytobenthos as indicators of ecological status in German lakes – a contribution to the implementation of the water framework directive. Limnologica 34:302–314

Scheffer M, Hosper SH, Meijer ML, Moss B, Jeppesen E (1993) Alternative equilibria in shallow lakes. Trends Ecol Evol 8:275–279

Schönfelder I (1997) Eine Phosphor-Diatomeen-Relation für alkalische Seen und Flüsse Brandenburgs und ihre Anwendung für die paläolimnologische Analyse von Auensedimenten der unteren Havel. Dissert Bot 283:1–148

Schönfelder I, Gelbrecht J, Schönfelder J, Steinberg CW (2002) Relationships between littoral diatoms and their chemical environment in northeastern German lakes and rivers. J Phycol 38:66–82

Siver PA (1999) Development of paleolimnological inference models for pH, total nitrogen and specific conductivity based on planktonic diatoms. J Paleolimnol 21:45–59

StatSoft (2003) STATISTICA (data analysis software system), Version 6. http://www.statsoft.com

ter Braak CJF, Šmilauer P (2002) CANOCO reference manual and CanoDraw for windows user's guide. Software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca

van Dam H, Mertens A (1993) Diatoms on herbarium macrophytes as indicators for water quality. Hydrobiologia 269/270:437–445

van Dam H, Mertens A, Sinkeldam J (1994) A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Neth J Aquat Ecol 28:117–133

Van der Grinten E, Janssen M, Simis SGH, Barranguet C, Admiraal W (2004) Phosphate regime structures species composition in cultured phototrophic biofilms. Freshw Biol 49:369–381

Verbruggen C, Denys L, Kiden P (1996) Belgium. In: Berglund BE, Birks HJB, Ralska-Jasiewiczowa M, Wright HE (eds) Palaeoecological events during the last 15000 years. Regional syntheses of palaeoecological studies of lakes and mires in Europe. John Wiley and Sons Ltd, Chichester, pp 553–574, 731

Vogel A, Beier T, Raeder U (2005) Does the process of drying macrophytes affect community structure and composition of epiphytic diatoms? Hydrobiologia 541:237–240

Werner P, Smol J (2005) Diatom-environmental relationships and nutrient transfer functions from contrasting shallow and deep limestone lakes in Ontario, Canada. Hydrobiologia 533:145–173

Wetzel RG (1996) Benthic algae and nutrient cycling in standing freshwater ecosystems. In: Stevenson RJ, Bothwell M, Lowe R (eds) Algal ecology: benthic algae in freshwater ecosystems. Academic Press, New York, pp 641–667