Journal of Paleolimnology (2006) 35: 763–787
DOI 10.1007/s10933-005-5459-5

# Calibration of littoral diatoms to water chemistry in standing fresh waters (Flanders, lower Belgium): inference models for historical sediment assemblages

Luc Denys[1,2]
*[1]Institute of Nature Conservation, Kliniekstraat 25, B-1070 Brussels, Belgium; [2]Biology Department, PLP, University of Antwerpen, Groenenborgerlaan 171, B-2020 Antwerpen, Belgium; e-mail: luc.denys@inbo.be*

Received 30 May 2004; accepted in revised form 8 November 2005

*Key words:* Bacillariophyceae, Herbarium macrophytes, Phytobenthos, Phytoplankton, Response models, Shallow waters, Water quality, Weighted averaging

## Abstract

Relationships between littoral surface-sediment diatom assemblages and ambient limnological conditions were examined in 186 lentic fresh waters throughout lower Belgium (Flanders). Most of these waters were small, unstratified, alkaline and rich in nutrients. Using weighted-averaging techniques, robust and accurate transfer functions were developed for median pH-values ranging from 3.4 to 9.3 and dissolved inorganic carbon concentrations from $<1.6$ to 63 mg $l^{-1}$ (jackknifed $r^2 \approx 0.87$, RMSEP $< 10\%$ of the observed range), while a less precise model was obtained for sodium (2–571 mg $l^{-1}$; jackknifed $r^2$ 0.69, RMSEP 9.9% of the range). Restricting the data set to circumneutral and alkaline sites ($pH \geq 6.5$) revealed the importance of additional variables, including calcium, silica, chemical oxygen demand and potential gross oxygen production (a proxy for metabolic activity and phytoplankton abundance). Calibration models for these variables were strong enough to be useful (jackknifed $r^2$ 0.57–0.59, RMSEP 13.1–16.4% of the observed range), although estimations should not always be considered entirely independent. Except for the predominant pH gradient, removal of all taxa with a distribution unrelated to the variable of interest improved model performance. In general, such taxa were proportionally represented among taxa classified according to their principal habitat. Application of the present models to diatom assemblages of shallow-water sediments obtained from historical samples and, most importantly, herbarium-macrophyte specimens, will improve hindsight into regional freshwater conditions and add to base-line setting of ecological quality standards in a highly impacted region.

## Introduction

Diatom assemblages from historical samples may be a valued alternative to sediment-core material for paleoenvironmental reconstruction when undisturbed sediment records are lacking. They can also provide supplementary information regarding limnological conditions and biodiversity in the littoral zone and bio-chronological correlation (Battarbee 1981; van Dam and Kooyman-van Blokland 1978; van Dam and Beljaars 1984; Arzet and van Dam 1986; van Dam and Mertens 1993; Denys 1997, 2001a, 2003; Sayer et al. 1999; Sayer 2001; Cocquyt and De Wever 2002). Contrary to core-derived samples, which may suffer from extensive sediment mixing and poor microfossil preservation, such historical samples potentially provide reliable and accurately dated diatom data for all kinds of water bodies, including small or periodic ponds. This may be particularly useful in many European regions, where small water bodies are far more numerous than large lakes and often of great importance to biodiversity and nature management. Although various types of old samples can be used, including those specifically collected for studying diatoms (e.g., Hürlimann et al. 2001), the most extensive archives probably consist of herbarium specimens of aquatic and riparian macrophytes. Obviously, these will yield epiphytic diatom communities, but in many cases, sediment assemblages can also be analysed from dried sediment adhering near the root base. Most archive material kept in public and private collections extends back to about two centuries and even older samples are sometimes available. This covers a significant part, if not all, of the history of many man-made ponds and lakes. Analysis of historical samples can offer an overview of former conditions in a large number of water bodies within a particular area. This provides opportunities to study former spatial patterns and geographic variability and, similar to the 'top versus bottom core sample' approach (Cumming et al. 1992; Hall and Smol 1996; Dixit et al. 1999; Betts-Piper et al. 2004), can be used to underpin a state-changed classification of water bodies in the sense of Moss et al. (1997), or to assess general background conditions preceding increased anthropogenic pressure. However, to allow better paleoenvironmental reconstructions from such samples, quantitative relationships between littoral sediment assemblages and environmental variables that may be relevant for freshwater monitoring and management need to be established.

Diatom-based regression and calibration modelling is an established method to estimate past environmental conditions (Reid 2005). Most often, surface-sediment samples collected close to the greatest lake depth are preferred for calibration purposes, as these are assumed to integrate whole-lake conditions best and to provide the proper analogues for diatom assemblages from cores taken in a corresponding position (Battarbee et al. 2001). Although relatively few transfer functions have been developed using littoral samples from sediments, rocks or macrophytes, a number of studies have demonstrated distinct potential in this area (e.g., ter Braak and van Dam 1989; Gasse et al. 1995; O'Connell et al. 1997; Schönfelder 1997; King et al. 2000; Schönfelder et al. 2002; DeNicola et al. 2004). Littoral assemblages are likely to differ significantly from those in deeper water, however, both in their species composition and response to ambient conditions (Moos et al. 2005). Typically, the proportion of non-planktonic taxa, but also the contribution of cells that actually lived at the site, will be comparatively larger, while recruitment from surrounding habitats (vegetations of helophytes and submerged macrophytes, shore areas, open water, etc.) will differ significantly from more profundal areas. Substrate relations can lead to a more pronounced decoupling of species distributions from water-column characteristics, for which reason planktonic taxa are sometimes favoured or certain non-planktonic diatoms excluded in developing transfer functions (Anderson et al. 1993; Bennion 1994; Siver 1999; Bennion et al. 2001). Apparent species optima derived from littoral assemblages may furthermore differ from those in deeper water due to greater variability of environmental conditions and stronger interaction of physical or biological factors. Clearly, all this necessitates the development of specific tools to allow environmental inferences from historical samples of littoral diatom assemblages. So far, only a fairly limited number of calibration efforts in NW-Europe have been made, and these have made use of mid-lake samples (Bennion et al. 1996, 2001; Bradshaw et al. 2002), combined epilithic, epiphytic and sediment assemblages from lakes and riverine sites to derive species characteristics (Schönfelder 1997; Schönfelder et al. 2002), or covered a narrower range of freshwater conditions (ter Braak and van Dam 1989), making them less appropriate for many collection materials.

This study explores calibration of diatom assemblages from littoral sediments in the region of Flanders, the low-lying (largely < 200 m a.s.l.) northern part of Belgium. In several respects, this region is fairly representative of the densely populated near-coastal regions of western Europe that extend from France to Denmark. If reliable transfer functions can be obtained here, these are likely of interest for a considerably larger area. In particular, their application to historical surface-sediment assemblages would be of considerable help in determining (more) pristine baseline conditions for standing waters, as required by the European Water Framework Directive (WFD;

European Parliament 2000). Furthermore, as the WFD established deviance in species composition and abundance from the pristine condition as key features for evaluating the phytobentos, results of inference models may also be considered to assess present-day benthic communities with respect to preset goals for particular physical–chemical characteristics or to interpret compositional changes (Philippi et al. 1998).

## Study area

Geographical characteristics of the study area (51°00′ N, 4°15′ E; 13.522 km$^2$; Figure 1) are summarized by Verbruggen et al. (1996) and Franklin et al. (2003). The region has a mild temperate maritime climate (average temperature 9.8 °C, precipitation 780 mm yr$^{-1}$). Soils are deep and varied with mineral textures ranging from heavy clay to sand; locally, peaty top soils occur. These developed in predominantly Quaternary surface deposits of marine, fluvial and niveo-eolian origin. Anthropogenic pressures on surface waters (eutrophication, fish stocking, acidification, physical perturbation) are usually quite marked (Denys 2001b) due to high population density (≈443 persons per km$^2$), intensive land use with much livestock farming and atmospheric deposition of acidifying substances, mostly ammonium (on average estimated at $4.3 \times 10^3$ acid equivalents ha$^{-1}$ yr$^{-1}$ for 2002, Van Avermaet et al. 2003). Few water bodies originated from natural processes and most have limited dimensions, except for those originating from extraction of sand or gravel. Bottom substrates mainly include peat, more or less organic muds and sand, often with substantial amounts of plant litter in the littoral areas. Natural stony substrates are lacking, but cobbles or boulders are sometimes used to stabilise shores. Development of aquatic macrophytes and helophytes, most commonly *Phragmites*, is highly varied.

## Material and methods

### Data collection

Following an extensive GIS-based map and field prospection, 186 standing waters were selected for sampling (Figure 1), with the intention to cover the range of permanent freshwater conditions. Canals, ditches and watercourses, as well as distinctly brackish waters were not considered in the survey. Most of the sampled waters were small, shallow, and well mixed. Surrounding land use included semi-natural heaths, shrubs and woodlands, as well as tree plantations (mostly conifers or poplar), lawns, pastures and agricultural fields.

Surface area was determined from recent aerial photographs. Ordinal scales were used for field estimates of maximum depth ( < 1.5 m, 1.5–3 m, 3–6 m, > 6 m) and underwater slope at the shore (≤1/20, 1/20– < 1/5, 1/5– < 1/3, > 1/3). Major soil-texture and land-use classes were assessed as proportions within a 50 m buffer around the water body using soil maps and the most recent ‘biological evaluation’ survey maps (De Blust et al. 1994).

A large number of environmental variables, related to water chemistry, trophic status, organic load and phytoplankton abundance, were measured repeatedly during the vegetation period (May–November) of a single year, either 1998 or

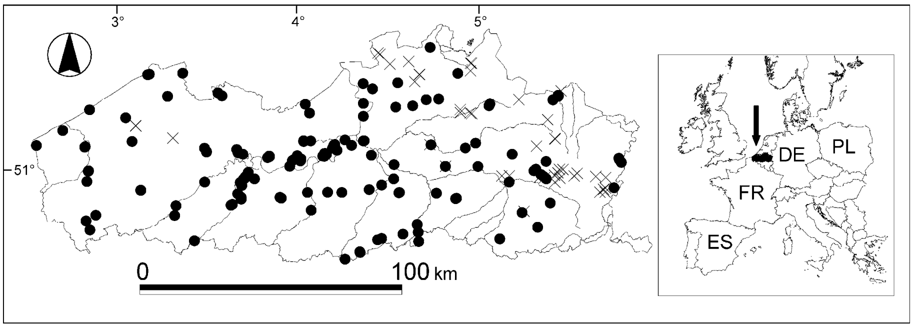

*Figure 1.* Map of Flanders with the main rivers and the location of the sampling sites (non-acid sites are represented by dots, acid sites by *x*).

1999. Water samples were taken with a horizontal 2.2 l Van Dorn sampler at a depth of 0.5 m, or halfway down the water column in case of a maximum depth of less than 1 m. Samples were taken preferably near the outflow, if one was present, or at a well-mixed site as far from the bank as could be reached in waders or from a jetty. Oxygen saturation ($O_2$%), pH and electric conductivity (EC) were measured 5–6 times in the field with portable meters (WTW Multiline P4) fitted with CellOx 325, TetraCon 325 and SenTix 97/T electrodes. Water samples for laboratory analysis were cooled during transport, stored at 4 °C and analysed within 24 h. Major ions, ammonium, nitrite, nitrate, Kjeldahl nitrogen, total phosphorus (TP), soluble reactive phosphorus and dissolved inorganic carbon (DIC) were usually analysed on three occasions. Analyses were carried out by a certified laboratory (ECOBE, University of Antwerpen) by means of segmented flow analysis ($SAN^{plus}$, SKALAR) or inductively coupled plasma mass spectrometry (ICP IRIS/CID, Thermo Jarrell Ash). Alkalinity was calculated from pH, DIC and EC. Total inorganic nitrogen (TIN) was determined as the sum of nitrate, nitrite and ammonium nitrogen, and total organic nitrogen (TON) as the difference between Kjeldahl and ammonium nitrogen. Variables reflecting biochemical and metabolic status or phytoplankton abundance were usually measured 4–5 times. Chemical oxygen demand (COD) was analysed following national standards (NBN 1974). The COD of dissolved substances and particulates was determined by analysing the filtrate through Whatman GF/C and subtraction from COD. Biochemical oxygen demand, potential gross oxygen production (pGOP) and potential net oxygen production were measured by dark and light bottle incubation for 24 h at 20 °C in controlled light conditions (Einheitsverfahren 1971). Chlorophyll *a* (chl *a*) and phaeopigments were extracted in 90% acetone and analysed following Golterman et al. (1978). Absorbance of organic matter and of yellow substances (gilvin) were measured at 254 nm and at 440 nm, respectively, before and after removal of particulates (A440, $A440_f$) by filtering through Whatman GF/C, with a Beckman DB-GT spectrophotometer using 1 cm quartz or 2 cm glass cuvettes. In view of the limited number of repeated measurements, site median values as well as maximum values for N- and P-fractions and chlorophyll *a* were used. Levels below detection thresholds were set at 50% of the threshold value for all calculations.

Surface sediments for diatom analysis were sampled between July and September of the same year environmental analyses were carried out, close to where water samples were taken, at the maximum water depth that could be reached in waders (no more than about 1.2 m) and where water was permanent. Only one sample was taken to maximize comparability with historical collections, which usually originate from a single location also. A plexiglass hand-held coring tube with a 3.4 cm diameter was used to retrieve the sediment/water interface. The upper 2–3 mm of sediment were removed by suctioning and fixated with dilute formaldehyde. Small aliquots of sediment were treated with concentrated hydrogen peroxide, potassium permanganate and dilute hydrochloric acid to decompose organic material and dissolve carbonates. After repeated addition of demineralised water followed by settling in test tubes for at least 48 h, the remaining slurry was used to prepare permanent slides with the highly refractive resin, Naphrax. Slides were examined at 1000–1250 × magnification with Leitz Orthoplan and Olympus BX 50 microscopes using differential interference optics. Diatoms were identified with the aid of standard floras and taxonomic literature (mainly Krammer 1997a, b, 2000; Krammer and Lange-Bertalot 1986–1991; Lange-Bertalot 1993; Lange-Bertalot and Moser 1994; Lange-Bertalot and Metzeltin 1996; Reichardt 1997, 1999). From each sample, 500 valves were counted to calculate percentage abundance of individual taxa. Additional taxa were inventoried and their abundance set to 0.02% (0.1 valves). All diatom analyses were carried out by the author to minimize the risk of taxonomic inconsistencies. Only taxa occurring with an abundance of 1% in at least one sample were considered in further analyses. Four marine species (*Cymatosira belgica* Grun., *Delphineis minutissima* (Hust.) Simonsen, *D. surirella* (Ehr.) Andrews and *Paralia sulcata* Cl.), that attained slightly more than 1% in a few samples from the coastal area, mostly due to reworking from older marine deposits, were also excluded. Species data sets are described in terms of 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 (Hill's N2; Hill 1973).

Species were classified as benthic, planktonic, epiphytic or plocon based on their predominant habitat. Taxa listed as benthic may live associated with sediments but also with other substrates, while epiphytes show a more distinct preference for plants. Plocon includes benthic araphid taxa forming unattached chains that mostly occur loosely associated with macrophytes or lie on the bottom sediments.

### *Data analysis*

Diatom species data were square-root transformed (sqrt) to stabilize variances and optimise the signal to noise ratio (Prentice 1980). A logarithmic (base 10) or square root transformation (silica) was applied to most environmental variables, except pH, $O_2$%, optical characteristics and variables measured on percentage or categorical scales, to obtain a more normal distribution and reduce the influence of extreme values.

In order to determine which variables could yield reliable calibration models, a series of multivariate analyses was carried out. Compositional gradient length of the species data was estimated by means of detrended correspondence analyses (DCA, detrending by segments, rare taxa down-weighted, non-linear rescaling) in order to determine whether ordination techniques assuming linear or unimodal species distributions were most appropriate. Canonical correspondence analysis (CCA, focussed on inter-species distances) with the forward selection option was used to identify a minimal set of environmental variables that significantly influenced diatom variation between sites. Variables with variance inflation factors (VIFs) exceeding 10 were removed to avoid colinearity (ter Braak 1987; ter Braak and Šmilauer 2002). The variance in species composition explained by each forward-selected variable (marginal effect) was estimated by a CCA constrained to the variable of interest (CCCA). In this case, the ratio of the eigenvalues for the first two axes ($\lambda_1/\lambda_2$) provided a measure of the relative importance of the constrained variable in explaining the species data (ter Braak 1987). Interaction with secondary gradients was examined by partial CCCAs with all other forward-selected variables as covariables. If the ratio of the principal eigenvalues ($\lambda_1'/\lambda_2'$) and the amount of variance explained in this analysis compares well relative to the CCCA, independent behaviour of the target variable and robust estimation can be assumed. The species gradient length associated to the selected variables was determined by detrended canonical correspondence analysis (DCCA) constrained to the variable of interest. The significance level in canonical analyses was set at $p \leq 0.05$ for 1000 unrestricted Monte Carlo permutations (full model), with application of a Bonferroni-type correction in variable selection ($p = 0.05/n+1$, where n is the number of forward-selected variables). All ordinations were performed with the CANOCO for Windows version 4.5 package (ter Braak and Šmilauer 2002).

To account for varying combinations of influential variables along the prominent environmental gradient affecting species composition, samples were classified by means of a minimum variance cluster analysis of the diatom data (Euclidean distance; Statistica 6.1, StatSoft 2003). This classification indicated that sites could be grouped according to their pH regime in a set of 141 samples from circumneutral to alkaline waters (pH $\geq$ 6.5) and 45 samples representing more or less acid waters. The latter originated mainly from the northeastern part of the study area where carbonate-poor sandy soils are common (Figure 1). The number of samples from acid sites was judged too small to attempt development of separate inference models and analyses were continued only with the entire data set and non-acid sites, respectively.

The response of taxa to the selected variables was assessed by fitting their distribution to a hierarchical set of models (Huisman et al. 1993) using the HOF version 2.3 program (http://cc.oulu.fi/~jarioksa/; Oksanen and Minchin 2002). This procedure uses maximum likelihood to select the simplest statistically significant solution from 5 possible models while progressively removing parameters: a skewed unimodal distribution (model V), a symmetric unimodal distribution (model IV), a monotonic relation with a plateau (model III), a monotonic increase or decrease (model II), and finally, a no-relationship null model (model I). Results are given for sqrt-transformed species data and a Poisson-approximation of the error structure. Response models were assessed only for taxa occurring at least 10 times in a data set.

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) were considered in model development (ter Braak and van Dam 1989; ter Braak and Juggins 1993; ter Braak et al. 1993), using the $C^2$ 1.3 program (Juggins 2003). Final model selection was based on the highest $r^2$ estimated by jackknifing ($r^2_{\text{jack.}}$), the lowest root mean squared error of prediction (RMSEP), and the residual structure (mean and maximum bias for observed minus estimated values; ter Braak and Juggins 1993). In agreement with Racca and Prairie (2004), maximum bias of the jackknifed residuals was estimated by dividing the gradient of predicted values into 10 equal segments. The performance of the models was also documented by the apparent explained variance ($r^2$) and root mean squared error (RMSE). The useful number of WA-PLS components was determined by requiring an improvement of RMSEP by at least 5% compared to the previous number of components (Birks 1998). Samples for which the estimate differed from the observed value by more than 1 standard deviation of the reconstructed variable and that showed an extreme $\chi^2$ distance in a constrained CCA were considered outliers and always removed to optimise the models (Birks et al. 1990; Jones and Juggins 1995; Lotter et al. 1997). Such outliers may result from flawed or inadequate measurement of environmental variables or non-equilibrium between species composition and ambient conditions. Stepwise removal of taxa presenting extreme tolerances allowed further fine-tuning of some models. Subsequently, all models were rerun without taxa that were not significantly related to the target variable according to the HOF analysis (type I response) in an attempt to eliminate deleterious noise resulting from their inclusion. Again following Racca and Prairie (2004), plots of predicted – observed values and residuals for selected models are drawn with predicted values on the $X$ axis. Cited correlations between variables or residuals are uncorrected Spearman rank coefficients (Statistica 6.1).

A list of all taxa included in the calibration models with their authorities, abundance characteristics, back-transformed WA optima and tolerances is given in Appendix 1 (available online as Electronic Supplementary Material).

## Results

### *Entire data set*

The sampled waters ranged from very small pools of a few hundred square meters to fairly large (74 ha) and deep (≈18 m) stratified sand and gravel pits (Table 1). Woody vegetation often fringed a substantial part of the shore and submerged vegetation cover varied from 0 to 100%, remaining low at most sites. The pH ranged from very acid to alkaline (pH 3.9–9.3) and ionic concentrations were low to fairly high (EC 24 to ≈3500 $\mu$S cm$^{-1}$). Conservative ions and nutrients also varied considerably (Table 1). With a median concentration of 130 $\mu$g l$^{-1}$ for TP and 21 $\mu$g l$^{-1}$ for chl *a*, most waters were considered (hyper) eutrophic. As evidenced by averages that are considerably higher than median values, distributions for sodium, iron, TIN, TP, silica, humic substances ($A440_f$), chl *a* and pGOP were positively skewed and data transformation was carried out as described in the previous section.

From a total of 1126, 374 taxa attained an abundance of 1% at least once in the 186 samples. An average of 39 ± 37 occurrences per taxon indicated high species-turnover (Table 1). The effective number of occurrences (Hill's N2) varied from 1 to more than 114 between taxa with a median of only 14.5, indicating that the relative abundance of the majority of taxa remained low in most samples. The species matrix was sparse, containing 78.9% empty cells. Assemblages were fairly rich in species (on average 79 ± 27 taxa per sample), but dominance (6.6–94.8%) and diversity (N2 1-57) varied considerably among samples. *Achnanthidium minutissimum*, *Amphora copulata*, *Encyonema silesiacum* aggr., *Eolimna minima*, *Gomphonema parvulum*, *G. truncatum* aggr., *Hippodonta capitata*, *Navicula cryptocephala*, *N. veneta*, *Nitzschia palea*, *Planothidium frequentissimum*, *Sellaphora pupula* aggr. and *Ulnaria ulna* were among the more important species with N2 $\geq$ 80 (Appendix 1; available as Electronic Supplementary Material). These are widespread diatoms, often associated with alkaline and

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

| | Unit | Entire datatset | | | | | 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 | 2.21 | 10.50 | 10.72 | 73.97 |
| Shoreline/surface | | 0.01 | 0.05 | 0.06 | 0.05 | 0.28 | 0.01 | 0.05 | 0.06 | 0.05 | 0.28 |
| Shoreline development | | 1.02 | 1.49 | 1.73 | 0.76 | 5.97 | 1.02 | 1.52 | 1.77 | 0.78 | 5.97 |
| Maximum depth | m | < 1 | 1.5–3 | – | – | ca. 18 | < 1 | 1.5–3 | – | – | ca. 18 |
| Shoreline with trees & shrubs | % | 0 | 60 | 55 | 36 | 100 | 0 | 60 | 54 | 35 | 100 |
| Submerged vegetation | % | 0 | 1 | 21 | 35 | 100 | 0 | 1 | 16 | 31 | 100 |
| pH | | 3.4 | 7.7 | 7.4 | 1.1 | 9.3 | 6.5 | 7.9 | 7.9 | 0.5 | 9.3 |
| EC | $\mu$S cm$^{-1}$ | 24 | 460 | 520 | 393 | 3520 | 192 | 550 | 632 | 380 | 3520 |
| Alkalinity | meq l$^{-1}$ | −0.33 | 1.25 | 1.29 | 1.05 | 5.20 | 0.10 | 1.56 | 1.68 | 0.91 | 5.20 |
| DIC | mg l$^{-1}$ | < 1.6 | 15.3 | 16.2 | 12.6 | 62.6 | 3.0 | 19.9 | 20.7 | 11.3 | 62.6 |
| Sodium | mg l$^{-1}$ | 1.9 | 21.0 | 32.6 | 52.5 | 571.0 | 7.0 | 24.0 | 38.8 | 57.6 | 571.0 |
| Calcium | mg l$^{-1}$ | 1.4 | 61.3 | 63.9 | 44.0 | 307.0 | 19.0 | 78.0 | 80.4 | 37.3 | 307.0 |
| Magnesium | mg l$^{-1}$ | 0.6 | 6.8 | 9.1 | 7.9 | 65.8 | 3.4 | 8.8 | 11.1 | 8.0 | 65.8 |
| Iron | mg l$^{-1}$ | 0.01 | 0.24 | 0.49 | 0.77 | 5.62 | 0.01 | 0.22 | 0.34 | 0.40 | 2.52 |
| Sulphate | mg l$^{-1}$ | < 4.0 | 38.0 | 52.9 | 61.2 | 390.0 | < 4.0 | 47.0 | 59.6 | 61.4 | 374.0 |
| Chloride | mg l$^{-1}$ | 3.0 | 37.0 | 51.6 | 79.8 | 921.0 | 9.0 | 42.0 | 62.7 | 88.7 | 921.0 |
| TIN | mg l$^{-1}$ | 0.07 | 0.24 | 0.78 | 1.38 | 7.95 | 0.07 | 0.27 | 0.88 | 1.52 | 7.95 |
| TP | mg l$^{-1}$ | < 0.07 | 0.13 | 0.29 | 0.45 | 2.89 | < 0.07 | 0.19 | 0.36 | 0.50 | 2.89 |
| Silica | mg l$^{-1}$ | 0.06 | 3.96 | 6.55 | 6.85 | 30.30 | 0.10 | 5.15 | 7.83 | 7.24 | 30.30 |
| A440 | m$^{-1}$ | 0.28 | 4.05 | 5.37 | 4.43 | 24.40 | 0.45 | 4.00 | 5.28 | 4.51 | 24.40 |
| A440$_f$ | m$^{-1}$ | < 0.1 | 1.18 | 1.81 | 2.31 | 19.85 | < 0.1 | 1.10 | 1.52 | 2.08 | 19.85 |
| Oxygen saturation | % | 12 | 92 | 91 | 29 | 206 | 15 | 92 | 92 | 31 | 206 |
| Chlorophyll *a* | $\mu$g l$^{-1}$ | < 3 | 21 | 42 | 56 | 310 | < 3 | 23 | 48 | 62 | 310 |
| pGOP | mg l$^{-1}$ | −1.0 | 3.3 | 6.6 | 8.4 | 41.8 | −1.0 | 4.1 | 7.9 | 9.3 | 41.8 |
| COD | mg l$^{-1}$ | < 1.5 | 37.4 | 40.7 | 27.5 | 309.0 | 9.3 | 38.4 | 42.5 | 29.5 | 309.0 |
| TON | mg l$^{-1}$ | 0.34 | 1.27 | 1.47 | 0.84 | 7.40 | 0.34 | 2.03 | 3.17 | 0.89 | 7.40 |
| Occurrences/taxon | | 1 | 26 | 39 | 37 | 167 | 1 | 22 | 35 | 34 | 133 |
| Hill's N2 (taxon) | | 1.0 | 14.5 | 21.6 | 21.7 | 114.1 | 1.0 | 12.8 | 20.2 | 20.6 | 104.2 |
| Dominance (taxon) | % | 1.0 | 3.8 | 9.7 | 14.4 | 94.4 | 0.0 | 2.8 | 7.7 | 13.0 | 79.4 |
| Taxa/sample | | 6 | 82 | 79 | 27 | 140 | 43 | 86 | 87 | 22 | 140 |
| Hill's N2 (sample) | | 1.0 | 5.5 | 9.1 | 9.7 | 57.1 | 1.0 | 5.3 | 8.7 | 9.5 | 64.7 |
| Dominance (sample) | % | 6.6 | 25.0 | 31.2 | 18.9 | 94.8 | 6.6 | 22.8 | 27.6 | 15.1 | 79.1 |

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

(highly) eutrophic water (van Dam et al. 1994), reflecting the strong representation of such conditions in the data set. Gradient lengths in DCA were 5.0 and 2.5 for the two major axes, representing 15.7% of the species variance, suggesting that ordination methods assuming a unimodal response were most appropriate.

CCA with forward selection indicated that 10 variables explained a significant amount of the variation in diatom data. In order of importance these were: pH, DIC, iron, pGOP, COD, heathland, sodium, surface area, silica and sand (Figure 2). The first two axes of a CCA with these variables captured 9.7 and 3.0% of the variance in species composition or 46.3 and 14.2% of the species-environment relation, respectively.

The inclusion of pH as well as DIC in the minimal model did not result in inflation factors above 5 (Table 2). Their correlation was not stronger than between sodium or silica and pH ($r \approx 0.6, p < 0.001$; Table 2). DIC was negatively correlated to the percentage of sandy soils ($r = -0.51$, $p < 0.001$) and heathland ($r = -0.41$, $p < 0.001$), while correlations were also significant for pH and iron ($r = -0.44$, $p < 0.001$) and for sand and silica ($r = -0.52$, $r < 0.001$).

In a series of CCCAs, individual water-chemistry variables explained slightly more than 8% (i.e., pH, DIC) to less than 2% (i.e., COD and iron) of the species variation (Table 3). The ratio of eigenvalues, $\lambda_1/\lambda_2$, ranged correspondingly from 1.37 to 0.17, exceeding 1 for pH and DIC, and

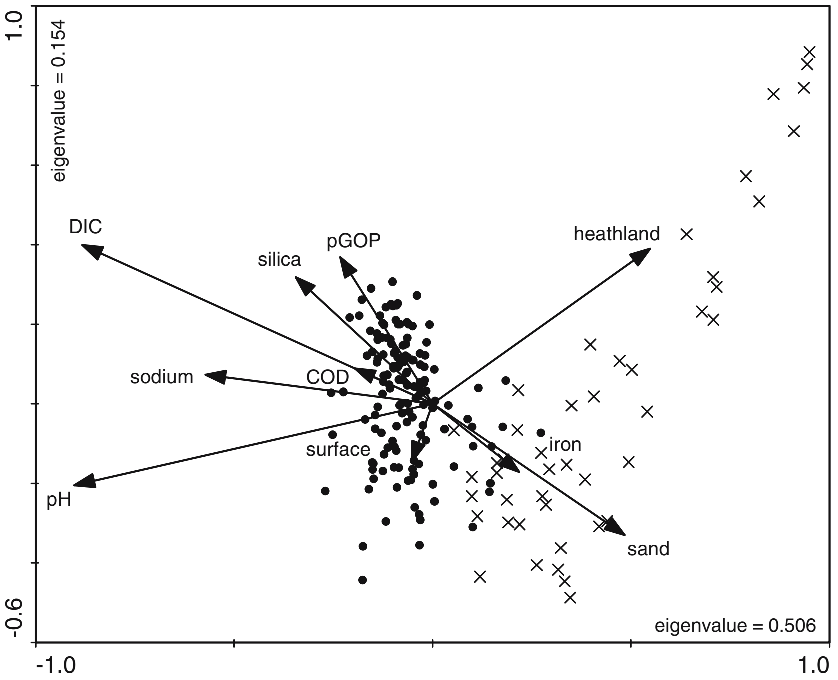

*Figure 2.* Canonical correspondence analysis (CCA) biplot of samples and forward-selected variables for the entire training set (non-acid sites are represented by dots, acid sites by x).

*Table 2.* Spearman rank correlations and VIFs for water chemistry variables included in the minimal CCA model with the complete training set (correlations with $p \leq 0.05$ are in italics).

| | DIC | pH | Sodium | Iron | pGOP | Silica | COD | Surface | Heathland | VIF |
|---|---|---|---|---|---|---|---|---|---|---|
| DIC | 1 | – | – | – | – | – | – | – | – | 4.7 |
| pH | *0.58* | 1 | – | – | – | – | – | – | – | 3.2 |
| Sodium | *0.60* | *0.54* | 1 | – | – | – | – | – | – | 1.9 |
| Iron | *−0.15* | *−0.44* | −0.12 | 1 | – | – | – | – | – | 1.9 |
| pGOP | *0.26* | *0.16* | *0.24* | *0.17* | 1 | – | – | – | – | 1.2 |
| Silica | *0.59* | 0.11 | *0.27* | *0.32* | 0.12 | 1 | – | – | – | 1.9 |
| COD | *0.29* | 0.09 | *0.36* | *0.34* | *0.39* | *0.14* | 1 | – | – | 1.6 |
| Surface | *−0.17* | *0.23* | 0.08 | *−0.16* | −0.03 | *−0.21* | *−0.19* | 1 | | 1.3 |
| Heathland | *−0.42* | *−0.35* | *−0.36* | −0.11 | *−0.16* | *−0.31* | *−0.19* | 0.10 | 1 | 1.3 |
| Sand | *−0.51* | *−0.25* | *−0.21* | −0.13 | *−0.17* | *−0.52* | −0.10 | 0.14 | *0.40* | 1.6 |

attaining 0.4 for sodium. This suggested that these variables would yield the best inference models because robustness can usually be assumed if this ratio exceeds 0.4–0.5 (Dixit et al. 1991; Hall and Smol 1992, 1996; Kingston et al. 1992; Schönfelder et al. 2002). In partial CCCAs, where the effects of the 9 remaining forward-selected variables were partialled out, the constrained axis remained highly significant for all variables, but the variance explained was only 3% for pH, and 2.1% for DIC. The proportion of the variance related to sodium and silica also suffered a considerable reduction (Table 3). The ratios of eigenvalues, $\lambda_1'/\lambda_2'$, were considerably lower for pH, DIC and sodium than in the preceding analysis providing evidence of important interactions. For the other variables, the eigenvalue ratios declined relatively less, or even increased (Table 3). In a DCCA, gradient lengths exceeded 4 S.D. in the case of pH, and were well over 2 for DIC, sodium and COD, suggesting that WA or WA-PLS would be most suited for model development.

*Table 3*. Results of constrained 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 (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\lambda_1$ | $\lambda_1/\lambda_2$ | % Species variance | $F$ | $p$ | $\lambda'_1$ | $\lambda'_1/\lambda'_2$ | % Species variance | $F$ | $p$ | |
| All sites | pH | 0.428 | 1.37 | 8.2 | 16.4 | $\leq 0.001$ | 0.126 | 0.54 | 3.0 | 5.3 | $\leq 0.001$ | 4.69 |
| | DIC (log $x$) | 0.423 | 1.09 | 8.1 | 16.3 | $\leq 0.001$ | 0.088 | 0.38 | 2.1 | 3.7 | $\leq 0.001$ | 3.04 |
| | Sodium (log $x$) | 0.211 | 0.44 | 4.1 | 7.8 | $\leq 0.001$ | 0.055 | 0.24 | 1.3 | 2.3 | $\leq 0.001$ | 2.95 |
| | Silica (log $x$) | 0.128 | 0.21 | 2.4 | 4.6 | $\leq 0.001$ | 0.042 | 0.18 | 1.0 | 1.8 | $\leq 0.002$ | 1.25 |
| | pGOP (log($x$ + 2)) | 0.111 | 0.19 | 2.1 | 4.0 | $\leq 0.001$ | 0.073 | 0.31 | 1.8 | 3.1 | $\leq 0.001$ | 1.51 |
| | COD (log $x$) | 0.100 | 0.17 | 1.9 | 3.6 | $\leq 0.001$ | 0.054 | 0.23 | 1.3 | 2.3 | $\leq 0.001$ | 2.68 |
| | Iron (log $x$) | 0.101 | 0.17 | 1.9 | 3.7 | $\leq 0.001$ | 0.049 | 0.21 | 1.2 | 2.1 | $\leq 0.001$ | 1.78 |
| Non-acid sites | DIC (log $x$) | 0.111 | 0.54 | 3.3 | 4.8 | $\leq 0.001$ | 0.050 | 0.33 | 1.8 | 2.4 | $\leq 0.001$ | 1.68 |
| | COD (log $x$) | 0.095 | 0.49 | 2.9 | 4.1 | $\leq 0.001$ | 0.053 | 0.35 | 1.9 | 2.5 | $\leq 0.001$ | 2.05 |
| | pGOP (log($x$ + 2)) | 0.096 | 0.45 | 2.9 | 4.1 | $\leq 0.001$ | 0.072 | 0.48 | 2.5 | 3.4 | $\leq 0.001$ | 1.39 |
| | Calcium (log $x$) | 0.092 | 0.45 | 2.8 | 3.9 | $\leq 0.001$ | 0.038 | 0.25 | 1.4 | 1.8 | $\leq 0.001$ | 1.67 |
| | Silica (sqrt) | 0.086 | 0.44 | 2.6 | 3.7 | $\leq 0.001$ | 0.050 | 0.21 | 1.8 | 2.4 | $\leq 0.001$ | 1.07 |
| | Sodium (log $x$) | 0.089 | 0.40 | 2.7 | 3.8 | $\leq 0.001$ | 0.051 | 0.34 | 1.8 | 2.4 | $\leq 0.001$ | 1.81 |
| | Magnesium (log $x$) | 0.064 | 0.29 | 1.9 | 2.7 | $\leq 0.001$ | 0.032 | 0.21 | 1.1 | 1.5 | $\leq 0.001$ | 1.29 |

Of the 286 taxa that occurred at least 10 times, the proportion that showed no significant relation (model I) was the lowest for DIC and pH (≈7%), but attained 27–37% for iron, silica, pGOP and COD (Table 4). A symmetric unimodal response (model IV) was most frequent for pH, DIC and sodium, while this type of relation was also relatively common for pGOP. A monotone increase or decrease of abundance (model II) was encountered most often for iron and silica (≈40%) and monotonic, mostly decreasing, relations were common for DIC (33%). Appendix 2 (available online as Electronic Supplementary Material) lists all taxa that were among the 5% with the highest average abundance in the data set and that displayed no significant relation to modelled variables. There were only five such taxa: the epiphytes *Cocconeis placentula* var. *euglypta* (sodium) and *Gomphonema parvulum* (DIC and sodium), and the plocon species *Staurosira construens* (DIC), *S. pinnata* (sodium) and *S. venter* (pH and sodium).

Inference models were developed only for the three variables expected to yield the most unconditional results according to the ordination analyses: pH, DIC and sodium. For pH and DIC, 2 samples were identified as outliers. For sodium, 7 samples (3.8%) deviated considerably and were discarded (Table 5). This possibly resulted from less precise estimates of ecologically meaningful concentrations of this ion due to temporal variation; e.g., peak concentrations may have been missed. Using all taxa, the strongest model was obtained for DIC by WA-PLS including 2 components. Its $r^2_{\text{jack.}}$ amounted to 0.88, which was only marginally lower than the apparent $r^2$ of 0.92, and the RMSEP represented 9.7% of the

*Table 4*. Proportion of taxa according to HOF response-model type for the complete training set (log-transformed variables, pH excepted).

| | pH | DIC | Sodium | Silica | pGOP | COD | Iron |
|---|---|---|---|---|---|---|---|
| I – Flat | 7.0 | 6.3 | 15.4 | 26.9 | 33.9 | 37.4 | 29.0 |
| II – Monotonic | 14.0 | 32.9 | 19.9 | 42.0 | 18.2 | 24.5 | 38.5 |
| III – Monotonic, plateau | 0.3 | – | – | 0.3 | – | 0.3 | 0.7 |
| IV – Symmetric unimodal | 66.8 | 52.4 | 47.9 | 24.8 | 32.9 | 27.6 | 25.9 |
| V – Skewed unimodal | 11.9 | 8.4 | 16.8 | 5.9 | 15.0 | 10.1 | 5.9 |

*Table 5.* Performance statistics for calibration models with the complete training set and non-acid sites only (most appropriate options in italics; a. all type I taxa, b. *Craticula riparia*, c. *Aneumastus tuscula*, *Fragilaria tenuistriata*, *Mastogloia lacustris*, *Navicula oppugnata*, d. *Aneumastus tuscula*, *Eunotia rhomboidea*, *Fragilaria tenuistriata*, *Navicula oppugnata*).

| Training set | Variable | Transformed range | Model | Taxa excluded | Number of taxa | % Outliers | $r^2$ | RMSE | $r^2_{\text{jack.}}$ | RMSEP | RMSEP % | Mean bias jackknifed | Mean bias % | Max. bias jackknifed | Max. bias % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| All sites | DIC (log(10$x$)) | 0.903–2.797 | *WA-PLS 2* | – | *376* | *1.1* | *0.92* | *0.151* | *0.88* | *0.183* | *9.7* | *0.003* | *0.2* | *0.062* | *3.3* |
| | | | WA-PLS 2 | a | 358 | 1.1 | 0.92 | 0.147 | 0.89 | 0.178 | 9.4 | −0.006 | −0.3 | 0.062 | 3.3 |
| | pH (10$x$) | 34–93 | $WA_{inv}$ | *b* | *375* | *1.1* | *0.87* | *4.058* | *0.87* | *4.32* | *7.3* | *−0.22* | *−0.4* | *6.74* | *11.4* |
| | | | $\mathrm{WA}_{\mathrm{inv}}$ | a + b | 355 | 1.1 | 0.87 | 4.06 | 0.85 | 4.33 | 7.3 | −0.11 | −0.2 | 7.33 | 12.4 |
| | sodium (log(10$x$)) | 1.279–3.219 | WA-PLS 2 | c | 371 | 3.8 | 0.83 | 0.143 | 0.66 | 0.201 | 10.4 | −0.008 | −0.4 | 0.266 | 13.7 |
| | | | *WA-PLS 2* | *a + c* | *327* | *3.8* | *0.84* | *0.138* | *0.69* | *0.193* | *9.9* | *−0.006* | *−0.3* | *−0.141* | *7.3* |
| Non-acid sites | DIC (log(10$x$)) | 1.519–2.797 | WA-PLS 2 | – | 348 | 5.0 | 0.87 | 0.083 | 0.68 | 0.128 | 10.0 | 0.001 | 0.1 | −0.126 | 9.9 |
| | | | $WA_{inv}$ | *a* | *261* | *5.0* | *0.77* | *0.107* | *0.71* | *0.123* | *9.6* | *<0.001* | *0.0* | *−0.114* | *8.9* |
| | Sodium (log(10$x$)) | 1.845–3.219 | WA-PLS 2 | d | 345 | 5.0 | 0.84 | 0.106 | 0.62 | 0.163 | 11.9 | −0.003 | −0.2 | 0.319 | 23.2 |
| | | | *WA-PLS 2* | *a + d* | *240* | *5.0* | *0.84* | *0.106* | *0.69* | *0.148* | *10.8* | *−0.002* | *−0.1* | *−0.170* | *12.4* |
| | COD (log(10$x$)) | 1.969–3.033 | WA-PLS 2 | – | 348 | 3.5 | 0.79 | 0.097 | 0.54 | 0.145 | 13.6 | −0.001 | −0.1 | 0.229 | 21.5 |
| | | | *WA-PLS 2* | *a* | *243* | *3.5* | *0.80* | *0.096* | *0.58* | *0.139* | *13.1* | *−0.002* | *−0.2* | *0.267* | *25.1* |
| | pGOP (log10($x$ + 2)) | 1–2.638 | WA-PLS 2 | – | 349 | 4.3 | 0.79 | 0.161 | 0.56 | 0.232 | 14.2 | −0.004 | −0.4 | 0.152 | 9.3 |
| | | | $WA_{inv}$ | *a* | *236* | *4.3* | *0.72* | *0.185* | *0.58* | *0.225* | *13.7* | *−0.007* | *−0.4* | *−0.101* | *6.2* |
| | calcium (log(10$x$)) | 2.279–3.228 | WA-PLS 2 | – | 349 | 2.8 | 0.83 | 0.081 | 0.57 | 0.129 | 13.6 | 0.005 | 0.5 | 0.187 | 19.7 |
| | | | $WA_{inv}$ | *a* | *235* | *2.8* | *0.70* | *0.107* | *0.59* | *0.126* | *13.3* | *< 0.001* | *0.1* | *−0.106* | *11.2* |
| | silica (sqrt(100$x$)) | 3.162–55.045 | WA-PLS 2 | – | 349 | 3.5 | 0.78 | 6.071 | 0.51 | 8.987 | 17.3 | −0.184 | −0.4 | 5.064 | 9.8 |
| | | | *WA-PLS 2* | *a* | *250* | *3.5* | *0.78* | *6.06* | *0.57* | *8.488* | *16.4* | *−0.573* | *−1.1* | *5.241* | *10.1* |

Relative RMSEP and bias estimates are calculated with respect to the range of transformed observed values.

transformed range. Maximum bias was 3.3% of this range, while average bias was low (0.2%). Removing only taxa with extreme tolerances did not result in any improvement. After exclusion of all taxa with a type I response in the HOF analysis (100+), the $r^2_{\text{jack.}}$ attained 0.89 and RMSEP decreased by only 0.3% of the range, which was not considered a substantial improvement. Plots of inferred vs. observed values and residuals showed no conspicuous trends, except for a somewhat neater fit at higher concentrations (Figure 3a). The strength of the pH model was comparable to that for DIC ($r^2_{\text{jack.}} = 0.87$), but here, simple WA yielded the best solution, while removal of one species (*Craticula riparia*) gave a slight improvement. The pH model was hardly affected when non-responsive taxa were not included and here also, preference was given to the model with the largest number of taxa. Even though the RMSEP was lower compared to that for DIC (7.3% of the range, 0.43 pH units), maximum bias was higher (11.4%). The relationship between estimated and observed pH values departed from linearity in the uppermost part of the range, such that the model consistently produced values close to 8 (Figure 3b). Although estimations for sodium should not be considered very robust, as discussed above, a moderately strong model was obtained by WA-PLS with two components. After deleting 7 outlying samples and 4 species with extreme tolerances (*Aneumastus tuscula*, *Fragilaria tenuistriata*, *Mastogloia lacustris* and *Navicula oppugnata*), the remaining 371 taxa returned an $r^2_{\text{jack.}}$ of 0.66 and an RMSEP equivalent to 10.4% of the transformed range. Amounting to 13.7% of the observed range, the maximum bias was larger than for DIC and pH. The $r^2_{\text{jack.}}$ and RMSEP were modestly improved (respectively with 0.03 and 0.5% of the range) by deleting all taxa with a type I response, while maximum bias decreased to 7.3% of the range. This option was preferred because it also resulted in better linearity at higher concentrations. At concentrations above 15 mg $\text{l}^{-1}$ a slight systematic underestimation appeared, but the number of observations in this area was rather limited (Figure 3c).

The residuals for DIC and pH ($r = 0.19$, $p < 0.01$) and for DIC and sodium ($r = 0.23$, $p < 0.01$) showed weak but significant correlations, and this increased slightly for the latter after discarding the type I taxa ($r = 0.30$, $p < 0.01$).

### *Non-acid sites*

The pH range was reduced to less than three units (pH 6.5–9.3) for this data set, covering only the circumneutral to alkaline part of the gradient. Ranges were only slightly restricted for EC, alkalinity, DIC, sodium, calcium, magnesium, sulphate, chloride, silica, A440, $O_2$%, COD and TON and not at all for DIC, TIN, TP, $A440_f$, chl *a* and pGOP (Table 1). Sodium, iron, chloride, TIN, TP, $A440_f$, chl *a*, pGOP and TON had positively skewed distributions. Environmental data transformation as indicated above remained necessary.

The species data included 348 taxa, which was hardly less than for all 186 samples together. The matrix was almost equally sparse (75.2% zero values) and diversity characteristics were also quite similar to the entire data set, although the minimum number of taxa per sample was higher (at least 43) and maximum dominance not as extreme (Table 1). The list of taxa that ranked highest according to their N2 was similar to that of the entire data set. Species gradient lengths along the first two DCA axes were 2.5 and 2.2 SD, respectively, suggesting that unimodal CCA was still appropriate.

The two principal axes of the CCA with forward selection accounted for 4.3 and 3.2% of the variance in species data and 24.9 and 18.7% of the species-environment relation, respectively (Figure 4). This was about half of the variance explained with the entire data set. The minimal set of variables determined by forward selection included DIC, pGOP, surface area, sodium, COD, silica, underwater slope, calcium and magnesium in order of the extra variance explained; the effect of pH was no longer significant. VIFs remained below 3 for all variables (Table 6).

Of the selected variables, DIC and calcium were most strongly correlated ($r = 0.78$, $p < 0.001$), but both were also positively related to magnesium and silica and negatively to surface ($p < 0.001$; Table 6). pGOP was only related to COD ($r = 0.41$, $p < 0.001$).

The relative importance of the forward-selected water-chemistry variables was of rather equal magnitude, with only magnesium having a considerably lower eigenvalue in the CCCA (Table 3). All variables remained significant ($p \leq 0.001$). The ratio of eigenvalues usually ranged from 0.54 (DIC) to 0.4 (sodium), but was only 0.3 for

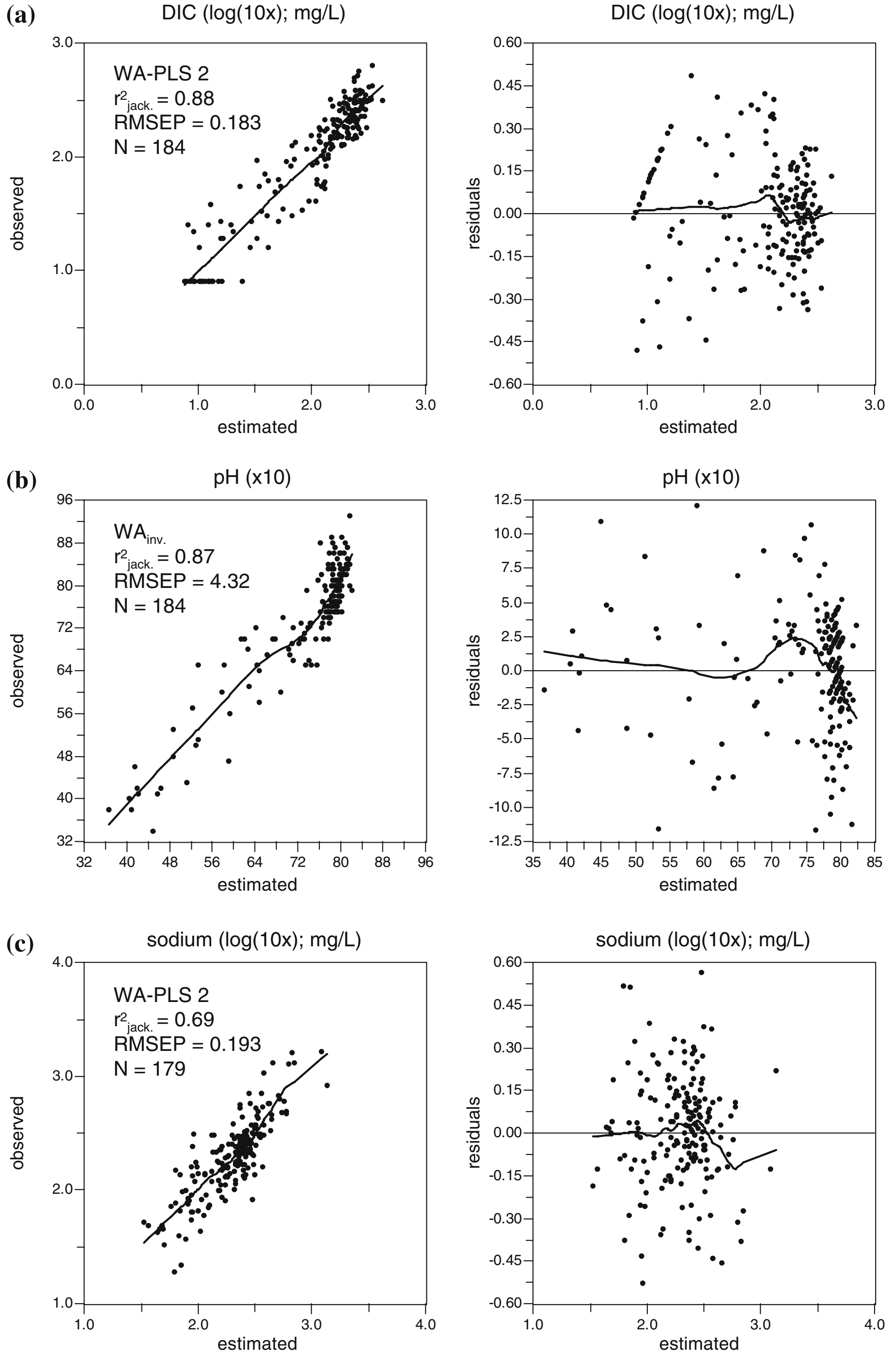

*Figure 3*. Scatter plots of observed values and residuals vs. jackknifed estimates for calibration models based on the entire data set for (a) DIC (type I taxa included), (b) pH (type I taxa included) and (c) sodium (type I taxa excluded). Trend lines are LOWESS smooth fits (stiffness = 0.4).

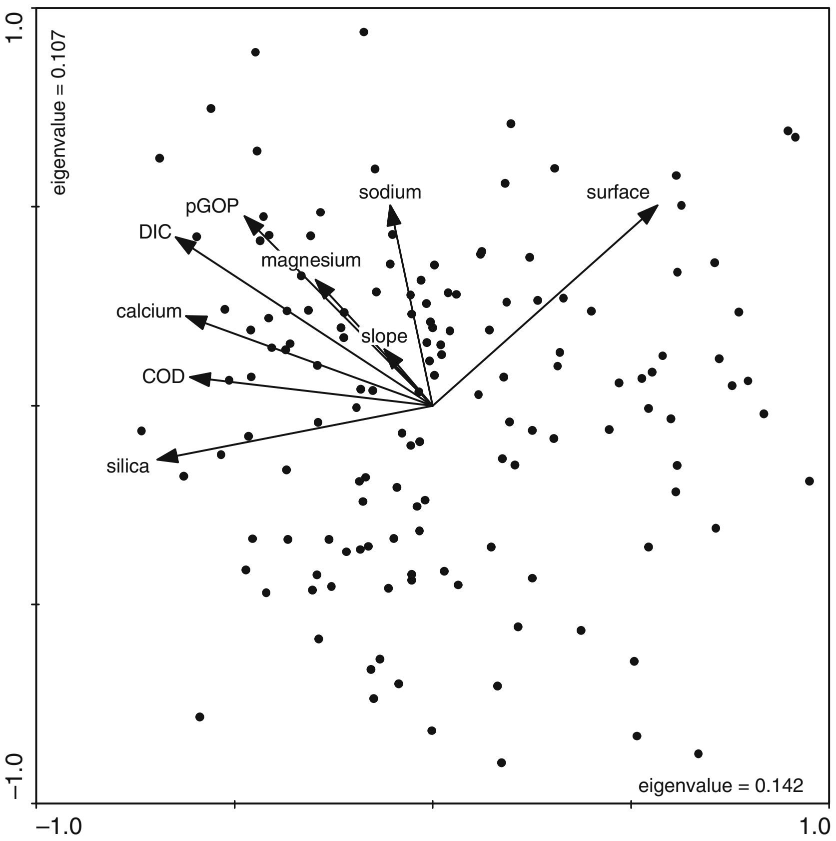

*Figure 4.* Canonical correspondence analysis (CCA) biplot of samples and forward-selected variables for the non-acid sites.

*Table 6.* Spearman rank correlations and VIFs for water-chemistry variables included in the minimal CCA model for the non-acid sites (correlations with $p \leq 0.05$ are in italics).

| | DIC | COD | pGOP | Calcium | Silica | Sodium | Magnesium | Surface | VIF |
|---|---|---|---|---|---|---|---|---|---|
| DIC | 1 | – | – | – | – | – | – | – | 2.6 |
| COD | *0.31* | 1 | – | – | – | – | – | – | 1.7 |
| pGOP | 0.15 | *0.41* | 1 | – | – | – | – | – | 1.3 |
| Calcium | *0.79* | 0.16 | 0.13 | 1 | – | – | – | – | 2.8 |
| Silica | *0.57* | *0.19* | 0.06 | *0.52* | 1 | – | – | – | 1.5 |
| Sodium | *0.31* | *0.44* | 0.14 | *0.26* | 0.04 | 1 | – | – | 2.1 |
| Magnesium | *0.61* | *0.23* | 0.07 | *0.59* | *0.31* | *0.46* | 1 | | 2.3 |
| Surface | *−0.34* | *−0.23* | −0.04 | *−0.29* | *−0.27* | 0.11 | −0.21 | 1 | 1.3 |
| Slope | 0.05 | −0.08 | 0.14 | 0.10 | 0.09 | 0.03 | 0.02 | −0.01 | 1.1 |

magnesium. Compared to the constrained analyses for the entire data set, the ratios for silica, pGOP and COD more than doubled, indicating that their effect was now much less modulated by pH status. The selected variables were also highly significant in the partial analyses. The unique contribution amounted to at least 50% of the marginal effect for all variables, with species distributions showing the strongest response to pGOP (2.5%). Magnesium explained only 1.1% of the species variation if all other variables were partialled out. The unique contribution of calcium (1.4%) was only slightly higher. DIC, calcium and silica explained considerably less variation if interactions with other variables were eliminated. For these variables, the ratios of the principal eigenvalues were reduced by ≈40–50% respective to those from the CCCA, suggesting that their estimation would be prone to interference by other gradients. Interactions were much less important for COD, pGOP, sodium and magnesium. As expected, compositional turnover along the gradients for the selected variables was considerably smaller for this restricted data set, with gradient length varying from 2.05 (COD) to no more than 1.07 S.D. (silica) in a DCCA.

Truncating the pH gradient notably altered the distribution of response characteristics among taxa. Absence of any relation (model I) became the most frequent response type for all variables, ranging from ≈35 (DIC) to 46% (calcium, pGOP) of the 249 fitted taxa (Table 7). A monotone increase or decrease (model II) was more common than a symmetric (model IV) or skewed (model V) unimodal distribution for silica, sodium and magnesium. For DIC and pGOP about 30% of all taxa displayed a symmetric unimodal distribution (model IV). Among the taxa with an average abundance in the upper 5th percentile there were 37 that showed no response to one or more variables (Appendix 2; available as Electronic Supplementary Material). A majority of them was classified as benthic, 9 as planktonic, 8 were epiphytes and 3 belonged to the plocon. The abundance of *Eolimna minima*, *F. capucina* var. *rumpens*, *F. capucina* var. *vaucheriae*, *Hippodonta capitata*, *Nitzschia palea* and *N. paleacea* appeared to be unrelated to most or all of the variables. Usually, species that were non-responsive to DIC or sodium along the entire pH gradient remained so for the restricted pH range, but this was not the case for *Staurosira pinnata* or *S. venter*. Of the other plocon species, *S. brevistriata* only responded to DIC and COD, while *S. construens* remained insensitive to DIC and silica, which was also the case for *S. venter* and COD or pGOP.

Characteristics of the calibration models for the non-acid sites ($pH \geq 6.5$) are given in Table 5. No calibration was attempted for magnesium in view of its low $\lambda_1/\lambda_2$ ratio in constrained ordination. In all instances error estimations were most favourable and parsimonious for WA-PLS with two components when all taxa were included. The proportion of outliers tended to be somewhat higher than with the entire data set (on average 3.6% vs. 2.8%), attaining 5% of the samples for DIC and sodium. This restrained the model range for DIC to $\geq 3.3$ mg $l^{-1}$, and to concentrations above 7 mg $l^{-1}$ for sodium. For the most part, the higher percentage of outliers was due to the smaller number of samples considered (24% less), but for DIC the number of outliers increased from 2 to 7, suggesting that truncation of the pH gradient resulted in less effective estimation of response characteristics for a number of taxa. Notably, parameter estimations were now clearly suspect for a number of sparsely represented diatoms from less alkaline habitats (e.g., various *Brachysira*, *Eunotia*, *Frustulia* and *Pinnularia* species; see Appendix 1, available as Electronic Supplementary Material).

The WA-PLS model for DIC with all taxa had an $r^2_{\text{jack.}}$ of 0.68. Its RMSEP corresponded to 10% of the range, thus remaining approximately the same as for the model spanning the entire pH gradient. Even though the maximum bias was higher than for the model using the entire data set, the apparent and jackknifed prediction errors were substantially lower. With exclusion of non-related taxa, simple WA became more efficient. In spite of a higher apparent error, all jackknifed statistics were modestly improved. A slightly more conspicuous tendency for underestimation developed at DIC concentrations above 30 mg $l^{-1}$ (Figure 5a) but comparison of the residuals for both DIC models (Figures 3a and 5a) suggests that estimations of higher concentrations may be somewhat more accurate with the non-acid sites option. The calibration model for sodium, obtained after discarding four highly tolerant taxa,

*Table 7.* Proportion of taxa according to HOF response-model type for the non-acid sites (log-transformed variables).

| | DIC | Sodium | Calcium | pGOP | COD | Silica | Magnesium |
|---|---|---|---|---|---|---|---|
| I – Flat | 34.9 | 42.2 | 45.8 | 45.4 | 42.2 | 39.8 | 45.0 |
| II – Monotonic | 28.1 | 29.7 | 26.5 | 22.1 | 27.3 | 34.1 | 32.1 |
| III – Monotonic, plateau | – | – | 0.4 | 0.4 | 0.8 | – | 0.4 |
| IV – Symmetric unimodal | 31.3 | 21.3 | 21.3 | 26.9 | 22.5 | 21.3 | 15.7 |
| V – Skewed unimodal | 5.6 | 6.8 | 6.0 | 5.2 | 7.2 | 4.8 | 6.8 |

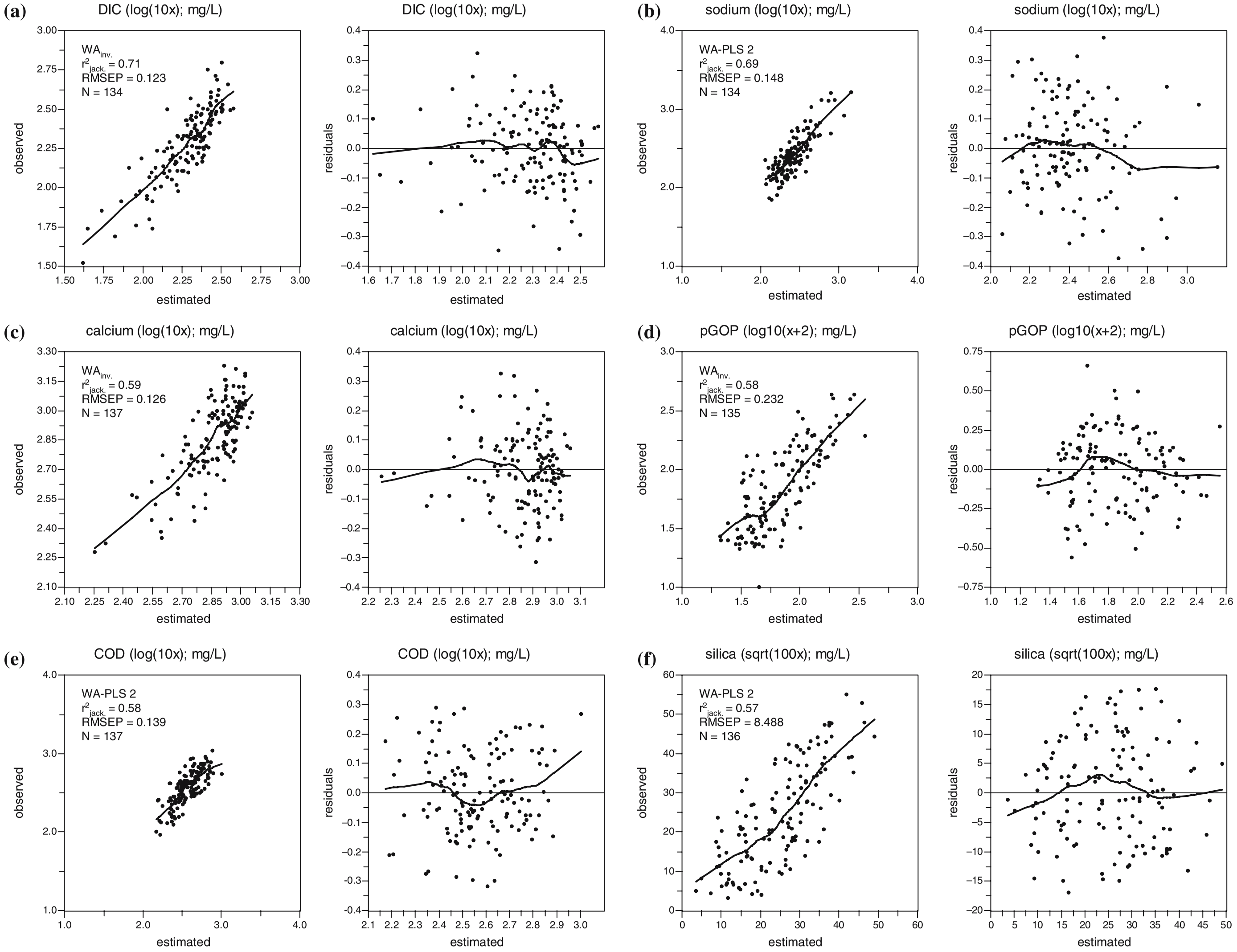

*Figure 5.* Scatter plots of observed values and residuals vs. jackknifed estimates for calibration models based on the non-acid sites for (a) DIC, (b) sodium, (c) calcium, (d) pGOP, (e) COD and (f) silica, always with exclusion of type I taxa. Trend lines are LOWESS smooth fits (stiffness = 0.4).

was moderately strong ($r^2_{\text{jack.}}$0.62). Its prediction error and bias estimates were lower than for the model spanning the entire pH range. Relative to the range, the RMSEP was comparable (11.9%), whereas average bias was lower and maximum bias higher (23.2%). The benefit of excluding unrelated taxa was more substantial in this case: $r^2_{\text{jack.}}$ increased to 0.69, while RMSEP and maximum bias declined slightly. The residuals showed less systematic bias at high concentrations than with the entire data set (Figure 5b). The models for calcium, pGOP and COD with inclusion of all taxa returned rather low apparent errors, but their strength was only moderate with cross-validation ($r^2_{\text{jack.}}$ 0.54 to 0.57 and RMSEP 13.6–14.2% of the range). Maximum bias was about 20% for calcium and COD, while for pGOP it amounted to 9.3%; average bias was no more than 0.5%. With type I taxa removed, WA was slightly more efficient than WA-PLS 2 for calcium and pGOP, but the latter remained superior for COD. Prediction strenght increased slightly and RMSEP was lower for all three variables, favouring this option. Maximum bias increased for COD only (25.1%), but this was due to a single site with an extremely high value (Figure 5e). There were no further obvious trends in the residual structure. Initially, with all taxa included, silica yielded the weakest model with an $r^2_{\text{jack.}}$ of only 0.51. In terms of relative RMSEP (≈17.3% of the range) or maximum bias, however, it was comparable to the other variables. With deletion of all type I taxa, $r^2_{\text{jack.}}$ increased to 0.57, while RMSEP declined slightly. Residuals showed no marked trend for this variable also (Figure 5f).

With all taxa included, correlation between residuals was fairly strong for DIC and calcium ($r = 0.61, p \leq 0.001$), and also significant for DIC and sodium ($r = 0.22, p < 0.01$) or silica ($r = 0.17$, $p < 0.05$), sodium and calcium ($r = 0.23, p < 0.01$) or COD ($r = 0.18$, $p < 0.05$), calcium and silica ($r = 0.26$, $p < 0.01$), as well as pGOP and COD ($r = 0.33, p < 0.001$). Exclusion of taxa with a type I response improved the correlation for calcium and DIC to 0.69. This possibly ensued from the WA option, which does not use structured information in the residuals to improve species parameters.

### *Insensitive taxa*

Table 8 shows the representation of the four habitat groups for taxa included in the HOF analyses and for those showing no response to modelled variables (see Appendix 1 for attribution of individual taxa; available as Electronic Supplementary Material). For both data sets about 2/3 of all taxa are classified as benthic, 1 in 5 as epiphytic, slightly less than 10% as planktonic and almost 5% as plocon. Overall, these proportions were very similar for insensitive taxa. Planktonic taxa were expected to be particularly good indicators of pGOP, as such diatoms were often abundant at high productivity sites. With 9.2% of all taxa considered as planktonic and only 3.5% showing no relation to this variable, there was indeed some support for this hypothesis ($p = 0.04$, Fisher exact, one-tailed).

## Discussion

### *Inferences*

Inference models were developed for seven environmental variables from littoral sedimentary diatom assemblages. These related to buffering conditions and base status (DIC, pH, calcium), salinity (sodium), the concentration of an essential

*Table 8*. Representation of taxa attributed to the categories benthic, epiphytic, planktonic and plocon in the HOF analyses and the proportion showing no response to modelled variables (pH untransformed, other log-transformed, except sqrt for silica).

| | All sites | | | | | Non-acid sites | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tested | | pH % | DIC % | Sodium % | Tested | | DIC % | Sodium % | Calcium % | pGOP % | COD % | Silica % |
| | Number | % | | | | Number | % | | | | | | |
| Benthic | 189 | 66.3 | 70.0 | 63.2 | 72.7 | 159 | 63.6 | 54.0 | 67.6 | 61.4 | 68.1 | 63.8 | 65.7 |
| Epiphytic | 58 | 20.4 | 5.0 | 21.1 | 27.3 | 55 | 22.0 | 31.0 | 26.7 | 26.3 | 23.0 | 24.8 | 21.2 |
| Planktonic | 24 | 8.4 | 15.0 | 10.5 | 2.3 | 23 | 9.2 | 12.6 | 4.8 | 10.5 | 3.5 | 5.7 | 10.1 |
| Plocon | 13 | 4.6 | 10.0 | 5.3 | 6.8 | 12 | 4.8 | 2.3 | 1.0 | 1.8 | 5.3 | 5.7 | 3.0 |

nutrient for diatoms (silica), organic matter (COD) and plankton metabolism (pGOP), thus including several key limnological variables. Considering that the training sets were heterogeneous and consisted predominantly of small and shallow water bodies, overall errors associated with these models seemed acceptable in view of expected strong variation in water chemistry. Moreover, they allowed considerably more accurate and variable-specific inferences than general indicator-value based assessments of water quality where pH, DIC and calcium were important confounding factors for estimating trophic status and organic loading (Denys 2004).

Diatom distributions presented the most precise and independent relationships to median DIC and pH, where error margins were less than 10% of the observed ranges. The strong relation of diatoms to pH has been documented extensively and cross-validated estimation errors of less than 0.5 pH units are not unusual for this variable (e.g., Birks et al. 1990; Korsman and Birks 1996; Cameron et al. 1999). The comparison of calibration models based on cross-validated error statistics remains difficult, however, because these may easily be constrained by strategic site selection (e.g., Walker et al. 1997; Racca et al. 2004) and different data transformations. Nevertheless, the results obtained here (RMSEP 0.4 pH units, 7.3% of the pH range) and in similar studies, such as by Schönfelder et al. (2002; RMSEP 0.35 pH units or 10.4% of the range) suggest that littoral sampling does not necessarily result in poorer predictability for principal environmental gradients compared to profundal-oriented approaches (Vyverman et al. 1996; Siver 1999). Extreme precision (RMSEP $\approx$0.2 pH units) may, however, only be obtainable in rather exceptional conditions where pH is governed by simple chemical relations with little biological interplay (e.g., Bigler and Hall 2002; Enache and Prairie 2002). The quality of the WA model for pH deteriorated at values above 7.5 and it seems that there is an upper limit to pH reconstructions based on the Flemish data using WA-based techniques. Although the number of sites presenting a pH of more than 7.5 was higher than in most other studies, making trends in this area more conspicuous, this feature was probably linked to a marked influence of photosynthetic activity on the pH of these waters and in consequence, more intense diurnal variation (Wetzel 1975; Kann and Smith 1999). Shallowness, allowing abundant macrophyte development, and alternatively hypereutrophic conditions with high phytoplankton biomass are likely to bear importance in this. Also, species turnover was very limited at higher pH values. Of the 80 taxa presenting WA pH optima $\geq$ 8.0 there were only 7 with an optimum pH $\geq$ 8.4.

DIC has also been identified as an influential variable explaining distribution patterns of littoral and sedimentary diatoms (Pienitz and Smol 1993; King et al. 2000; Rühland and Smol 2002; Schönfelder et al. 2002). That reliable and independent calibration appeared possible for DIC and pH simultaneously, may seem unexpected considering their rather close chemical relation. Schönfelder et al. (2002) also observed important and independent contributions of DIC and pH to variation in diatom assemblage composition, whereas Philibert and Prairie (2002a) found $CO_2$ to be a prime candidate for developping a calibration model besides pH. The inclusion of both DIC and calcium in the minimal CCA for non-acid sites further illustrated that the relevance of DIC to diatom distribution should not be interpreted solely as an alkalinity effect (cf. Niederhauser and Schanz 1993). Niche separation among diatoms comprises uptake efficiency of inorganic carbon species, whilst DIC also reflects the availability of a potentially limiting resource (Talling 1976). Although the Flemish data also allowed accurate modelling of (calculated) alkalinity, only results for DIC are reported because it relates to a concentration gradient and was actually measured. It is acknowledged, however, that for some applications, such as in acidification studies, or for estimating background nutrient levels from regression models using this variable (Vighi and Chiaudani 1985), reconstruction of alkalinity may be considered.

As for calcium, few calibration models have been documented so far because of colinearity problems. Bootstrapped statistics for poorly buffered Tasmanian mountain lakes (Vyverman et al. 1996) appear to agree well with the present results. Also emphasizing more dilute conditions, Dixit et al. (1991) obtained a WA regression with an $r^2$ of 0.73 for $\ln(x+1)$ transformed concentrations (only apparent statistics were reported), whereas jackknifed $r^2$ values of 0.59 were obtained for epilithic algae in the English Lake district

(King et al. 2000). The strength of the present model for non-acid sites was similar, but its range was beyond any of those reported previously. However, ordination analyses indicate that it cannot be considered as truly independent and consequently estimates should be treated cautiously.

Inferences of sodium concentrations were equally less robust, but, nevertheless, may give some additional information that is useful for management, e.g., to point out agricultural influences or other forms of pollution leading to higher levels and to document salinity conditions accompanying biological restoration targets. Although the resulting models were not as accurate as those for pH or DIC and of modest predictive power (Prairie 1996), they should still allow assessment of major trends. Sodium was an even more influential variable in north-eastern Germany, where $\lambda_1/\lambda_2$ reached 0.85 in a constrained CCA (Schönfelder et al. 2002), but the jackknifed RMSEP obtained by WA for chloride, which was strongly correlated to sodium and preferred for pragmatic reasons, was slightly higher (15.5% of the transformed range) than in this study (10.4 and 11.9%). The $r^2$ values of the Flemish sodium models also surpassed some for chloride from the north-eastern U.S. (Dixit and Smol 1994, $r^2 = 0.61$; Dixit et al. 1999, $r^2 = 0.46$). Notably, diatom communities showed a stronger response to calcium or sodium than to conductivity. Potapova and Charles (2003) recently highlighted the importance of individual cations for the distribution of benthic river diatoms.

Silica was the last inorganic substance describing a statistically significant portion of the variation in diatom assemblages among non-acid sites. Compositional turnover occurred along the entire gradient, even at concentrations far exceeding limiting conditions. This may be because availability of silica relative to other nutrients, such as phosphorus, was more influential than its actual concentration (Tilman et al. 1982; Sommer 1988), or because of other indirect relations to variables influencing diatom distribution. Unfortunately, even the best inference model for this variable was rather weak and only capable of differentiating between more extreme values with relative confidence. With increased precision, however, it could become a useful paleohydrological tool to estimate the contribution of silica-rich river or groundwater to the water budget of surface waters and assess hydrological isolation. Such improvement may necessitate increasing sampling intensity to a level that is more apt for a biologically reactive component than was possible in this study.

Although diatoms are widely considered as sensitive to organic loads and are commonly used in pollution assessment in running waters for this reason, there are no known inference models for COD in lentic environments. This is probably because few studies have included sufficient waters with elevated levels of easily degradable organic substances and this aspect was seldom considered. Repeatedly, however, fairly reliable transfer functions could be constructed for more refractory organic compounds, usually measured as DOC or colour, in less productive, often acid waters (Kingston and Birks 1990; Korsman and Birks 1996; Rosén et al. 2000; Dixit et al. 2001). Surrogate measures for organic acids, such as yellow substance, were not significant in this study, whereas COD remained rather unimportant as long as the entire pH range was considered. COD incorporates a broad spectrum of compounds, including particulate matter in suspension, and its correlation to diatom distribution may comprise diverse links, for instance with the abundance of planktonic taxa, but also with oxygen regime, light availability and availability of nutrients (Cholnoky 1968; Lange-Bertalot 1979; Köster and Hübener 2001). Effects of DOC on diatoms in humic-acid waters more likely pertain to buffering, metal chelation and UV irradiation (Alles et al. 1991; Vinebrooke and Leavitt 1999). Although COD attained the second highest constrained $\lambda_1/\lambda_2$ ratio of the variables that significantly affected diatom distribution for the non-acid sites, the most efficient model was also of moderate quality. Nevertheless, even with jackknifing, model strength ($r_{\text{jack.}} = 0.76$) compared favourably to the correlation of river pollution indices to COD (Prygiel and Coste 1993; Kwandrans et al. 1998).

A better inference model proved possible for pGOP than for other variables reflecting trophic status. TP, in particular, was found to be an important explanatory variable for diatom distribution and could be calibrated successfully on many occasions (Hall and Smol 1999). There are several possible explanations why this was not the case in this study. First of all, the limited number of TP measurements may have confounded estimation of ecologically meaningful concentrations.

Among others, Bennion and Smith (2000) and Bradshaw et al. (2002) concluded that high frequency sampling may be essential for good TP calibration. Furthermore, the detection threshold for TP analyses was quite high in this study (70 $\mu g\ l^{-1}$), and species turnover at the lower end of the gradient was therefore not reflected by corresponding variation in TP. Often, TP calibration is more successful at lower concentrations (Anderson et al. 1993; Reavie et al. 1995; Kauppila et al. 2002), although notable exceptions exist (Bennion et al. 1996). More fundamental reasons may be the complex behaviour of TP in shallow waters, typically including strong interactions with macrophytes, sediment, suspended matter and zooplankton, as well as a weaker relation of benthic diatoms to TP in the water column due to phosphorus supply from the sediment (Bennion 1994; Bennion et al. 2001; Sayer 2001). Stratified waters were a small minority in the Flemish training set, whereas hypereutrophic sites were well represented. Under such conditions, phosphorus may no longer be limiting diatom production and other variables, including nitrogen, light, organic matter or toxic substances, come into play. A metabolic variable such as pGOP may be more effective than TP in capturing associated variation in diatom species composition (cf. Jones and Juggins 1995; DeNicola et al. 2004). Finally, nutrient concentrations in the water column may be less relevant to benthic communities than factors directly influenced by phytoplankton abundance, e.g., light climate (Anderson et al. 1993; Anderson 1994; Jones and Juggins 1995; Philibert and Prairie 2002b), although it should be noted that there was no apparent relation to any of the measured optical characteristics in this study. The correlation of pGOP to chlorophyll ($r = 0.87$ and 0.89 for all and $pH \geq 7.5$ sites, respectively) was considerably stronger than to TP ($r = 0.32$ and 0.23, all $p \leq 0.01$). Considering that increased phytoplankton productivity and organic loading characterise the eutrophication process, reconstruction tools for pGOP and COD are promising assets to assess ecosystem responses.

### *Model development*

WA-PLS performs better than WA in the presence of a structured secondary gradient, as it makes use of the residual variation associated to variables that are not entirely independent (ter Braak and Juggins 1993; Birks 1998). As such, it was no surprise to find that with this complex data set, simple WA only proved most appropriate for the predominant variable, pH, with the entire training set and in a few cases where both pH differences and the number of taxa were reduced. WA-PLS also consistently outperformed PLS, even with short gradients and monotonic diatom responses outnumbering symmetric relations, which may again point to interactions between variables.

Evaluation of the $r^2$ between observed and (back-transformed) jackknifed estimates obtained with and without transformation of the environmental data, but using the same model options otherwise, indicated that this had little or no effect in most cases. For the non-acid sites, however, transformation improved the fit by about 5% for DIC, sodium and silica. The compositional gradient length was quite short for silica (1.07 SD) while its eigenvalue ratio was only 0.44 in a constrained CCA, so, conversely to the suggestion by Köster et al. (2004), it seems that even weak gradients may be sensitive to such data manipulation.

Several studies point out that inadequate estimation of optima for sparsely occurring taxa is a major cause of reduced model performance in tolerance-adjusted WA (Wilson et al. 1996; Köster et al. 2004). For weighted-averaging techniques without tolerance down-weighting, the bootstrapped RMSEP is expected to be minimal with inclusion of as many taxa as possible (Birks 1994), while Cameron et al. (1999) and Philibert and Prairie (2002b) found that performance was comparable for WA-PLS with all taxa included or those with few occurrences deleted. Deletion of taxa based on their number of occurrences or Hill's N2 did indeed not result in any appreciable improvement with the Flemish data. Removing taxa with exceptionally wide tolerances had limited, if not a negative effect, although this proved beneficial on other occasions (Racca et al. 2001; Enache and Prairie 2002; Philibert and Prairie 2002b). Elimination of taxa that were unrelated to the variable of interest was more adequate, particularly with models pertaining to non-acid sites only, where their number was more important. This involved up to 1/3 of all taxa, including several abundant ones, and will obviously have strong repercussions for determining sample analogy. Selective species deletion, although following a more sophisticated pruning procedure based on the relative contribution of individual taxa, was even more efficient for modelling with artificial neural networks and also has the attractive potential to reduce effects of other environmental influences (Racca et al. 2004). There was little ground to exclude certain groups of taxa because of their growth form or preferred habitat, and even widespread *Staurosira* spp., which are notoriously more dependent of physical habitat than water chemistry (Bennion 1995; Bennion et al. 2001), added useful information to most of the models. In general, non-responsive taxa were proportionally represented among taxa classified as principally benthic, planktonic, epiphytic or plocon, but planktonic diatoms were particularly good indicators of pGOP, mainly because high phytoplankton concentrations implied abundance of such taxa (e.g., *Aulacoseira granulata* and its morphotype *curvata*, *A. muzzazensis*, *Cyclotella atomus*, *C. meduanae*, *C. scaldensis*, *Stephanodiscus* spp., *Thalassiosira pseudonana)*. In view of the differences between shallow and deep lakes in assemblage composition and their behaviour towards eutrophication, Bradshaw et al. (2002) suggested elaboration of separate TP models for both lake categories, whereas Philibert and Prairie (2002b) observed little effect of lake depth. The pGOP models deteriorated slightly if the 11 water bodies with a maximum depth allowing stable stratification ($>6$ m) were removed. Although stratified water bodies represented less than 10% of the training sets and a different result might have been obtained if they had been more numerous, it may be that differentiation according to water depth is less essential for this variable when sampling focus is on shallow littoral areas, as in this investigation. It is highly unlikely, however, that the present calibration would allow valid inferences for assemblages originating from greater water depth.

In this study, up to 5% of the samples were considered outliers. Although this high frequency may be due to inherently more noisy relationships between littoral diatom assemblages and measured conditions, the intensity of human impact should be considered also, as noted by Seppä et al. (2004) for pollen-climate calibration models. Although the mechanisms are different for diatoms or other aquatic organisms than for terrestrial vegetation, a similar relation to disturbance may exist due to an increased likelihood of hydrochemically unstable conditions (resulting from liming, baiting, short-lived pollution events, temporary drawdown or flushing, etc.) or physical disturbance of surface sediments.

Composing a training set involves balancing between increasing data heterogeneity and avoiding non-analogue problems in future application of the resulting inference models. Contrary to many other studies, the Flemish sampling sites were not selected with a specific gradient in mind, but chosen to reflect the variation in regional surface water characteristics. While this approach reduces the strength of models for less important variables, it makes them less susceptible to changing interactions between variables along the main environmental gradients and broadens their possible field of application (Kauppila et al. 2002; Racca et al. 2004). Considering the large regional variation in surface-water conditions and the often sparse documentation of environmental conditions for historical samples, this is essential to allow assessment of former freshwater conditions throughout the study area.

A trade-off exists between expenditure for data collection and improving predictive ability of inference models. The point for more frequent water sampling was already made. Although the rate at which precision increases slows down beyond a certain training-set size (Wilson et al. 1996), this aspect also requires further elaboration. Particularly, the representation of sites with a pH $< 6.5$, and correspondingly lower ionic concentrations, needs to be augmented. Such waters are characterized by a highly specific diatom flora and require further sampling efforts. To some extent, it may be difficult to fill in this 'biodiversity gap', however, as some acidification-sensitive taxa, e.g. *Kobayasiella* spp. and several *Eunotia*, have become extremely rare and now occur with lower abundances than in the past (Denys 2001a). Ongoing studies indicate that even in the more densely sampled high pH range, the account of species distributions is still quite incomplete, while the often considerable difference between apparent and cross-validated model errors also suggested that further appreciation of the species variation could improve certain transfer functions. Final optimisation may necessitate incorporation of data from less impacted, yet geographically similar and nearby regions. Some improvement may also follow from refining taxonomic precision. Several taxa used as basic entities here consist of morphologically distinct species, e.g., the *Gomphonema truncatum* aggr. (Reichardt 2001) or the *Sellaphora pupula* aggr. (Mann et al. 2004). As evidenced already by the different optima observed for morphotypes of *Achnanthidium minutissimum* (cf. Appendix 1; available as Electronic Supplementary Material), this lack of taxonomic precision may result in the loss of information.

So far, only the more 'traditional' methods for developing inference models have been explored with the present data. It seems worthwhile to explore alternatives, such as artificial neural networks or Bayesian estimation, that may be more suited to handle strongly heterogeneous training sets with short and secondary gradients (Birks 1998; Vasko et al. 2000; Toivonen et al. 2001; Köster et al. 2004). Moreover, more precise estimations may be possible by joint application of reconstruction techniques based on different algorithms (Racca et al. 2001) and by considering inferences from several contemporaneous sediment samples or from epiphytic assemblages, which may be provided by the same specimens in case herbarium macrophytes are used.

## Conclusions

Quantitative inferences of former water-chemistry from sediment diatom assemblages obtained from herbarium macrophytes or other samples preserved in museum collections require models that are specific to littoral habitats and applicable without much prior site knowledge or assumptions on influential environmental gradients. Using WA and WA-PLS methods, a set of useful inference models could be developed for this purpose from a region-wide assessment of littoral freshwater diatom assemblages and corresponding environmental conditions in lower-Belgium. Models for pH and dissolved inorganic carbon appeared to be relatively robust and were most accurate according to cross-validation, whereas for other variables, i.c., sodium, calcium, silica, chemical oxygen demand and potential gross oxygen production, inferences were less precise. At least for some of these variables, performance was compatible with calibrations based on sampling the deepest part of the basin. Further testing using independent data is required to asses their actual prediction accuracy. *A posteriori* reduction of the principal gradient influencing diatom distribution, pH, and removal of taxa that were not statistically related to the variable of interest were useful to expand the number of variables that could be modelled and reduce errors. The results show that, if training sets are sufficiently large to account for substantial between and within site variation, considerable potential exists for using littoral diatom assemblages from historical samples to reconstruct environmental conditions in relation to anthropogenic change even in water bodies lacking *in situ* sediment records of sufficient quality. A substantial amount of data on littoral sediment diatom assemblages from c. 1850 to 1945 is already available from the study area and application of the calibration models will improve hindsight into a less impacted status of standing waters for individual sites and on a more regional scale.

## Appendix 1

Taxa included in the training sets with number of observations (obs.), maximum abundance (max.) and Hill's N2, as well as WA optima (opt.), tolerances (tol.) and HOF response model (see text) for selected variables. Attribution to habitat group is given for taxa included in HOF analyses. View online at www.springerlink.com

## Appendix 2

Taxa with average abundance in the upper 5th percentile of the training sets and showing no significant relation to inferred variables according to HOF analyses.
View online at www.springerlink.com

## Acknowledgements

Data used in this paper were obtained in the research project VLINA C97/02, granted by the Flemish Government to the Biology Department, University of Antwerpen, UIA (ECOBE) and RUCA (PLP). Thanks are due to A. Das,

V. Clement, M. Coenen, V. Moons, J. Packet, D. Van Pelt, B. Veraart and L. Weiss for their diverse and substantial contributions to this project and to W. De Smet for discussions. Both anonymous reviewers are acknowledged for their appreciated comments and suggestions.

## References

Alles E., Nörpel-Schemp M. and Lange-Bertalot H. 1991. Zur Systematik und Ökologie charakteristischer *Eunotia*-Arten (Bacillariophyceae) in elektrolytarmen Bachoberläufen. Nova Hedwigia 53: 171–213.

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

Anderson N.J., Rippey B. and Gibson C.E. 1993. A comparison of sedimentary and diatom-inferred phosporus profiles: implications for defining pre-disturbance nutrient conditions. Hydrobiologia 253: 357–366.

Arzet K. and 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 R.W. 1981. Changes in the diatom microflora of a eutrophic lake since 1900 from a comparison of old algal samples and the sedimentary record. Holarctic Ecol. 4: 73–81.

Battarbee R.W., Jones, V.J., Flower R.J., Cameron N.G., Bennion H., Carvalho L. and Juggins S. 2001. Diatoms. In: Smol J.P., Birks H.J.B. and Last W.M. (eds), Tracking Environmental Change Using Lake Sediments. Vol. 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 H. and Smith M.A. 2000. Variability in the 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., Appleby P.G. and Phillips G.L. 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.

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

Betts-Piper A.B., Zeeb A. and Smol J.P. 2004. Distribution and autecology of chrysophyte cysts from high Arctic Svalbard lakes: preliminary evidence of recent environmental change. J. Paleolimnol. 31: 467–481.

Bigler C. and Hall R.I. 2002. Diatoms as indicators of climatic and limnological change in Swedish Lapland: a 100-lake calibration set and its validation for paleoecological reconstructions. J. Paleolimnol. 27: 97–115.

Birks H.J.B. 1994. The importance of pollen and diatom taxonomic precision in quantitative palaeoenvironmental reconstructions. Rev. Palaeobot. Palyno. 83: 107–117.

Birks H.J.B. 1998. Numerical tools in palaeolimnology – Progress, potentialities and problems. J. Paleolimnol. 20: 307–332.

Birks H.J.B., Juggins S. and Line J.M. 1990. Lake surface-water chemistry reconstructions from paleolimnological data. In: Mason B.J. (ed.), The Surface Waters Acidification Programme. Cambridge University Press, Cambridge, pp. 301–313.

Bradshaw E.G., Anderson N.J., Jensen J.P. and Jeppesen E. 2002. Phosphorus dynamics in Danish lakes and the implications for diatom ecology and palaeoecology. Freshwater Biol. 47: 1963–1975.

Cameron N.G., Birks H.J.B., Jones V.J., Berge F., Catalan J., Flower R.J., Garcia J., Kawecka B., Koinig K.A., Marchetto A., Sánchez-Castillo, Schmidt R., Šiško M., Solovieva N., Štefková E. and 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.

Cholnoky B.J. 1968. Die Ökologie der Diatomeen in Binnengewässern. J. Cramer Verlag, Lehre, 699 pp.

Cocquyt C. and 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.

Cumming B.F., Smol, J.P., Kingston J.C., Charles D.F., Birks H.J.B., Camburn K.E., Dixit S.S., Uutala A.J. and Selle A.R. 1992. How much acidification has occurred in Adirondack region lakes (New York, USA) since preindustrial times? Can. J. Fish. Aquat. Sci. 49: 128–141.

De Blust G., Paelinckx D. and 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 D.M., de Eyto E., Wemaere A. and Irvine K. 2004. Using epilithic algal communities to assess trophic status in Irish lakes. J. Phycol. 40: 481–495.

Denys L. 1997. Diatom assemblages from herbarium macrophytes and old samples – potential for historical limnology and studies from Flanders (Belgium). Biol. Jaarb. Dodonaea 65: 137–138.

Denys L. 2001a. Historical distribution of 'Red List' diatoms in Flanders (Belgium). Syst. Geogr. Plants 70: 409–420.

Denys L. 2001b. Stilstaande zoete wateren. In: Kuijken E., Boeye D., De Bruyn L., De Roo K., Dumortier M., Peymen J., Schneiders A., Van Straaten D. and Weyembergh G. (eds), 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.

Dixit S.S. and Smol J.P. 1994. Diatoms as indicators in the Environmental Monitoring and Assessment Program-Surface Waters (EMAP-SW). Environ. Monit. Assess. 31: 275–306.

Dixit S.S., Dixit A.S. and Smol J.P. 1991. Multivariable environmental inferences based on diatom assemblages from Sudbury (Canada) lakes. Freshwater Biol. 26: 251–266.

Dixit S.S., Keller W., Dixit A.S. and Smol J.P. 2001. Diatom-inferred dissolved organic carbon reconstructions provide assessments of past UV-B penetration in Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 58: 543–550.

Dixit S.S., Smol J.P., Charles D.F., Hughes R.M., Paulsen S.G. and Collins G.B. 1999. Assessing water quality changes in the lakes of the northeastern United States using sediment diatoms. Can. J. Fish. Aquat. Sci. 56: 131–152.

Einheitsverfahren 1971. L 12 Der Assimilations-Zehrungstest. L13. Die Bestimmung der Biogenen Belüftungsrate (Hell-Dunkelflashen Methode). L14. Die Bestimmung des Sauerstoff-Produktions-Potentials. In: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-Untersuchung II. Physikalische, chemische, biologische und bacteriologische Verfahren. Verlag Chemie Gmbh, Weinheim, 13pp.

Enache M. and Prairie Y.T. 2002. WA-PLS diatom-based pH, TP and DOC inference models from 42 lakes in the Abitibi clay belt area (Quebec, Canada). J. Paleolimnol. 27: 151–171.

European Parliament 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. L327: 1–73.

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

Gasse F., Juggins S. and Ben Khelifa L. 1995. Diatom-based transfer functions for inferring past hydrochemical characteristics of African lakes. Palaeogeogr. Palaeocl. 117: 31–54.

Golterman H.L., Clymo R.S. and Ohnstad M.A.M. 1978. Methods for Physical and Chemical Analysis of Fresh Waters. Blackwell Scientific Publ, Oxford, 315 pp.

Hall R. and Smol J.P. 1992. A weighted-averaging regression and calibration model for inferring total phosphorus concentration from diatoms in British Columbia (Canada) lakes. Freshwater Biol. 27: 417–434.

Hall R. and Smol J.P. 1996. Palaeolimnological assessment of long-term water-quality changes in south-central Ontario lakes affected by cottage development and acidification. Can. J. Fish. Aquat. Sci. 53: 1–17.

Hall R. and Smol J.P. 1999. Diatoms as indicators of lake eutrophication. In: Stoermer E.F. and Smol J.P. (eds), The Diatoms. Applications for the Environmental and Earth Sciences. Cambridge University Press, Cambridge, pp. 128–168.

Hill M.O. 1973. Diversity and evenness: a unifying notation and is consequences. Ecology 54: 427–432.

Huisman J., Olff H. and Fresco L.F.M. 1993. A hierarchical set of models for species response analysis. J. Veg. Sci. 4: 37–46.

Hürlimann J., Elber F., Niederberger K., Straub F., Stöckli A. and Niederhauser P. 2001. Historische Kieselalgenproben als biologische Referenzen zur Bewertung von Fliessgewässern des Schweizer Mittellandes. In: Jahn R., Kociolek J.P., Witkowski A. and Compère P. (eds), Lange-Bertalot Festschrift. Studies on Diatoms. A.R.G. Gantner Verlag, Ruggel, pp. 401–416.

Jones V.J. and Juggins S. 1995. The construction of a diatom-based chlorophyll *a* transfer function and its application at three lakes on Signy Island (maritime Antarctic) subject to differing degrees of nutrient enrichment. Freshwater Biol. 34: 433–445.

Juggins S. 2003. $C^2$. Software for Ecological and Palaeoecological Data Analysis and Visualisation. User guide version 1.3. University of Newcastle, Newcastle upon Tyne, 69 pp.

Kann J. and Smith V.H. 1999. Estimating the probability of exceeding elevated pH values critical to fish populations in a hypereutrophic lake. Can. J. Fish. Aquat. Sci. 56: 2262–2270.

Kauppila T., Moisio T. and Salonen V.-P. 2002. A diatom-based inference model for autumn epilimnetic total phosphorus concentration and its application to a presently eutrophic lake. J. Paleolimnol. 27: 261–273.

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

Kingston J.C. and Birks H.J.B. 1990. Dissolved organic carbon reconstructions from diatom assemblages in PIRLA project lakes, North America. Philos. T. Roy. Soc. B 327: 279–288.

Kingston J.C., Birks H.J.B., Uutala A.J., Cumming B.F. and Smol J.P. 1992. Assessing trends in fishery resources and lake water aluminium from paleolimnological analyses of siliceous algae. Can. J. Fish. Aquat. Sci. 49: 116–127.

Korsman T. and Birks H.J.B. 1996. Diatom-based water chemistry reconstructions from northern Sweden: a comparison of reconstruction techniques. J. Paleolimnol. 15: 65–77.

Köster D. and Hübener T. 2001. Application of diatom indices in a planted ditch constructed for tertiary sewage treatment in Schwaan, Germany. Internat. Rev. Hydrobiol. 86: 241–252.

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

Krammer K. 1997a. Die cymbelloiden. Diatomeen Eine Monographie der weltweit bekannen Taxa. Teil 1. Algemeines und *Encyonema* Part. Bibl. Diatomol. 36: 1–382.

Krammer K. 1997b. Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannen Taxa. Teil 2. *Encyonema* part., *Encyonopsis* and *Cymbellopsis*. Bibl. Diatomol. 37: 1–469.

Krammer K. 2000. Diatoms of Europe. Vol 1. The genus *Pinnularia*. A.R.G. Gantner Verlag K.G, Ruggel, 703 pp.

Krammer K. and Lange-Bertalot H. 1986–1991. Bacillariophyceae. In: Ettl H., Gerloff J., Heynig H. and Mollenhauer D. (Hrsgb), Süßwasserflora von Mitteleuropa Bd 2(1–4). Fischer Verlag, Stuttgart, 473, 596, 576, 876 pp.

Kwandrans J., Eloranta P., Kawecka B. and Wojtan K. 1998. Use of benthic communities to evaluate water quality in rivers of southern Poland. J. Appl. Phycol. 10: 193–201.

Lange-Bertalot H. 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia Beih. 64: 285–304.

Lange-Bertalot H. 1993. 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süsswasserflora von Mitteleuropa. Vol. 2/1–4. Bibl. Diatomol. 27: 1–454.

Lange-Bertalot H. and Metzeltin D. 1996. Indicators of oligotrophy. 800 taxa representative of three ecologically distinct lake types, carbonate buffered – oligodystrophic – weakly buffered soft water. Iconogr. Diatomol. 2: 1–390.

Lange-Bertalot H. and Moser G. 1994. *Brachysira.* Monographie der Gattung. Bibl. Diatomol. 29: 1–212.

Lotter A.F., Birks H.J.B., Hofmann W. and Marchetto A. 1997. Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. I. Climate. J. Paleolimnol. 18: 395–420.

Mann D.G., McDonald S.M., Bayer M.M., Droop S.J.M., Chepurnov V.A., Loke R.E., Ciobanu A. and du Buf J.M.H. 2004. The *Sellaphora pupula* species complex (Bacillariophyceae): morphometric analysis, ultrastructure and mating data provide evidence for five new species. Phycologia 43: 459–482.

Moos M.T., Laird K.R. and Cumming B.F. 2005. Diatom assemblages and water depth in Lake 239 (Experimental Lakes Area, Ontario): implications for paleoclimatic studies. J. Paleolimnol. 34: 217–227.

Moss B., Johnes P. and Phillips G. 1997. New approaches to monitoring and classifying standing waters. In: Boon P.J. and Howell D.L. (eds), Freshwater Quality: Defining the Indefinable? Scottish Natural Heritage, Edinburgh, pp. 118–133.

NBN 1974. T91-201. Wateronderzoek. Bepaling van het chemisch zuurstofverbruik (COD). Kaliumdichromaatmethode. Belgisch Instituut voor Normalisatie, Brussel, 4 pp.

Niederhauser P. and Schanz F. 1993. Effect of nutrient (N, P, C) enrichment upon the littoral diatom community of an oligotrophic mountain lake. Hydrobiologia 269/270: 453–462.

O'Connell J.M., Reavie E.D. and Smol J.P. 1997. Assessment of water quality using epiphytic diatom assemblages on *Cladophora* from the St Lawrence River (Canada). Diatom Res. 12: 55–70.

Oksanen J. and Minchin P.R. 2002. Continuum theory revisited: what shape are species responses along ecological gradients? Ecol. Model. 157: 119–129.

Philibert A. and Prairie Y.T 2002a. Diatom-based transfer functions for western Quebec lakes (Abitibi and Haute Mauricie): the possible role of epilimnetic $CO_2$ concentration in influencing diatom assemblages. J. Paleolimnol. 27: 456–480.

Philibert A. and Prairie Y.T 2002b. 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.

Philippi T.E., Dixon P.M. and Taylor B.E. 1998. Detecting trends in species composition. Ecol. Appl. 8: 300–308.

Pienitz R. and Smol J.P. 1993. Diatom assemblages and their relationship to environmental variables in lakes from the boreal forest-tundra ecotone near Yellowknife, Northwest Territories, Canada. Hydrobiologia 269/270: 391–404.

Potapova M. and Charles D.F. 2003. Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. Freshwater Biol. 48: 1311–1328.

Prairie Y.T. 1996. Evaluating the predictive power of regression models. Can. J. Fish. Aquat. Sci. 53: 490–492.

Prentice I.C. 1980. Multidimensional scaling as a research tool in Quaternary palynology: a review of theory and methods. Rev. Palaeobot. Palyno. 3: 71–104.

Prygiel J. and Coste M. 1993. Utilisation des indices diatomiques pour la mesure de la qualité des eaux du basin Artois-Picardie: bilan et perspectives. Ann. Limnol. 29: 255–267.

Racca J.M.J. and Prairie Y.T. 2004. Apparent and real bias in numerical transfer functions in paleolimnology. J. Paleolimnol. 31: 117–124.

Racca J.M.J., Gregory-Eaves I., Pienitz R. and Prairie Y.T. 2004. Tailoring palaeolimnological diatom-based transfer functions. Can. J. Fish. Aquat. Sci. 61: 2440–2454.

Racca J.M.J., Philibert A., Racca R. and Prairie Y.T. 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.

Reavie E.D., Hall R. and Smol J.P. 1995. An expanded weighted-averaging model for inferring past total phosphorus concentrations from diatom assemblages in eutrophic British Columbia (Canada) lakes. J. Paleolimnol. 14: 49–67.

Reichardt E. 1997. Taxonomische Revision des Artenkomplexes um *Gomphonema pumilum* (Bacillariophyta). Nova Hedwigia 65: 99–129.

Reichardt E. 1999. Zur Revision der Gattung *Gomphonema.* Die Arten um *G. affine*/*G. insigne*, *G. angustatum*/*G. micropus*, *G. acuminatum* sowie gomphonemoide Diatomeen aus dem Oberoligozän in Böhmen. Iconogr. Diatomol. 8: 1–203.

Reichardt E. 2001. Revision der Arten um *Gomphonema truncatum* und *G. capitatum.* In: Jahn R., Kociolek J.P., Witkowski A. and Compère P. (eds), Lange-Bertalot Festschrift. Studies on Diatoms. A.R.G. Gantner Verlag, Ruggel, pp. 187–224.

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

Rosén P., Hall R., Korsman T. and Renberg I. 2000. Diatom transfer-functions for quantifying past air-temperature, pH and total organic carbon concentration from lakes in northern Sweden. J. Paleolimnol. 24: 109–123.

Rühland K.M. and Smol J. 2002. Freshwater diatoms from the Canadian arctic treeline and development of paleolimnological inference models. J. Phycol. 38: 249–364.

Sayer C.D. 2001. Problems with the application of diatom-total phosphorus transfer functions: examples from a shallow lake. Freshwater Biol. 46: 743–75.

Sayer C.D., Roberts N., Sadler J., David C. and Wade P.M. 1999. Biodiversity changes in a shallow lake ecosystem: a multi-proxy palaeolimnological analysis. J. Biogeogr. 26: 97–114.

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. and Steinberg C.W. 2002. Relationships between littoral diatoms and their chemical environment in northeastern German lakes and rivers. J. Phycol. 38: 66–82.

Seppä H., Birks H.J.B., Odland A., Poska A. and Veski S. 2004. Modern pollen surface sample set from northern Europe:

developing and testing a tool for palaeoclimatological reconstructions. J. Biogeogr. 31: 251–267.

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

Sommer U. 1988. Growth and survival strategies of plankton diatoms. In: Sandgren C.D. (ed.), Growth and Reproductive Strategies of Freshwater Phytoplankton. Cambridge University Press, Cambridge, pp. 227–260.

StatSoft 2003. STATISTICA (data analysis software system), version 6. www.statsoft.com

Talling J.F. 1976. The depletion of carbon dioxyde from lake water by phytoplankton. J. Ecol. 64: 79–121.

ter Braak C.J.F. 1987. CANOCO – a FORTRAN program for canonical community ordination by (partial detrended) (canonical) correspondence analysis, principal components analysis and redundancy analysis (version 2.1). TNO Institute of Applied Computer Science, Wageningen, 95 pp.

ter Braak C.J.F. and Juggins S. 1993. Weighted averaging partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. Hydrobiologia 260: 458–502.

ter Braak C.J.F. and Šmilauer P. 2002. CANOCO Reference Manual and CanoDraw for Windows User's. Guide Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, 500 pp.

ter Braak C.J.F. and van Dam H. 1989. Inferring pH from diatoms: a comparison of old and new calibration methods. Hydrobiologia 178: 209–223.

ter Braak C.J.F., Juggins S., Birks H.J.B. and der Voet H. 1993. Weighted averaging partial least squares regression (WA-PLS): definition and comparison with other methods for species-environment calibration. In: Patil G.P. and Rao C.R. (eds), Multivariate Environmental Statistics. North-Holland, Amsterdam, pp. 525–560.

Tilman D., Kilham S. and Kilham P. 1982. Phytoplankton community ecology: the role of limiting nutrients. Annu. Rev. Ecol. Syst. 13: 439–372.

Toivonen H.T.T., Mannila H., Korhola, A. and Olander H. 2001. Applying Bayesian statistics to organism-based environmental reconstruction. Ecol. Appl. 11: 618–630.

Van Avermaet P., Denys L., Brouwers S. and Overloop S. 2003. Verzuring. In: Van Steertegem M. (red.), Milieu- en natuurraport Vlaanderen. Thema's. LannooCampus, Leuven, pp. 251–262.

van Dam H. and Beljaars K. 1984. Nachweis von Versauerung in West-Europäischen kalkarmen stehenden Gewässern durch Vergleich von alten und rezenten Kieselalgenproben. In: Wieting J., Lenhart B., Steinberg C. Hamm A. and Schoen R. (eds), Gewässerversauerung in der Bundesrepublik Deutschland. Schmidt, Berlin, pp. 184–186.

van Dam H. and Kooyman-van Blokland H. 1978. Man-made changes in some Dutch moorland pools, as reflected by historical and recent data about diatoms and macrophytes. Int. Rev. Ges. Hydrobio. 63: 587–607.

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

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

Vasko K., Toivonen H.T.T. and Korhola A. 2000. A Bayesian multinomial Gaussian response model for organism-based environmental reconstruction. J. Paleolimnol. 24: 243–250.

Verbruggen C., Denys L. and Kiden P. 1996. Belgium. In: Berglund B.E., Birks H.J.B., Ralska-Jasiewiczowa M. and Wright H.E. (eds), Palaeoecological Events During the Last 15,000 Years Regional Syntheses of Palaeoecological Studies of Lakes and Mires in Europe. J. Wiley and Sons Ltd, Chichester, pp. 553–574, 731.

Vighi M. and Chiaudani H. 1985. A simple method to estimate lake phosphorus concentrations resulting from natural background loadings. Water Res. 19: 987–991.

Vinebrooke R.D. and Leavitt P.R. 1999. Differential responses of littoral communities to ultraviolet radiation in an alpine lake. Ecology 80: 223–237.

Vyverman W., Vyverman R., Rajendran V.S. and Tyler P. 1996. Distribution of benthic diatom assemblages in Tasmanian highland lakes and their possible use as indicators of environmental changes. Can. J. Fish. Aquat. Sci. 53: 493–508.

Walker I.R., Levesque A.J., Cwynar L.C. and Lotter A.F. 1997. An expanded surface-water palaeotemperature inference model for use with fossil midges from eastern Canada. J. Paleolimnol. 18: 165–178.

Wetzel R.G. 1975. Limnology. W.B. Saunders Co, Philadelphia, 743 pp.

Wilson S.E., Cumming B.F. and Smol J.P. 1996. Assessing the reliability of salinity inference models from diatom assemblages: an examinatuon of a 219-lake data set from western North America. Can. J. Fish. Aquat. Sci. 53: 1580–1594.