New Jersey Periphyton Bioassessment Development Projects

Trophic Diatom Inference Models and Index Development for New Jersey Wadeable Streams

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Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams

Final Report Year 3

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Main Subject: Diatom-based inference models and trophic indices as tools for monitoring nutrients in New Jersey streams

Abstract. There is an increasing need for effective techniques to meet specific nutrient monitoring and assessment requirements in the United States. This study evaluates the use of diatoms as indicators of nutrient conditions in New Jersey streams and rivers. We assessed the relationship between benthic diatom and water chemistry samples (*n*=101) collected from 45 sites in three New Jersey ecoregions: Northern Piedmont, Northeastern Highlands and Ridge and Valley. Multivariate analysis showed that nutrient concentrations explained significant proportions of the variation in diatom species composition. Diatom assemblages were dominated by pollution tolerant taxa. A weighted-averaging partial least square (WA-PLS) total phosphorus (TP) and a dissolved nitrate + nitrite (NO3-N) inference model (*n*= 91) showed good predictive ability (TP model: $r^2_{apparent}$= 0.87; r^2_{boot}= 0.72; $RMSE_{boot}$= 0.23 log 10 µg L^{-1} TP and NO3-N model: $r^2_{apparent}$= 0.89; r^2_{boot}= 0.51; $RMSE_{boot}$= 0.35 log 10 µg L^{-1} NO3-N). Diatom TP and NO3-N Indices were created by rescaling the inference model results from 0 – 100. They are meant to express response of diatom species composition to nutrient concentrations. This study shows that diatom inference models can be used reliably to assess late-summer river nutrient conditions, based on one-time nutrient measurements taken at the time of algal sampling.

Key words: diatoms, nutrients, inference model, index, periphyton, benthic algae, streams, phosphorus, nitrogen, water-quality criteria, New Jersey, USA.

Introduction

Cultural eutrophication is a major cause of impairment of US surface waters (U.S. EPA 2000a). Addition of nitrogen and phosphorus can increase benthic algal biomass in rivers (Blum 1956, Francoeur 2001), which can cause water quality problems (Dodds and Welch 2000, U.S. EPA 2000b, ENSR 2001). Section 304(a) of the Clean Water Act (FWPCA 2002) directs the U.S. Environmental Protection Agency (EPA) to develop and publish criteria guidance to assist states and authorized tribes in developing water quality standards that are protective of designated uses. The U.S. EPA has formulated a "National Strategy for the Development of Regional Nutrient Criteria" (U.S. EPA 1998) and provided technical guidance (U.S. EPA 2000d) to assist States in adopting criteria (e.g., numeric concentration levels) for nutrients, including nitrogen and phosphorus, as part of enforceable State water quality standards. It has recommended eco-regional nutrient criteria, and has encouraged the States to refine these to better reflect local and regional geophysical conditions and relationships between nutrient levels and biological effects. The research results presented here are part of New Jersey's response to these recommendations.

New Jersey is the most densely populated state with 1,158 persons per square mile (U.S. Census Bureau 2002). Rivers and streams in New Jersey receive high inorganic nutrient loadings coming from a variety of urban, residential and agricultural sources (USGS 2001). These loadings and resulting algal growth can render the waters unsuitable for the states' designated uses, such as potable water supply, recreational use, fisheries and aquatic life (NJ DEP 2001).

The current New Jersey Surface Water Quality Standard for flowing surface water (N.J.A.C. 7:9B-1.14(c)) states that "phosphorus as total P shall not exceed 0.1 (mg/L) in any stream, unless it can be demonstrated that total P is not a limiting nutrient and will not otherwise render the waters unsuitable for the designated uses" (NJ DEP 2001). However, the narrative standard for "rendering waters unsuitable" due to the impact of nutrient inputs on algal biomass and species composition in New Jersey streams and rivers has not been studied sufficiently to develop specific guidelines.

Monitoring of nutrient levels in rivers and streams is problematic because of periodic and diffuse input from non-point sources (Cattaneo and Prairie 1995, Chételat and Pick 2001). New Jersey has monitored river quality since 1976 through an extensive Surface Water Quality Monitoring Network, and since 1997, chemistry parameters are measured 4 times a year at 115 stations statewide (NJ DEP 2000). However, because chemical monitoring does not inform about 'biological health' and provides only 'snapshot' information on river pollution, biological monitoring was introduced. Biological indicators such as benthic macroinvertebrates have been used to monitor river health since 1992 (NJDEP 1993) using the extensive Ambient Biomonitoring Network (AMNET). A fish Index of Biotic Integrity (IBI) has supplemented this since 2000 (NJ DEP 2000). Although a multitude of approaches has been used to monitor general river health, it remains unclear how increased loadings of phosphorus and nitrogen impact the biota in river ecosystems, and how to effectively monitor and regulate those impacts. The State of New Jersey has a need for additional effective techniques to meet specific nutrient monitoring and assessment requirements. The research presented in this paper demonstrates that diatom monitoring can help meet that need.

Diatoms are widely recognized and used as indicators of river and stream water quality, including trophic state conditions (Stevenson and Pan 1999). Benthic diatom species composition responds directly to nutrients (Pan and Lowe 1994, Pan et al. 1996), and can be a more stable indicator of trophic state than measurements of nutrient concentrations or algal biomass (U.S. EPA 2000d). The US EPA's Environmental Monitoring and Assessment Program (EMAP) and the USGS National Water Quality Assessment Program (NAWQA), the two largest U.S. surface water monitoring programs, both use diatom and other algae indicators, as do several state agencies.

The most effective diatom indicators of trophic state incorporate information on the relative abundance of taxa in a sample and measures of the association of those taxa with nutrient concentrations. The basic idea is that if a sample contains a high abundance of taxa generally found in waters with high nutrient concentrations and algal biomass, then the site from which the sample was collected probably has a high trophic state. Many procedures have been developed to quantify these two types of information and

express them as indicators. They range from percentage of taxa in categories such as "eutrophic" to metrics calculated using nutrient response models (Potapova et al. 2004).

Two of the most common and effective approaches for the development of diatom indicators are weighted average indices and inference models. One approach, weighted average indices, is based on assignment of sensitivity and indicator values to each species. Final indices are calculated using Zelinka and Marvan's (1961) weighted averaging equation. Values for most of these indices range from 1 (very low nutrient concentrations) to 5 (very high nutrient concentrations). In Europe, a large variety of such indicators has been developed and are widely used, including several diatom indices for monitoring nutrient enrichment mostly referred to as 'Trophic Diatom Indices' (TDI's) (Descy and Coste 1990, Schiefele and Schreiner 1991, Kelly and Whitton 1995, Coring et al. 1999, Rott et al. 2003). Other commonly applied indices, such as the Biological Diatom Index (IBD) (Prygiel and Coste 1999) and the Specific Polluosensitivity Index (IPS) (Coste 1982) reflect overall impairment conditions, including those caused by organic and inorganic nutrients. In North America, several states are currently developing diatom indices for rivers and streams using similar approaches. Many of these are multimetric indices, or indices of biotic integrity (IBI's), and were developed using a combination of variables reflecting human disturbance, including nutrient pollution (Hill et al. 2000, Fore and Grafe 2002, KDOW 2002).

A second approach for establishing species-environment relationships is the use of 'inference' models. This approach is common in paleolimnological studies to monitor cultural eutrophication in lakes based on diatoms. Several models for reconstruction of TP concentrations have been developed successfully for lakes in North America and Europe (Hall and Smol 1992, Dixit and Smol 1994, Reavie et al. 1995, Bennion et al. 2001, Bradshaw and Anderson 2001). Weighted averaging (WA) regression and calibration techniques are used to calculate species optima and tolerances from modern samples and to then 'infer' past nutrient conditions from fossil diatom species assemblages (ter Braak and Juggins 1993). The optima and tolerances calculated are values of the variable estimated (e.g. TP).

More recently, the same technique is being used to develop diatom inference models for monitoring TP and TN in rivers (Pan et al. 1996, Winter and Duthie 2000,

Soininen and Niemelä 2002). Model performances show a strong response in diatom assemblage composition along nutrient gradients, and demonstrate that WA estimated species optima and tolerances can be used to reliably infer nutrient concentrations. In this study, we wanted to investigate how successfully the inference model approach could be applied to the study area.

The goal of this study was to develop diatom indices that can be used as regulatory tools to effectively monitor nitrogen and phosphorus concentrations and periphyton responses in New Jersey rivers. It was therefore important that the indices a) accurately characterize nutrient concentrations, b) represent the biological response of benthic algae, c) are consistent with NJ nutrient criteria categories, and d) can be used in conjunction with other indicators being used by the state.

To meet this goal, we needed indicators of both nutrient concentrations (e.g., ug/L TP) and response of algal species composition to those concentrations. We chose weighted average inference modeling as our fundamental approach because it incorporates the most accurate method for quantifying species response to nutrients. We used the nutrient concentrations inferred from the models in two ways. One, directly, by using the inferred values as estimates of the nutrient concentrations prevailing at the site during the time the algal assemblages were developing. Two, by rescaling the concentrations from 0 - 100 to create Diatom TP and NO3-N Indices that would represent species composition response to nutrient conditions and related trophic state conditions, and that would be more easily interpretable by non-specialists.

The study approach was to select sites, collect algae and water chemistry samples, prepare diatom samples for analysis, identify and count diatom taxa, use multivariate analysis to understand relationships between assemblage composition and water chemistry, and develop and test diatom inference models using WA regression and calibration techniques. Diatom indices were developed by creating nutrient categories and subsequently assigning the inferred nutrient values, converted to an ordinal scale, to those categories.

Study Sites

This study was conducted in northern and central New Jersey, USA, in the Northern Piedmont, the Northeastern Highlands and the Ridge and Valley ecoregions (Omernik 1995) (Fig. 1). The Northern Piedmont is characterized by irregular plains with low to moderately high hills and tableland (USEPA 2000b, Tedrow 1986). Late Triassic and Early Jurassic age sedimentary rocks, siltstone, shale, sandstone and conglomerate are interlayered with basalt and diabase intrusions (NJDEP 1999). The hilly uplands of the Northeastern Highlands consist mostly of Precambrian gneiss and granite. Paleozoic sedimentary rocks and glacial deposits are also present. In the Ridge and Valley ecoregion, erosion-resistant sedimentary Cambrian and Devonian sandstone alternate with easily eroded shale and limestone. As a result of extreme folding and faulting, and following erosion, parallel ridges and valleys characterize the region. Elevations vary in Northern New Jersey from 150 to 550 m above sea level (Ludlum 1985).

Northern and central New Jersey has a temperate and continental climate (Tedrow 1986, Ludlum 1983). Land-use in the NJ Piedmont is primarily a mix of urban areas and farmland. Most of the state's urban and industrial areas are concentrated in the northeastern, and to a lesser degree, the southwestern portion of the Piedmont. These intensive agricultural, residential and urban land-uses are the sources of the generally high nutrient concentrations in Piedmont rivers (U.S. EPA 2000b). Both the Highlands and the Ridge and Valley ecoregions are more sparsely populated; land-use is predominantly forest and agriculture (U.S. EPA 2000c), the remainder being in residential use (U.S. EPA 2001). Compared to the rivers of the Northern Piedmont, those in the Highlands and the Ridge and Valley receive lower nutrient loadings, mainly from agricultural and a few urban sources (U.S. EPA 2001 and 2000c).

Because a goal of our research was to develop algal indicators of anthropogenic nutrient increases, it was important to select study sites with relatively similar natural environmental conditions (e.g., geology, geomorphology), but with a wide range of nutrient concentrations. We based our selection of sites on chemistry data available from the NJ and USGS monitoring networks. All sites were part of the State's Ambient Surface Water Monitoring Network (AMNET) (NJDEP 2000). We selected sites with a

range of known biological impairments ranging from unimpaired to severely-impaired, based on AMNET macroinvertebrate data collected between 1992 and 1999 (NJDEP 1993a, 1993b, 1993c, 1994, 1998a, 1998b, 1999). All sites selected are 1st to 6th order wadeable rivers and streams.

During the summers of 2000 through 2002 we sampled 45 AMNET sites, 12 in the Highlands, 5 in the Ridge and Valley and 28 in the Piedmont (Fig. 1). Table 1a presents site information including site name and location. Values of the most important environmental variables for each sample collected are shown in Table 1b. The most important physical characteristics, water chemistry and other site information are summarized in Tables 2 and 3.

Methods

Field and laboratory procedures

Samples of epilithic diatoms were collected from August through October of 2000, 2001 and 2002. The sampling area at each site was divided into three sections, so that within-site variability could be assessed. At two sites, only one section could be established, because the flow, geomorphology, substrate type, or other characteristics upstream from the first sampling reach were unsuitable. For each section, we estimated the percent of each substrate type (boulder, cobble, gravel, sand, silt, bedrock) to the nearest percent using habitat assessment protocols adapted from Barbour et al. (1999). We categorized flow velocity as slow, moderate, or fast and measured light conditions (percent open canopy cover) using a spherical densiometer. In addition, one quantitative composite biomass sample was collected from 3 rocks per section for measurement of chlorophyll *a* (chl *a*) and ash-free dry mass (AFDM). Rock surfaces were sampled using the 'top-rock scrape method' (Moulton et al. 2002).

Water chemistry samples were collected by Academy of Natural Sciences of Philadelphia (ANSP) staff at the time of algal sampling. One unfiltered and one filtered sample (250 ml each) were taken for nutrient analysis. Filtering was done using a plastic syringe with an attached filtration device. In addition, one unfiltered (500ml) sample was collected for analysis of major anions and cations. Laboratory analysis of dissolved nitrate+ nitrite (NO_3+NO_2, here referred to as NO_3-N), total Kjeldahl Nitrogen (TKN), dissolved ammonia (NH_3-N), orthophosphate (O-P), total phosphorus (TP), chloride (Cl^-), total alkalinity, total hardness and conductivity was performed by the PCER Geochemistry Section under the direction of Dr. D. Velinsky following USGS methods (Fishman 1993). Chl *a* and AFDM samples were analyzed by the PCER Geochemistry Section using standard methods (APHA, AWWA and WPCF 1992) and US EPA method 445 (U.S. EPA 1992). Additional water chemistry data for water quality monitoring stations were provided by NJDEP and USGS staff. In 2002, ANSP staff measured conductivity and pH in the field using a portable meter (Oakton pH/CON 10 Multiparameter Meter). All other chemistry and land-use data (percent land-use type per

watershed) were provided by the NJ-DEP's AMSN program. We used water chemistry data that was measured closest to the time of algal sampling.

Diatom samples were collected from natural rock substrates using techniques consistent with those used in the USGS NAWQA program (Moulton et al. 2002) and those recommended by the U.S. EPA (Barbour et al. 1999). A composite diatom sample was created by randomly selecting 4-5 rocks of ca. 5 cm diameter from mid-stream. Diatoms were removed from the rocks by scraping and brushing. Diatoms were permanently mounted on microscope slides and counted following USGS NAWQA protocols (Charles et al. 2002). Per slide, 600 valves were identified to lowest taxonomic level and their relative abundance was recorded. For the sites visited in 2000, samples from all three sections at each site were analyzed, resulting in 44 additional 'within-site replicate samples' from sections 2 and 3 at 22 sites. All replicate samples were included in the analysis. In 2001 and 2002, only the sample from the first sampling section was counted. Nine of the sites located in the Piedmont and Highlands ecoregions that had been sampled once in 2000 were revisited during the summers of 2001 and/or 2002 to provide a two- to three-year record of species composition for comparison. These 'repeat' samples were included in the analysis. In total we collected 101 samples from 45 sites.

Numerical analysis

Dataset and data transformations. The complete dataset used in the analyses contained 101 samples including a total of 44 within-site replicate samples and 10 repeat samples. We included the replicate and repeat samples to capture a bigger range of within-site as well as between-year variability. Potapova and Charles (2002) found the benefit of including these kinds of samples to outweigh the negative effect of a possible bias towards the sites where multiple samples were taken. Diatom species were included if their abundance was = 0.5% in at least two samples. All relative abundance data were square-root transformed. The water chemistry and physical parameters included in exploratory ordinations (PCA and DCA) were pH, conductivity, total alkalinity, total hardness, chloride, TP, O-P, TN, NO_3-N, NH_3-N, chl *a*, AFDM, dissolved oxygen (DO), basin size, land-use (% forested, % agriculture, % urban), % open canopy cover, flow

estimate (slow, medium, fast), stream width and percent substrate type. The estimated substrate type was grouped in three categories, each formed by combining the percentages of two or three substrate types: % bedrock and boulder (BRBD), % cobble and gravel (CBGR), % sand silt and clay (SDSTCL). Environmental variables were transformed to reduce skewed distributions: all water chemistry variables, except pH, were log10-transformed; all data expressed in percentages (land-use, substrate and open canopy cover) were square-root transformed.

Ordination analysis. Ordinations were produced using Canoco for Windows version 4.5 (ter Braak, C.J.F., and P. Šmilauer, 2002). Principal components analysis (PCA) was performed to detect major gradients and principal patterns of variation within the environmental variables (ter Braak and Prentice 1998). The environmental variables were centered and standardized. In the same PCA, "outliers" or "rogues" were defined as samples with extreme sample scores on any of the first four axes of the PCA of the environmental data (Birks et al. 1990). Extreme sample scores were defined as scores falling above the 95% confidence interval of all sample score means (Winter and Duthie 2000). Detrended correspondence analysis (DCA) with detrending by segments and down-weighting of rare taxa was used to examine patterns in the diatom data, and to determine the maximum amount of variation within the species composition data (ter Braak, 1995). We used the calculated gradient length of the main DCA ordination axes to determine whether linear or unimodal techniques were to be applied for modeling the relationship between diatoms and environmental variables (ter Braak and Prentice 1988). Furthermore, DCA was used to determine outliers, e.g. samples that showed extreme sample scores on any of the first four axes of the DCA of the species data (Birks et al. 1990). Outliers were screened using the same criteria as applied in PCA. All samples determined as outliers by PCA and DCA were excluded from all subsequent ordinations and from the development of calibration models. A series of canonical correspondence analyses (CCA's) with down-weighting of rare taxa were performed, in order to determine the variables that independently explained a significant amount of variation in diatom species composition (ter Braak 1995). First, an initial CCA was run to determine the strength of the correlations among all 24 environmental variables and all 131 species,

as well as to identify variables that were intercorrelated, based on weighted correlations and variance inflation factors (VIF's). Variables with high variance inflation factors (VIF's >5) indicated strong co-linearity among environmental variables and were removed from all subsequent analyses. The data from the remaining 13 variables and all sites were included in a second CCA analysis with forward selection in order to identify the minimal number of variables that explained the largest amount of variation in the diatom species data. Unrestricted Monte Carlo permutation tests (999 permutations) were used to assess the statistical significance of each forward selected variable ($p < 0.05$). As a last step, to assess the strength of the relationship between diatom species composition and the forward selected variables, we ran CCA constrained to one variable at a time and partial CCA. A high ratio between the first (constrained) eigenvalue and the second (unconstrained) eigenvalue (e.g. $?_1/?_2$) indicated strong influence of these variables on diatom species composition, and justified development of inference models (Bigler and Hall 2002, Winter and Duthie 2000).

Species optima and tolerances and inference models. Weighted averaging (WA) regression and calibration techniques were used to calculate diatom species optima and tolerances as well as to develop and test diatom inference models for TP and NO3-N. These were run using the program C^2, version 1.3 (Juggins 2003). Several models were developed, based on WA inverse and classical deshrinking (Birks et al. 1990) and on weighted-averaging partial least square regressions (WA-PLS) (ter Braak and Juggins 1993). Model error estimation was performed by bootstrapping with 1000 cycles (Birks 1995). We selected the best inference model based on a combination of the following characteristics: a) the highest prediction accuracy e.g. the lowest root mean square error of prediction (RMSEP) based on cross-validation; b) the highest coefficient of determination (r^2) between observed and inferred values and c) the minimum number of WA-PLS components (Birks 1995).

Index development. We created Diatom Trophic State Indices for TP and NO3-N based on values inferred for each diatom sample using the two- and three-component WA-PLS models. The inferred nutrient values (log 10) obtained for each sample were converted to

a scale from 0-100 to provide a continuous measure of biological response to TP and NO3-N. In addition to being indicators of response of species composition, they also serve as general indicators of trophic state conditions potentially supported by the inferred nutrient concentrations (e.g., algal biomass, dissolved oxygen fluctuations). Following U.S. EPA guidelines (Barbour et al. 1999), the 95th percentile of all inferred nutrient values (log 10) was given the score 100.

We divided the 0 – 100 scale into trophic state categories ranging from 1 (low) to 4 (very high) with respect to the range of values in the calibration dataset. The category boundaries are those suggested by Wetzel (2001) for trophic classification of temperate streams, based on the biomass-nutrient relationships established in Dodds et al. 1998. We set the boundaries between low and moderate nutrient concentrations (i.e. diatom index categories 1 and 2) at 0.025 mg L^{-1} TP and at 0.7 mg L^{-1} NO3-N and between moderate and high concentrations (e.g. diatom index categories 2 and 3) at 0.075 mg L^{-1}TP and at 1.5 mg L^{-1} NO3-N. Finally we used the state nutrient criteria of 0.1 mg L^{-1} TP to determine the boundary between high and very high nutrient levels (e.g. diatom TP index categories 3 and 4). Because the NO3-N gradient was shorter, we established only three categories for the NO3-N diatom index, from 1 (low) to 3 (high). The final TP categories established were 1) less than 0.025, 2) 0.025- 0.075, 3) 0.075- 0.1 and 4) above 0.1 mg L^{-1} TP. These correspond to the following $\log_{10}$ TP values: 1) less than 1.4, 2) 1.4- 1.9, 3) 1.9- 2.0 and 4) above 2.0 and to the index scores of 1) 0-60, 2) 60-81, 3) 81-86 and 4) above 86. The final NO3-N categories established were 1) less than 0.7, 2) 0.7-1.5 and 3) above 1.5 mg L^{-1} NO3-N. These correspond to the following $\log_{10}$ NO3-N values: 1) less than 2.8, 2) 2.8-3.2 and 3) above 3.2 and to the index scores of 1) less than 81, 2) 81- 91 and 3) above 91.

Results

Diatom species composition

A total of 399 diatom species were identified; pollution-tolerant taxa dominated. The 10 most abundant species, determined by high abundances and high numbers of occurrences, had high TP and TN WA-optima (Table 4). These taxa were *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot, *Navicula minima* Grunow, *Nitzschia amphibia* Grunow, *Navicula gregaria* Donkin, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Nitzschia inconspicua* Grunow, *Sellaphora seminulum* (Grunow) Mann, *Gomphonema parvulum* (Kützing) Kützing, *Nitzschia palea* (Kützing) W. Smith and *Melosira varians* Agardh. After applying our selection criteria (= 0.5% abundance in = 2 samples), 131 taxa were included in the analyses.

Environmental gradients and species distribution

PCA detected two major gradients in the environmental data (Fig. 2). The first PCA axis (eigenvalue $?_1$= 0.31) reflected a chemical gradient driven mainly by all inorganic nutrients measured, % urban land-use, conductivity and chloride. The second axis (eigenvalue $?_2$= 0.17) revealed a physical gradient strongly influenced by average river width and basin size, % open canopy cover, and % sand, silt and clay substrates. The first two PCA axes explained 47.3% of the cumulative percentage of variance in the environmental variables. Compared with sites in other ecoregions, those in the Northern Piedmont generally had higher nutrient concentrations, higher % urban land-use, high conductivity and chloride, and a higher proportion of sand, silt and clay substrate (Fig. 2). In contrast, sites located in the Highlands and the Ridge and Valley generally had lower nutrients, lower conductivity and chloride, but higher % forest land-use, higher dissolved oxygen and a higher proportion of larger-sized substrate (BRBD) (Fig. 2). The largest pH gradient was found within the Northern Piedmont sites, ranging from the lowest (6.5) to the highest (9.0) values in the dataset. In the DCA of diatom data, the eigenvalues of the first two axes were $?_1$= 0.3 and $?_2$= 0.17, accounting for 20.1% of the variance in the

species data. The gradient length of the first two DCA axes of 2.7 and 1.8 standard deviation (SD) units indicate that either linear or unimodal species response models would be effective (ter Braak and Prentice 1988). As determined by two DCA's, with TP and NO3-N as the only external predictors, respectively, the gradient lengths for the first two axes were above 2 SD in both analyses. We therefore chose to use techniques based on assumptions of unimodal species distribution (ter Braak 1995).

Data screening

Ten samples were identified as outliers, based on PCA and DCA, and were removed from CCA analysis and inference model development. Five of these samples were from three sites, and were determined to be outliers because of their high sample score means on the PCA axes: samples 31, 32 and 33 (site 291, sections 1-3), sample 75 (site 231, section 1), and sample 71 (site 192, section 1) (Fig. 2). All three sites had high conductivity values (983-595 µS cm^{-1}) and a high percentage of sand, silt and clay sediments (Table 1b). The nutrient values for sites 291 and 231 were high (TP: 0.73 and 0.69 mg L^{-1}; TN: 6.1 and 5.4 mg L^{-1}). Although it was important to keep samples with high nutrient values in the dataset, the extreme values of conductivity were considered to be a potential problem for the development of nutrient inference models. Eight samples from 6 sites were identified as outliers because of their high sample score means on the DCA axes: sample 83 (site 006, section 1), sample 96 (site 318, section 1), 86 (site 29, section 1), samples 6–8 and 70 (site 115, sections 1-3, section 1 sampled in 2000 and 2001) and sample 89 (site 245, section 1) (Fig. 3A). Because all sites had low-nutrient concentrations, and it was important that sites in the calibration dataset have a wide nutrient gradient, we decided to exclude only samples from sites 115 and 245 since they were the two most acidic sites with the lowest pH's (6.5 and 6.7) in the dataset (Table 1b). In addition, site 115 had a high % of sand substrate. Despite the fact that both sites represented the low end of the phosphorus gradient, we decided to exclude them from further model development to avoid the influence of a strong pH and alkalinity gradient. In the following, species numbers in parentheses correspond to numbers used in Figure 3B. Diatom species names and numbers for the most common species are also listed in

Table 4. Site 115 had high abundances of *Eunotia exigua* Rabenhorst (29), *Psammothidium subatomoides* Bukhtiyarova et Round (117), *Gomphonema patrickii* Kociolek & Stoermer (44), *Eunotia pectinalis* var. *minor* (Kützing) Rabenhorst (31) and *Eunotia implicata* Nörpel, Lange-Bertalot et Alles (32). At site 245 *Cocconeis placentula* var. *euglypta* Cleve (18) was most abundant, accompanied by several acidophilous *Eunotia*, *Fragilaria* and *Gomphonema* species (Fig. 3B). Removing the 10 outlier samples reduced the dataset from 101 samples to 91.

Relationships between diatom assemblages and environmental variables

CCA was used to determine the environmental variables explaining most of the variation in diatom species composition, especially the importance of nutrients as compared with other environmental characteristics. In a CCA including all 24 environmental variables and diatom data for 91 sites, 18.4% of the total variance of the diatom data was explained by CCA axis 1 ($?_1 = 0.23$) and CCA axis 2 ($?_2 = 0.16$). The two CCA axes showed high species-environment correlations for axis 1 ($r = 0.93$) and for axis 2 ($r = 0.94$), accounting for 36.3% of the variance in the species-environment relationships. This indicates a strong correlation between the 24 environmental variables and the 131 species included in the complete CCA. Nevertheless, strong weighted correlations and high variance inflation factors (VIF's >5) indicated strong co-linearity among several environmental variables. Consequently, we omitted the following 11 variables from further analysis: TN, NH3-N, O-P, average river width, % urban land-use, % open canopy cover, % cobble and gravel, AFDM, hardness and conductivity. CCA with forward selection that excluded these variables, and included only 13 variables in total, identified 5 environmental variables that significantly ($p<0.05$) explained the variance in diatom species composition. These were TP, basin size, % forested land-use, NO3-N and % bedrock and boulder. These results are consistent with Kennen and Ayers (2002) study of New Jersey streams. The five variables together explained 14.7% of the total variance in the diatom data. Species-environment correlations of CCA axes were high for axis 1 ($r = 0.88$) and for axis 2 ($r = 0.85$) and accounted for 14% of the variance in the species data and for 69.3% of the variance in the species-environment

relationships. In comparison with the CCA that included all 23 variables, species-environment correlations on the first two axes were only slightly lower, but explained most of the variance in the species-environment relationship.

CCA's constrained to one variable at a time were run on all remaining five variables to assess the strength of their relationship with diatom species composition, and to determine which had a strong enough relationship with diatom assemblages to justify development of inference models. Of the five variables, TP had the highest $?_1/?_2$ ratio (0.78), capturing 7.2% of the variation in the species data. All other variables (% basin size, % forest land-use, NO3-N and % bedrock and boulder) showed $?_1/?_2$ ratios below 0.6 and explained variances below 6.1% (Table 5). Because we were particularly interested in modeling nutrient variables, we needed to test if TP and NO3-N had independent influences on diatom species composition. Partial CCA's constrained to TP or NO3-N, with each of the four significant variables entered as covariables respectively, did not significantly decrease the ratio $?_1/?_2$ or the % variance explained by the first (constrained) variable (Table 5). This confirmed that both variables had significant and independent influence on the diatom assemblages and that development of inference models for both variables was justified.

Species WA-optima and tolerances

TP and NO3-N WA species optima were calculated using the reduced dataset (*n*=91). The results are presented for the species with effective numbers of occurrences (Hill's N2) > 20 (Table 4). Species apparent WA TP optima ranged from 13 µg L^{-1} for *Achanthes deflexa* Reimer to 163 µg L^{-1} for *Luticula goeppertiana* (Bleisch) Mann. Weighted-average NO3-N optima ranged from 102 µg L^{-1} for *Navicula reichardtiana* Lange-Bertalot to 3446 µg L^{-1} for *Nitzschia coarctata* Grunow. Taxa with high and low TP optima had values comparable to those calculated in other studies (Potapova et al. 2004; Winter and Duthie 2000). Species with high TP optima were *Navicula recens* Lange-Bertalot, *Gomphonema kobayasii* Kociolek and Kingston, *Nitzschia capitellata* Hustedt and *Nitzschia inconspicua* Grunow. Taxa with low TP optima were *Achnanthidium rivulare* Potapova and Ponader, a species reported as *Achnanthidium* sp.

10 NAWQA in Potapova et al. 2004, *Encyonema minutum* (Hilse) Mann, *Navicula cryptocephala* Kützing and *Navicula cryptotenella* Lange-Bertalot in Krammer & Lange-Bertalot.

Inference models

Models to infer TP and NO3-N ($\log_{10}$ µg L^{-1}) were developed and tested using WA and WA-PLS. The models were run on the full (*n*=101) and the reduced (*n*=91) calibration dataset, which included 131 taxa. Table 6 shows performance measures for all inference models. The best TP model was a two-component WA-PLS model using the reduced dataset (*n*=91), showing the highest r^2_{boot} (0.72) and the lowest $RMSE_{boot}$ (0.23). For NO3-N, a three-component model using the reduced dataset (*n*=91) showed best performance, with an r^2_{boot} of 0.51 and an $RMSE_{boot}$ of 0.35. For both variables and both modeling techniques, the reduced dataset always gave better results than when all samples were included in the model. In the reduced-model dataset, the TP gradient was 6-458 µg L^{-1}(as compared to 6-732 µg L^{-1} in the full dataset - see Table 1b) and the NO3-N range was 10-7992 µg L^{-1} for both the full and the reduced dataset.

When comparing the WA-PLS models (*n*=91), the difference between apparent and bootstrapped r^2 was less (0.87 - 0.72) for TP than for NO3-N (0.89 - 0.51) (Figs 4A and 5A). The residuals plot (Fig. 4B) shows that the TP model using WA-PLS (*n*=91) tends to underestimate TP values at above > 100 µg L^{-1}. This trend commonly occurs with models developed on samples with an uneven distribution along the TP gradient (Soininen and Niemela 2002, Reavie et al. 1995). It is often referred to as “edge-effect” (Hall and Smol 1999), and causes the WA estimates of species optima to be biased. Despite the rather wide range of TP included in our dataset, the majority of samples fall below concentrations of 100 µg L^{-1} TP. This might explain why the model is less reliable at concentrations of > 100 µg L^{-1}. Another explanation may be that above higher concentrations of TP (e.g. concentrations of > 100 µg L^{-1}) only subtle changes occur in the diatom species composition and that therefore the species response to higher TP concentrations is weaker (Reavie et al. 1995, Pan et al. 1996). A similar trend is shown in the NO3-N model using WA-PLS (*n*=91), where the difference between bootstrapped

versus inferred NO3-N values is higher above 1000 µg L^{-1} NO3-N (Figs 5A and 5B). Generally, both graphs show that the NO3-N data have a highly uneven distribution along the nitrogen gradient. The average TN in the Piedmont (2241 µg L^{-1}) and the combined dataset (1987 µg L^{-1}) (Table 2) is low compared to the average TN (4940 µg L^{-1}) in the EPA Northern Piedmont aggregate nutrient ecoregion (USEPA 2000b), or compared to similar studies where nitrogen inference models have been developed (Winter and Duthie 2000). The model therefore is built mainly on sites with moderate nitrogen concentrations, and underrepresents sites with low and high nitrogen. This explains the decrease of the model performance, especially at the higher end of the gradient.

Diatom TP and NO3-N Indices

Because they are simply rescaled values of inferred nutrient concentrations, the TP and NO3-N diatom indices correspond directly with those concentrations (Figs 4A and 5A). All measures of the performance of inference models are also applicable to the relationships between the indices and measured nutrient concentrations. The index values might also be compared with other trophic state characteristics such as algal biomass or dissolved oxygen fluctuations, but there are insufficient data for this analysis to be meaningful.

The diatom indices were able to separate samples into nutrient categories reasonably well (Fig 6A and 6B), but not without error. To provide a measure of the error, we evaluated how accurately the index values based on bootstrapped inferred nutrient concentrations (Fig 4A and 5A) could be assigned to the nutrient categories. The Diatom TP Trophic State Index correctly assigned the majority of the samples. Categories 1, 3 and 4 had the highest proportion of samples correctly assigned, whereas category 2 had the highest proportion of samples assigned to a neighboring category. The Diatom NO3-N Trophic State Index correctly assigned the majority of the samples to category 1 and 3, but placed a high proportion of samples belonging to category 2 into neighboring categories. The indices showed least accuracy placing samples in the diatom inferred TP category 2 (0.025- 0.075 mg L^{-1}) and the diatom inferred NO3-N category 2 (0.7-1.5 mg L^{-1}), which are the categories with the highest number of samples.

Discussion

Diatoms as nutrient indicators- inference model performance

The diatom-based TP and NO3-N inference models developed for the full dataset performed well and were further improved by removing outliers. When compared to similar studies conducted to develop benthic diatom nutrient indicators for streams in eastern North America (Pan et al. 1996, Winter and Duthie 2000, Potapova et al. 2004) using one-time chemistry measurements, the TP and NO3-N WA-PLS model presented here (*n*=101 and *n*=91) perform better, with higher apparent and bootstrapped r^2 and lower apparent and bootstrapped RMSE. Differences in model performances between our study and previous studies may be explained by several factors.

First, site selection was limited to three ecoregions in NJ with a rather limited range in water chemistry and geology. Development of nutrient models is known to be difficult for datasets where pH is a variable strongly influencing diatom species composition (Reavie and Smol, 2001). Sites in this study were selected based on knowledge of their chemistry to avoid wide ranges of pH, alkalinity and conductivity. The pH gradient included in our study was relatively short, 6.5-9 (*n*=101) and 6.9-9 (*n*=91), and excluded acidic sites, comparable to the range included in the Winter and Duthie (2000) dataset (pH 7.1-8.7). In contrast, a large pH range of 4.2-8.6 (Pan et al. 1996) and of 5.7-8.6 (Potapova and al. 2004) and the influence of related variables (e.g. alkalinity, hardness, conductivity etc.) could be responsible for a weaker performance of their TP models. Our results suggest that strong model performance can be obtained for nutrient models only if large gradients in pH are avoided during site selection. Therefore, to ensure the reliability of inferred nutrient variables, the TP and NO3-N models presented here should only be applied to sites that fall in the pH range of 6.5-9 for the models based on *n*=101 samples, and pH of 6.9-9, for the models based on *n*=91 sites).

Second, we tried to capture a wide range of concentrations along the nutrient gradients. Our dataset included a TP range of 6-458 µg L^{-1} (*n*= 91) and 6-732 µg L^{-1} (*n*=101), and the range in NO3-N was 10-7992 µg L^{-1} for both, the full and the reduced

dataset. The nutrient gradients included in all models (developed on the full and reduced dataset) were relatively large, and the model error is lower when compared to other studies based on comparable or shorter nutrient gradients. The TP models by Pan et al. (1996) and Potapova et al. (2004) had higher RMSE's than our TP models, although they included TP gradients of 3-472 µg L^{-1} and 6-761 µg L^{-1} respectively, which are comparable to the TP ranges included in our reduced and full datasets. The Winter and Duthie (2000) dataset included a shorter TP gradient (5-215 µg L^{-1}) and a shorter NO3-N gradient (300-6900 µg L^{-1}), but higher RMSE's when using one-time chemistry measurements.

Finally, water samples for nutrient analysis were taken at the same time diatom samples were collected. This ensures that diatom-inferred WA- optima and tolerances accurately reflect water chemistry conditions at the time of sampling.

There are a multitude of other factors that might be responsible for differences in model performances between our models and previous studies. These include the influence of temporal variability in nutrient concentrations in streams (Cattaneo and Prairie 1994, Pan et al. 1997) and the indirect influence of nutrients on diatom species composition through increasing competition with soft-algae species (e.g. *Cladophora* sp.) (Winter and Duthie, 2000). So far, only a few TP diatom inference models have been developed for rivers in the eastern US, and future studies are necessary to help understand the influence of these factors on diatom species composition in relationship with nutrients.

Despite the wide ranges in TP and NO3-N captured in our models, model performance could certainly be improved by adding samples to the dataset. For example, having fewer samples at the low and the extremely high total phosphorus range makes the TP model performance less reliable towards the ends of both gradients. Addition of more samples toward the ends of the gradients might help improve the TP model performance, although significant improvement is expected to be more probable at the lower nutrient range of the model: our results show that species assemblages respond less strongly to TP values over 100 µg L^{-1}. Improvement of the model at the lower end of the TP gradient would be useful especially with regard to management purposes. To improve the NO3-N model performance, it would be important to add samples to both ends, but especially to

the higher end of the nitrogen gradient. The current model includes mostly samples with moderate nitrogen concentrations and only captures a range up to a maximum NO3-N concentration of 7792 µg L^{-1}. This does not reflect the full range of NO3-N concentrations of 225-8362 µg L^{-1} (USEPA 2000b) found in the EPA Northern Piedmont nutrient ecoregion, where the majority of our samples were taken.

In general, our TP and NO3-N inference models have high predictive power and showed reliable results (e.g. inferred nutrient concentrations) when tested using bootstrapping, and especially when compared to existing nutrient models. When applying the models to samples in any of the three ecoregions of NJ, it is important to select sites with water chemistry values that are within the ranges included in the model calibration set, especially for pH, conductivity, hardness and alkalinity (Table 1b).

Use of diatom inference model and trophic indices for management purposes

Our results show that diatom WA-PLS models can reliably and accurately infer late summer nutrient concentrations in NJ rivers. We recommend applying the inference models when accurate estimates of diatom inferred TP and NO3-N are needed. The good performance of the models presented here confirms the conclusion of another study conducted in the Northern Piedmont ecoregion, that models based on WA-equation can be used reliably to model regional diatom-nutrient responses (Potapova et al. 2004).

The Diatom TP and NO3-N indices, which are based on inference model relationships between nutrients and diatom assemblage composition, are meant to represent response of diatom species composition to nutrient concentrations. In addition to being biological response indicators, these indices have the practical advantage of being more comparable than inferred nutrient values to indices commonly used by the state, e.g. the Fish IBI (Karr 1981), the Macroinvertebrate index and the Habitat Assessment Index (NJ DEP 1993, NJ DEP 2000). Nevertheless it is important to keep in mind that although they are presented in the same way, the Diatom TP and N03-N indices are calculated differently than IBI's. Common IBI's are a combination of metric scores and therefore represent a multitude of variables, whereas the diatom indices presented here reflect only one variable (e.g. TP or NO3-N). In consequence, they are more similar

to commonly used diatom indices, such as Trophic Diatom Indices (TDI's), with the main difference that our indices are based on WA-PLS inference models. When tested, the Diatom TP and NO3-N Indices assigned the samples to the correct water chemistry category in the majority of the cases, for both TP and NO3-N. The boundaries between the categories assigned are based on correlations between nutrients and high algal biomass as established by Dodds et al. 1998, and as suggested by Wetzel (2001) for trophic classification of temperate streams. Therefore, the categories reflect not only nutrient concentrations but also their potential impact on algal biomass.

In addition to the categories, there are other useful reference points for evaluating the inferred nutrient concentrations, and that could serve as a basis for alternative category boundaries. Several studies conducted on large and small scale datasets on streams in the US, including high proportions of eastern US streams, confirm that levels of TP above 0.05 mg L^{-1} and TN above 0.47 mg L^{-1} lead to mean benthic chl *a* values of >50 mg m^{-2} (Dodds and Welch 2000, Dodds et al. 2002). The results of three years of this study showed that TP values above 0.05 mg L^{-1} and NO3-N values above 0.2 mg L^{-1} can lead to nuisance algal biomass, defined here as measured periphyton chl *a* >100 mg m^{-2} (Ponader and Charles 2003). The establishment of category boundaries could be refined through more extensive exploration of relationships between nutrients and biomass.
We believe that application of the index scores assigned to categories, as presented, is a useful applied method for the purpose of routine monitoring. Both methods, the inference models as well as the two trophic indices, the Diatom TP and NO3-N Indices, present reliable and useful tools for monitoring nutrients and can be applied in a regulatory context. Further improvement of the inference models could be achieved by adding more samples to the model calibration dataset, especially at the lower and the higher ends of the nutrient gradients.

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Table 1a. Site names and location. NP: Northern Piedmont, HL: Highlands, RV: Ridge and Valley, Lat: Latitude (decimal), Long: Longitude (decimal).

Site ID	Ecoregion	River Name	Lat	Long	year(s) sampled
AN0318	HL	Spruce Run	40.72484444	-74.90944278	2000, 2002
AN0321	HL	Mulhockaway Creek	40.64746917	-74.96879917	2000, 2002
AN0299	HL	Wallkill River	41.13339167	-74.57879028	2002
AN0255	HL	Wanaque River	41.16354667	-74.31665639	2002
AN0259	HL	Pequannock River	41.07805611	-74.48907	2002
AN0265	HL	Pequannock River	41.00343639	-74.33517111	2002
AN0213	HL	Passaic River	40.77178306	-74.56989139	2002
AN0245	HL	Beaver Bk	40.94695944	-74.46031306	2002
AN0313	HL	Stony Bk	40.80512333	-74.75083556	2002
AN0315	HL	S Br Raritan River	40.78501417	-74.78001889	2002
AN0346	HL	N Br Raritan River	40.77123083	-74.62578694	2002
AN0115	NP	Miry Run	40.24709611	-74.687155	2000, 2001
AN0396	NP	Heathcote Brook	40.36998667	-74.61582722	2000
AN0339	NP	Pleasant Run	40.56192806	-74.79364389	2000
AN0370	NP	Lamington River	40.63466778	-74.68672139	2000
AN0405	NP	Pike Run	40.42011333	-74.64046194	2000, 2001
AN0374	NP	N Br Raritan River	40.56972278	-74.6781925	2000, 2001, 2002
AN0341	NP	S Br Raritan River	40.54684972	-74.69648556	2000
AN0413	NP	Royce Brook	40.53704528	-74.58963	2000
AN0081	NP	Nishisakawick Creek	40.54896528	-75.03443139	2000
AN0326	NP	S Br Raritan River	40.57242111	-74.86786389	2000
AN0215	NP	Primrose Brook	40.76823694	-74.53400139	2000, 2002
AN0424	NP	Bound Brook	40.58069361	-74.49928167	2000
AN0429	NP	Mile Run	40.50556722	-74.46724194	2000
AN0195	NP	Rahway River	40.61817861	-74.27835444	2000
AN0281	NP	Saddle River	41.03191861	-74.10015778	2000
AN0111	NP	Shipetaukin Creek	40.29611194	-74.70484806	2000
AN0267	NP	Ramapo River	41.03576167	-74.23739944	2000
AN0234	NP	Whippany River	40.80136556	-74.46628333	2000, 2001, 2002
AN0211	NP	Coles Brook	40.91110611	-74.03999472	2000, 2001
AN0291	NP	Saddle River	40.86408806	-74.10156417	2000
AN0274	NP	Passaic River	40.88747556	-74.22373556	2000
AN0118	NP	Assunpink Creek	40.21720528	-74.76637194	2000
AN0333	NP	Neshanic River	40.47343333	-74.82763417	2001
AN0235	NP	Whippany River	40.81954639	-74.44017	2001
AN0192	NP	Rahway River	40.76972833	-74.28322361	2001
AN0231	NP	Passaic River	40.82762667	-74.33508056	2001
AN0237	NP	Troy Brook	40.854405	-74.38987889	2001
AN0207	NP	Pascack Bk	40.99288417	-74.02121583	2001
AN0209	NP	Tenakill Brook	40.97850861	-73.96730833	2001
AN0016	RV	UNT to Paulins Kill	41.06688278	-74.96218194	2002
AN0006	RV	Big Flat Bk	41.200015	-74.81550889	2002
AN0008	RV	Flat Bk	41.10155722	-74.81550889	2002
AN0029	RV	Jacksonburg Ck	40.98741222	-74.6877675	2002
AN0032A	RV	Paulins Kill	40.96679167	-74.97866056	2002

Table 1b. Values of selected environmental variables for each sample collected during 2000-2002 ordered by Sample numbers (#) used in ordinations and corresponding Sanple ID's. NO3-N: dissolved nitrate, TN: Total Nitrogen, O-P: orthophosphate, TP: total phosphorus, Chl a: Chlorophyll a, AFDM, Ash free dry mass, Alk: total alkalinity, DO: dissolved oxygen, Cond: Conductivity, Cl⁻: chloride, BASIN: basin size, URB: %urban land-use, AG: %agriculture land-use, FOR: %forest land-use, Open: % open canopy cover, BRBD: % bedrock and boulder, CBGR: % cobble and gravel, SDSTCL: % sand silt and clay, AvgW: average stream width.

#	Sample ID	Section	Year	NO3-N (mg L^{-1})	TN (mg L^{-1})	O-P (µg L^{-1})	TP (µg L^{-1})	Chl a (mg m^{-2})	AFDM (g m^{-2})	Alk (mg L^{-1})	DO (mg L^{-1})	pH	Cond (µS cm^{-1})	BASIN (km^2)	URB (%)	AG (%)	FOR (%)	Open (%)	SDSTCL (%)	BRBD (%)	CBCR (%)	AvgW (m)
1	AN081	1	2000	1.58	2.1	41.0	51.0	153	16	40	8.2	7.6	144	25	16	56	21	100	10	17	73	8.0
2	AN081	2	2000	1.58	2.1	41.0	51.0	83	9	40	8.2	7.6	144	25	16	56	21	62	12	35	67	8.0
3	AN081	3	2000	1.58	2.1	41.0	51.0	37	5	40	8.2	7.6	144	25	16	56	21	12	8	33	38	8.0
4	AN111	1	2000	1.74	2.0	40.0	71.0	73	17	37	6.0	7.6	298	16	39	33	13	17	30	5	65	7.0
5	AN111	2	2000	1.74	2.0	40.0	71.0	64	13	37	6.0	7.6	298	16	39	33	13	6	50	5	45	7.0
6	AN115	1	2000	1.15	1.9	4.0	25.5	98	79	20	5.0	6.9	93	28	49	27	3	24	80	0	20	4.0
7	AN115	2	2000	1.15	1.9	4.0	25.5	31	11	20	5.0	6.9	93	28	49	27	3	31	73	0	27	4.0
8	AN115	3	2000	1.15	1.9	4.0	25.5	33	32	20	5.0	6.9	93	28	49	27	3	26	85	0	15	4.0
9	AN118	1	2000	2.49	3.2	268.5	322.5	1115	153	48	7.5	7.5	292	237	45	25	10	82	4	51	47	20.0
10	AN118	2	2000	2.49	3.2	268.5	322.5	64	12	48	7.5	7.5	292	237	45	25	10	86	1	22	78	20.0
11	AN118	3	2000	2.49	3.2	268.5	322.5	14	8	48	7.5	7.5	292	237	45	25	10	23	0	5	95	20.0
12	AN195	1	2000	0.97	1.4	41.0	75.0	129	23	87	6.0	7.6	407	108	79	0	16	22	10	0	90	11.5
13	AN195	2	2000	0.97	1.4	41.0	75.0	229	33	87	6.0	7.6	407	108	79	0	16	26	10	0	90	11.5
14	AN195	3	2000	0.97	1.4	41.0	75.0	152	23	87	6.0	7.6	407	108	79	0	16	77	5	0	95	11.5
15	AN211	1	2000	1.10	1.4	19.5	50.5	68	19	50	7.1	7.9	658	16	92	0	5	5	30	10	60	5.0
16	AN211	2	2000	1.10	1.4	19.5	50.5	75	22	50	7.1	7.9	658	16	92	0	5	17	60	10	30	5.0
17	AN211	3	2000	1.10	1.4	19.5	50.5	81	17	50	7.1	7.9	658	16	92	0	5	15	40	5	55	5.0
18	AN215	1	2000	0.27	0.4	8.0	20.0	37	6	45	8.3	7.0	143	1	6	0	93	53	50	5	45	3.0
19	AN215	2	2000	0.27	0.4	8.0	20.0	3	5	45	8.3	7.0	143	1	6	0	93	3	45	5	50	3.0
20	AN215	3	2000	0.27	0.4	8.0	20.0	7	6	45	8.3	7.0	143	1	6	0	93	5	75	5	20	3.0
21	AN234	1	2000	1.97	2.6	115.5	153.0	179	18	55	2.8	6.9	209	73	48	3	43	11	5	5	90	9.0
22	AN234	2	2000	1.97	2.6	115.5	153.0	48	8	55	2.8	6.9	209	73	48	3	43	10	15	15	70	9.0

23	AN234	3	2000	1.97	2.6	115.5	153.0	87	9	55	2.8	6.9	209	73	48	3	43	22	5	15	80	9.0
24	AN267	1	2000	1.24	1.5	88.0	110.0	82	11	56	8.0	7.6	303	357	19	2	73	80	15	23	62	34.0
25	AN267	2	2000	1.24	1.5	88.0	110.0	81	9	56	8.0	7.6	303	357	19	2	73	88	19	17	64	34.0
26	AN267	3	2000	1.24	1.5	88.0	110.0	45	6	56	8.0	7.6	303	357	19	2	73	82	23	5	72	34.0
27	AN274	1	2000	3.09	3.6	450.0	510.0	111	22	70	6.3	8.0	595	1962	34	2	49	64	0	33	67	45.0
28	AN281	1	2000	1.92	2.2	11.0	32.5	25	10	108	9.3	7.8	493	40	82	1	14	5	32	7	62	4.5
29	AN281	2	2000	1.92	2.2	11.0	32.5	29	4	108	9.3	7.8	493	40	82	1	14	14	38	10	52	4.5
30	AN281	3	2000	1.92	2.2	11.0	32.5	202	19	108	9.3	7.8	493	40	82	1	14	79	32	5	67	4.5
31	AN291	1	2000	5.52	6.1	662.5	730.5	593	74	160	5.2	7.5	595	158	82	1	11	65	5	20	75	16.5
32	AN291	2	2000	5.52	6.1	662.5	730.5	263	45	160	5.2	7.5	595	158	82	1	11	78	65	0	35	16.5
33	AN291	3	2000	5.52	6.1	662.5	730.5	523	45	160	5.2	7.5	595	158	82	1	11	74	53	2	45	16.5
34	AN318	1	2000	0.34	0.4	6.0	11.0	70	10	46	9.3	7.6	161	15	14	19	51	17	0	72	28	8.0
35	AN318	2	2000	0.34	0.4	6.0	11.0	78	12	46	9.3	7.6	161	15	14	19	51	20	0	70	30	8.0
36	AN318	3	2000	0.34	0.4	6.0	11.0	80	9	46	9.3	7.6	161	15	14	19	51	38	0	50	50	8.0
37	AN321	1	2000	0.82	1.0	15.0	34.0	127	17	86	9.0	7.8	244	31	24	21	43	41	30	13	57	8.5
38	AN321	2	2000	0.82	1.0	15.0	34.0	132	16	86	9.0	7.8	244	31	24	21	43	77	27	7	67	8.5
39	AN321	3	2000	0.82	1.0	15.0	34.0	47	9	86	9.0	7.8	244	31	24	21	43	16	38	3	60	8.5
40	AN326	1	2000	1.31	1.7	22.5	42.0	200	25	64	9.8	8.2	269	389	25	22	41	93	15	15	70	27.0
41	AN326	2	2000	1.31	1.7	22.5	42.0	178	21	64	9.8	8.2	269	389	25	22	41	87	15	15	70	27.0
42	AN326	3	2000	1.31	1.7	22.5	42.0	135	18	64	9.8	8.2	269	389	25	22	41	84	15	15	70	27.0
43	AN339	1	2000	1.10	1.1	60.0	68.5	145	29	66	8.2	7.5	210	10	32	30	33	29	5	7	88	5.0
44	AN339	2	2000	1.10	1.1	60.0	68.5	51	12	66	8.2	7.5	210	10	32	30	33	11	22	5	73	5.0
45	AN339	3	2000	1.10	1.1	60.0	68.5	66	9	66	8.2	7.5	210	10	32	30	33	29	17	0	83	5.0
46	AN341	1	2000	1.28	1.7	79.0	103.0	60	9	64	9.8	8.2	269	692	26	30	33	100	8	2	90	25.0
47	AN341	2	2000	1.28	1.7	79.0	103.0	141	20	64	9.8	8.2	269	692	26	30	33	100	25	0	75	25.0
48	AN341	3	2000	1.28	1.7	79.0	103.0	112	15	64	9.8	8.2	269	692	26	30	33	100	35	8	57	25.0
49	AN370	1	2000	1.05	1.4	64.0	82.0	117	31	64	8.4	7.2	255	257	24	24	43	100	27	0	73	20.0
50	AN370	2	2000	1.05	1.4	64.0	82.0	288	72	64	8.4	7.2	255	257	24	24	43	100	26	0	74	20.0
51	AN370	3	2000	1.05	1.4	64.0	82.0	129	20	64	8.4	7.2	255	257	24	24	43	100	25	0	75	20.0
52	AN374	1	2000	0.52	1.0	12.5	32.5	153	7	44	8.8	7.7	251	486	29	22	42	100	30	17	53	35.0
53	AN374	2	2000	0.52	1.0	12.5	32.5	214	29	44	8.8	7.7	251	486	29	22	42	100	20	2	78	35.0
54	AN374	3	2000	0.52	1.0	12.5	32.5	325	48	44	8.8	7.7	251	486	29	22	42	100	25	0	75	35.0
55	AN396	1	2000	4.56	5.1	14.0	24.0	104	55	34	6.9	7.1	212	24	32	15	25	24	30	15	55	5.0
56	AN396	2	2000	4.56	5.1	14.0	24.0	35	5	34	6.9	7.1	212	24	32	15	25	8	47	2	52	5.0
57	AN396	3	2000	4.56	5.1	14.0	24.0	63	34	34	6.9	7.1	212	24	32	15	25	10	27	7	67	5.0
58	AN405	1	2000	3.11	3.3	77.0	103.0	120	118	73	9.3	7.6	285	56	30	26	31	12	45	10	45	12.0
59	AN405	2	2000	3.11	3.3	77.0	103.0	94	65	73	9.3	7.6	285	56	30	26	31	35	25	10	65	12.0

60	AN405	3	2000	3.11	3.3	77.0	103.0	100	76	73	9.3	7.6	285	56	30	26	31	24	50	10	40	12.0
61	AN413	1	2000	1.34	2.0	87.0	109.5	100	11	53	9.4	7.7	282	41	51	17	17	15	25	7	68	8.0
62	AN413	2	2000	1.34	2.0	87.0	109.5	143	23	53	9.4	7.7	282	41	51	17	17	23	20	10	70	8.0
63	AN413	3	2000	1.34	2.0	87.0	109.5	52	7	53	9.4	7.7	282	41	51	17	17	29	32	3	65	8.0
64	AN424	1	2000	0.97	1.5	67.0	100.5	85	23	107	4.9	7.6	460	62	80	1	3	37	30	0	70	9.0
65	AN424	2	2000	0.97	1.5	67.0	100.5	46	17	107	4.9	7.6	460	62	80	1	3	28	72	3	25	9.0
66	AN424	3	2000	0.97	1.5	67.0	100.5	136	23	107	4.9	7.6	460	62	80	1	3	31	58	2	40	9.0
67	AN429	1	2000	1.31	2.0	76.0	93.0	81	8	66	8.5	7.7	461	15	91	0	5	55	30	5	65	6.0
68	AN429	2	2000	1.31	2.0	76.0	93.0	100	10	66	8.5	7.7	461	15	91	0	5	15	10	3	87	6.0
69	AN429	3	2000	1.31	2.0	76.0	93.0	57	6	66	8.5	7.7	461	15	91	0	5	17	17	0	83	6.0
70	AN115	1	2001	2.57	3.3	10.0	12.0	70	23	20	5.0	6.5	192	28	49	27	3	9	80	0	20	4.0
71	AN192	1	2001	0.40	0.8	34.0	56.0	41	9	80	5.4	7.4	983	10	78	0	20	53	70	5	25	2.8
72	AN207	1	2001	2.54	2.9	232.0	337.0	37	4	90	7.5	7.8	552	74	83	1	12	70	95	0	5	15.0
73	AN209	1	2001	1.53	1.8	15.0	55.0	86	16	100	8.1	8.0	453	23	85	0	11	1	40	0	60	3.5
74	AN211	1	2001	1.13	1.5	42.5	81.5	52	17	48	5.8	7.2	214	16	92	0	5	9	30	10	60	5.0
75	AN231	1	2001	5.03	5.4	573.0	699.0	29	30	90	7.2	7.5	670	340	49	5	23	100	100	0	0	50.0
76	AN234	1	2001	1.37	2.0	70.0	125.0	82	13	55	8.0	7.8	327	73	48	3	43	90	5	5	90	9.0
77	AN235	1	2001	2.31	2.5	78.0	118.0	183	23	70	11.3	7.6	434	83	51	2	39	59	30	15	55	20.0
78	AN237	1	2001	0.23	2.0	19.5	64.5	43	5	95	8.7	9.0	436	27	62	0	23	2	20	30	50	5.0
79	AN274	1	2001	2.59	3.6	359.0	458.0	87	10	70	6.3	7.9	478	1962	34	2	49	100	10	30	60	45.0
80	AN333	1	2001	1.09	1.4	10.0	35.0	281	26	78	9.2	8.5	275	66	24	46	22	66	15	0	85	11.0
81	AN374	1	2001	0.65	1.0	44.0	63.0	92	13	73	7.1	8.3	298	486	29	22	42	86	30	17	53	35.0
82	AN405	1	2001	0.57	6.2	45.0	30.0	42	7	80	9.2	7.6	529	56	30	26	31	13	45	10	45	3.5
83	AN006	1	2002	0.10	0.2	4.0	9.0	42	15	30	8.8	7.8	121	76	2	0	86	53	10	60	30	8.0
84	AN008	1	2002	0.01	0.2	2.0	8.0	15	5	90	10.0	7.7	291	170	3	5	81	46	10	50	40	9.0
85	AN016	1	2002	0.22	0.5	4.0	14.0	248	39	236	2.8	8.0	741	10	8	29	30	61	20	30	50	2.5
86	AN029	1	2002	0.36	0.6	10.0	15.0	47	7	37	8.9	7.7	161	21	11	11	70	29	20	30	50	2.0
87	AN032A	1	2002	0.20	0.6	6.0	29.0	15	5	153	6.5	8.1	530	417	14	18	52	75	30	10	60	12.5
88	AN213	1	2002	0.13	0.7	38.0	38.0	30	12	64	4.9	7.4	326	5	60	10	20	11	50	0	50	2.0
89	AN245	1	2002	0.11	0.3	8.0	18.0	7	6	7	7.6	6.8	51	18	4	0	76	2	55	0	45	3.5
90	AN255	1	2002	0.18	0.8	13.0	57.0	11	4	32	7.9	7.7	103	79	16	1	65	33	35	15	50	6.0
91	AN259	1	2002	0.02	0.2	2.0	6.0	20	7	74	7.2	8.2	363	49	4	0	76	6	15	55	30	5.0
92	AN265	1	2002	0.62	0.8	16.0	14.0	24	8	61	9.4	7.7	245	190	10	0	72	27	15	50	35	10.0
93	AN299	1	2002	0.26	0.7	8.0	32.0	108	15	182	5.8	7.9	663	104	21	7	54	26	20	10	70	3.5
94	AN313	1	2002	1.31	1.4	26.0	34.0	10	4	35	10.0	7.2	154	9	32	8	41	1	25	30	45	4.0
95	AN315	1	2002	1.58	1.8	146.0	156.0	34	9	85	11.3	8.0	311	113	32	11	40	57	30	10	60	10.0
96	AN318	1	2002	0.44	0.6	8.0	14.0	73	12	38	8.4	7.6	161	15	14	19	51	13	0	72	28	8.0

97	AN321	1	2002	1.09	1.2	7.0	21.0	74	10	88	8.6	7.8	275	31	24	21	43	52	30	13	57	10.0
98	AN346	1	2002	7.99	8.5	135.0	177.0	97	9	66	8.0	7.6	440	20	39	6	45	19	30	10	60	3.5
99	AN215	1	2002	0.25	0.4	8.0	13.0	4	6	56	8.6	7.3	114	1	6	0	93	53	50	5	45	3.0
100	AN234	2	2002	1.83	2.5	144.0	212.0	66	24	186	8.0	7.7	528	73	48	3	43	9	5	5	90	9.0
101	AN374	1	2002	0.85	1.2	52.0	62.0	166	124	178	8.0	7.6	353	486	29	22	42	100	30	17	53	35.0
			min	0.01	0.2	2.0	6.0	3	4	7	2.8	6.5	51	1	2	0	3	1	0	0	0	2.0
			max	7.99	8.5	662.5	730.5	1115	153	236	11.3	9.0	983	1962	92	56	93	100	100	72	95	50.0
			mean	1.52	2.0	81.3	107.3	111	22	70	7.7	7.6	327	163	41	14	34	45	29	13	57	12.5
			median	1.15	1.7	41.0	62.0	81	13	64	8.2	7.6	282	56	32	11	33	31	26	7	60	8.5

Table 2. Water chemistry parameters of all 45 sampling sites by ecoregion. NO3-N: dissolved nitrate, TN: Total Nitrogen, O-P: orthophosphate, TP: total phosphorus, Chl a: Chlorophyll a, AFDM: Ash free dry mass, Hard: total hardness, Alk: total alkalinity, DO: dissolved oxygen, Cond: Conductivity, Cl^-: chloride;

Ecoregion		NO3-N	NH3-N	TN	O-P	TP	Chl a	AFDM	Hard	Alk	DO	pH	Cond	Cl^-
	unit	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	mg m^{-2}	g m^{-2}	mg L^{-1}	mg L^{-1}	mg L^{-1}		µS cm^{-1}	mg L^{-1}
NP	mean	1719	47	2241	98	127	124	25	102	69	7.5	7.6	341	50.0
	median	1307	24	1856	60	75	85	17	90	64	8.0	7.6	285	42.7
	min	232	3	362	4	12	3	4	45	20	2.8	6.5	93	2.8
	max	5525	177	6200	663	731	1115	153	280	186	11.3	9.0	983	240.0
HL	mean	1013	12	1272	28	41	60	10	100	66	8.5	7.6	253	27.5
	median	441	5	818	13	32	70	9	107	64	9.0	7.7	244	21.4
	min	22	2	247	2	6	7	4	25	7	4.9	6.8	51	3.6
	max	7992	52	8547	146	177	132	17	240	182	11.3	8.2	663	69.0
RV	mean	178	4	416	5	15	73	14	157	109	7.4	7.9	369	21.5
	median	198	4	538	4	14	42	7	136	90	8.8	7.8	291	17.3
	min	10	2	170	2	8	15	5	46	30	2.8	7.7	121	1.4
	max	361	7	577	10	29	248	39	306	236	10.0	8.1	741	55.8
all three ecoregions combined	mean	1524	39	1987	81	107	111	22	105	70	7.7	7.6	327	44.8
	median	1155	23	1653	41	62	81	13	90	64	8.2	7.6	282	37.5
	min	10	2	170	2	6	3	4	25	7	2.8	6.5	51	1.4
	max	7992	177	8547	663	731	1115	153	306	236	11.3	9.0	983	240.0

Table 3. Physical site characteristics of all 45 sampling sites by ecoregion. BASIN: basin size, URB: %urban land-use, AG: %agriculture land-use, FOR: %forest land-use, Open: % open canopy cover, BRBD: % bedrock and boulder, CBGR: % cobble and gravel, SDSTCL: % sand silt and clay, AvgW: average stream width, SecL: Section length, Flow: flow estimate, mean per section (1=slow, 2=medium, 3=fast).

Ecoregion		BASIN	URB	AG	FOR	Open	SDSTCl	BRBD	CBGR	AvgW	SecL	Flow
unit		km^2	%	%	%	%	%	%	%	m	m	estimate
NP	mean	190	47	14	29	48	31	9	60	14.1	47.8	1.9
	median	62	45	15	23	31	27	5	65	9.0	40.0	2.0
	min	1	6	0	3	1	0	0	0	2.8	5.0	1.0
	max	1962	92	56	93	100	100	51	95	50.0	125.0	3.0
HL	mean	45	22	12	51	27	23	28	48	6.8	30.6	1.8
	median	31	21	11	51	20	27	13	50	8.0	30.0	2.0
	min	5	4	0	20	1	0	0	28	2.0	15.0	1.0
	max	190	60	21	76	77	55	72	70	10.0	40.0	2.5
RV	mean	139	8	13	64	53	18	36	46	6.8	26.0	1.7
	median	76	8	11	70	53	20	30	50	8.0	20.0	1.5
	min	10	2	0	30	29	10	10	30	2.0	20.0	1.5
	max	417	14	29	86	75	30	60	60	12.5	40.0	2.0
all three ecoregions combined	mean	163	41	14	34	45	29	13	57	12.5	43.8	1.9
	median	56	32	11	33	31	26	7	60	8.5	40.0	2.0
	min	1	2	0	3	1	0	0	0	2.0	5.0	1.0
	max	1962	92	56	93	100	100	72	95	50.0	125.0	3.0

Table 4. Species apparent optima and tolerances estimated by WA.Only species with effective numbers of occurrence (Hill's N2) > 20 are shown. Species are sorted by increasing TP optima.

Taxon #	Taxon name	Hill's N2	NO3-N µg L-1		TP µg L-1	
			Opt	Tol	Opt	Tol
2	*Achanthidium rivluare* Potapova and Ponader	27.3	684.01	3.28	25.26	2.62
1	*Achnanthidium minutissimum* (Kützing) Czarnecki	48.2	563.91	4.06	32.54	2.62
107	*Encyonema minutum* (Hilse) Mann	25.0	646.98	3.51	35.05	2.33
54	*Navicula cryptocephala* Kützing	21.2	676.27	4.80	37.25	2.52
70	*Navicula cryptotenella* Lange-Bertalot	43.6	664.72	3.72	38.84	2.35
20	*Cocconeis pediculus* Ehrenberg	26.3	534.85	5.85	40.30	2.72
28	*Diatoma vulgaris* Bory	23.0	733.08	3.44	43.49	2.17
57	*Navicula tripunctata* (O. F. Müller) Bory	26.5	747.76	3.92	45.84	2.50
100	*Reimeria sinuata* (Gregory) Kociolek and Stoermer	49.4	871.06	2.68	47.31	2.24
43	*Gomphonema minutum* (C.A. Agardh) C.A. Agardh	24.4	874.06	3.03	47.99	2.39
103	*Synedra ulna* Ehrenberg	26.9	876.27	2.30	48.13	2.13
76	*Navicula capitatoradiata* Germain	25.2	745.26	3.68	50.34	2.17
90	*Nitzschia liebethruthii* Rabenhorst	35.0	998.80	2.99	51.73	2.50
122	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot	49.1	1095.49	2.24	52.22	2.14
41	*Gomphonema parvulum* (Kützing) Kützing	53.5	882.69	2.64	52.68	2.32
10	*Amphora pediculus* (Kützing) Grunow	42.4	972.57	2.43	54.09	2.08
4	*Achnanthes subhudsonis* var. *kraeuselii* Cholnoky	33.7	1114.88	2.49	55.43	3.14
71	*Navicula perminuta* Grunow	27.4	1239.85	1.90	56.02	2.11
50	*Melosira varians* Agardh	51.2	1047.10	2.50	58.49	2.25
16	*Caloneis bacillum* (Grunow) Cleve	39.7	974.77	3.11	59.36	2.53
73	*Navicula germainii* Wallace	41.6	943.69	2.14	60.50	2.06
53	*Mayamaea atomus* (Kützing) Lange-Bertalot	22.3	1097.13	1.55	60.67	1.66
126	*Nitzschia dissipata* (Kützing) Grunow	30.6	955.56	2.65	62.56	2.42
17	*Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck	48.3	1126.70	2.05	63.29	2.24
123	*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot	56.4	1149.19	2.43	63.92	2.25
129	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot	70.3	1077.31	2.43	64.46	2.36
124	*Achnanthes conspicua* Mayer	34.5	1202.87	1.97	67.18	1.77
60	*Navicula canalis* Patrick	21.1	1371.77	2.36	69.13	2.50
87	*Nitzschia palea* (Kützing) W. Smith	52.8	1138.31	2.32	69.92	2.34
66	*Navicula rostellata* Kützing	27.3	1172.28	1.79	71.52	1.82

56	*Eolimna minima* (Grunow) Lange-Bertalot	67.1	1111.68	2.21	71.86	2.21
63	*Navicula symmetrica* Patrick	45.5	1035.62	2.18	72.39	2.21
23	*Cyclotella meneghiniana* Kützing	33.1	888.67	2.85	73.71	2.74
72	*Eolimna subminuscula* (Manguin) Lange-Bertalot	32.6	1124.63	1.70	74.07	1.98
55	*Navicula gregaria* Donkin	58.4	1256.03	1.97	74.07	2.23
79	*Navicula lanceolata* (Agardh) Ehrenberg	44.5	1362.76	1.93	74.94	2.27
81	*Nitzschia amphibia* Grunow	60.0	1326.94	2.24	75.42	2.06
89	*Nitzschia inconspicua* Grunow	56.3	1318.86	1.82	75.84	1.87
82	*Nitzschia capitellata* Hustedt	20.2	1255.63	2.13	76.30	1.89
125	*Navicula ruttnerii* var. *capitata* Hustedt	23.0	1212.16	2.35	78.85	1.89
25	*Cyclotella pseudostelligera* Hustedt	20.6	1334.90	2.38	85.75	2.50
112	*Sellaphora seminulum* (Grunow) Mann	54.7	1248.33	2.09	88.40	2.11
121	*Sellaphora pupula* (Kützing) Meresckowsky	24.3	1250.98	2.01	88.91	2.21
45	*Gomphonema kobayasii* Kociolek & Kingston	38.5	1134.80	2.39	90.67	2.58
75	*Navicula recens* Lange-Bertalot	20.7	1191.90	1.71	101.10	2.28

Table 5. Results of all CCA's constrained to 1 variable at a time and partial CCA's. All analyses were run on 5 environmental variables (TP, NO3-N, Basin size, %Forest, % BRBD) that significantly ($p < 0.05$) explained the variance in diatom species composition in the reduced ($n = 91$) dataset. $?_1$: eigenvalue axis 1, $?_2$: eigenvalue axis 2.

variable	covariable	$?_1$	$?_2$	$?_1/?_2$	permutation test (999 permutations)	% variance explained by axis 1
TP	no covar.	0.152	0.195	0.78	0.001	7.2
	NO3-N	0.101	0.195	0.52	0.001	5.1
	Basin size	0.152	0.194	0.78	0.001	7.6
	BRBD	0.117	0.185	0.63	0.001	5.8
	%Forest	0.106	0.192	0.55	0.001	5.4
NO3-N	no covar.	0.112	0.228	0.49	0.001	5.3
	TP	0.062	0.195	0.32	0.001	3.2
	Basin size	0.112	0.222	0.51	0.001	5.6
	BRBD	0.087	0.185	0.47	0.001	4.4
	%Forest	0.077	0.208	0.37	0.001	3.9
Basin size	no covar.	0.109	0.268	0.41	0.001	5.2
	TP	0.109	0.194	0.56	0.001	5.6
	NO3-N	0.109	0.222	0.49	0.001	5.5
	BRBD	0.111	0.192	0.58	0.001	5.5
	%Forest	0.112	0.19	0.59	0.001	5.7
%Forest	no covar.	0.128	0.216	0.59	0.001	6.1
	TP	0.082	0.192	0.43	0.001	4.2
	NO3-N	0.092	0.208	0.44	0.001	4.6
	Basin size	0.131	0.19	0.69	0.001	6.6
	BRBD	0.086	0.186	0.46	0.001	4.3
BRBD	no covar.	0.107	0.193	0.55	0.001	5.1
	TP	0.072	0.185	0.39	0.001	3.7
	NO3-N	0.082	0.185	0.44	0.001	4.1
	Basin size	0.108	0.192	0.56	0.001	5.4
	%Forest	0.065	0.186	0.35	0.001	3.3

Table 6. Performance of weighted-averaging (WA) and weighted-averaging partial least (WA-PLS) squares regression and calibration models developed for the full (n=101) and the reduced (n=91) sample dataset. All TP and NO3-N units are in log 10 µg L^{-1}. WA-inv.desh.: WA-inverse deshrinking, WA-class.desh.: WA-classical deshrinking. The r^2 and RMSE in parentheses were derived from bootstrapping. n = Number of samples included in model.

n	method	variable	r^2 (r^2_{boot})	RMSE ($RMSEP_{boot}$)	Mean bias $_{boot}$	Max bias$_{boot}$
n=101	WA-inv. desh.	TP	0.63 (0.55)	0.27 (0.31)	-0.010	0.77
n=91	WA-inv. desh.	TP	0.70 (0.61)	0.22 (0.26)	-0.009	0.57
n=101	WA-inv. desh.	NO3-N	0.57 (0.39)	0.31 (0.37)	-0.012	1.50
n=91	WA-inv. desh.	NO3-N	0.63 (0.42)	0.28 (0.35)	-0.014	1.51
n=101	WA-class. desh.	TP	0.63 (0.55)	0.35 (0.35)	-0.012	0.64
n=91	WA-class. desh.	TP	0.70 (0.61)	0.26 (0.28)	-0.012	0.47
n=101	WA-class. desh.	NO3-N	0.57 (0.41)	0.40 (0.41)	-0.017	1.17
n=91	WA-class. desh.	NO3-N	0.63 (0.44)	0.35 (0.38)	-0.019	1.27
n=101	WA-PLS-2 components	TP	0.81 (0.64)	0.20 (0.29)	0.006	0.68
n=91	WA-PLS-2 components	TP	0.87 (0.72)	0.15 (0.23)	-0.010	0.45
n=101	WA-PLS-2 components	NO3-N	0.76 (0.50)	0.23 (0.36)	-0.015	1.40
n=91	WA-PLS-3 components	NO3-N	0.89 (0.51)	0.15 (0.35)	-0.020	1.59

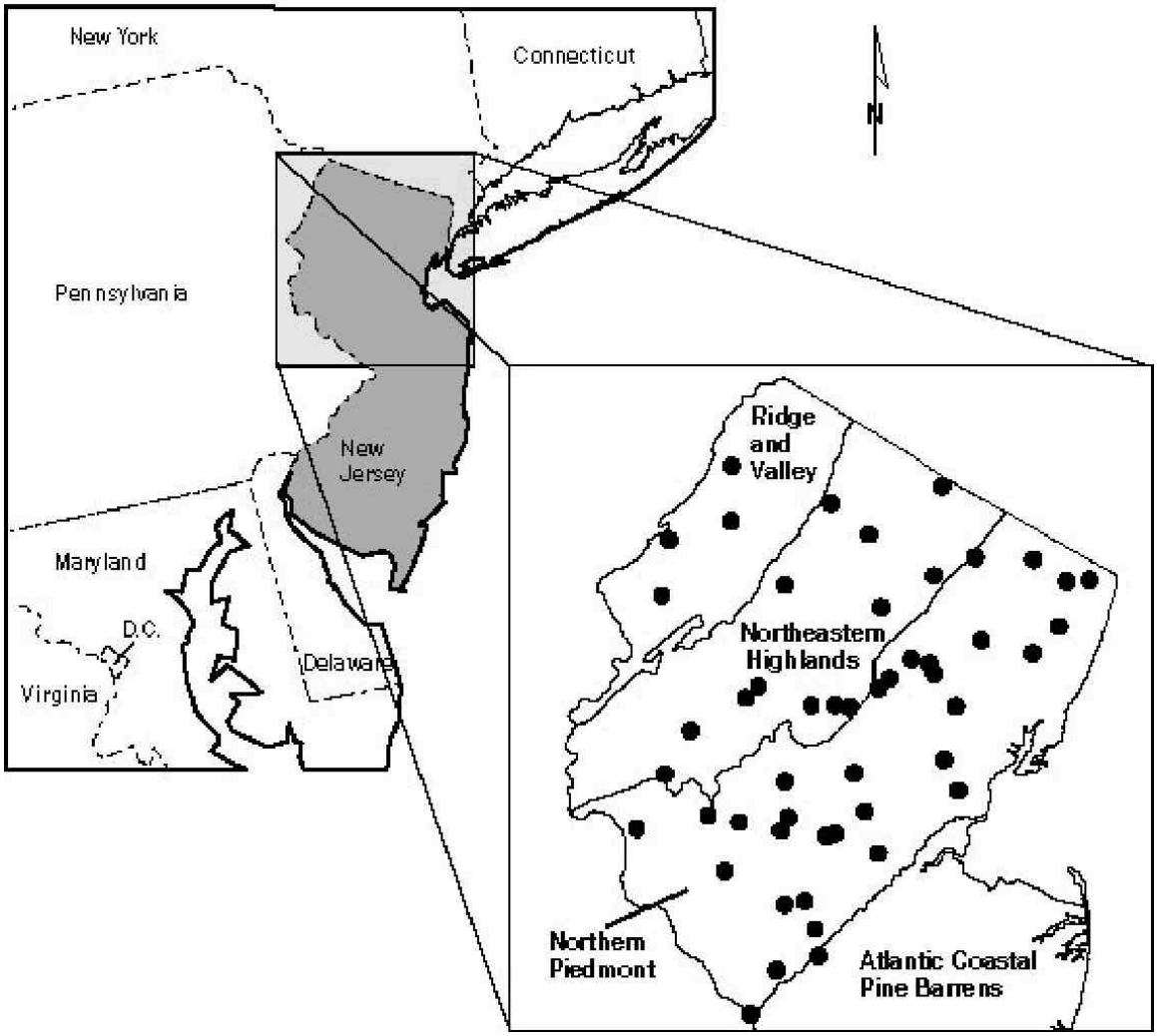

FIG. 1: Geographic location of study sites and ecoregions.

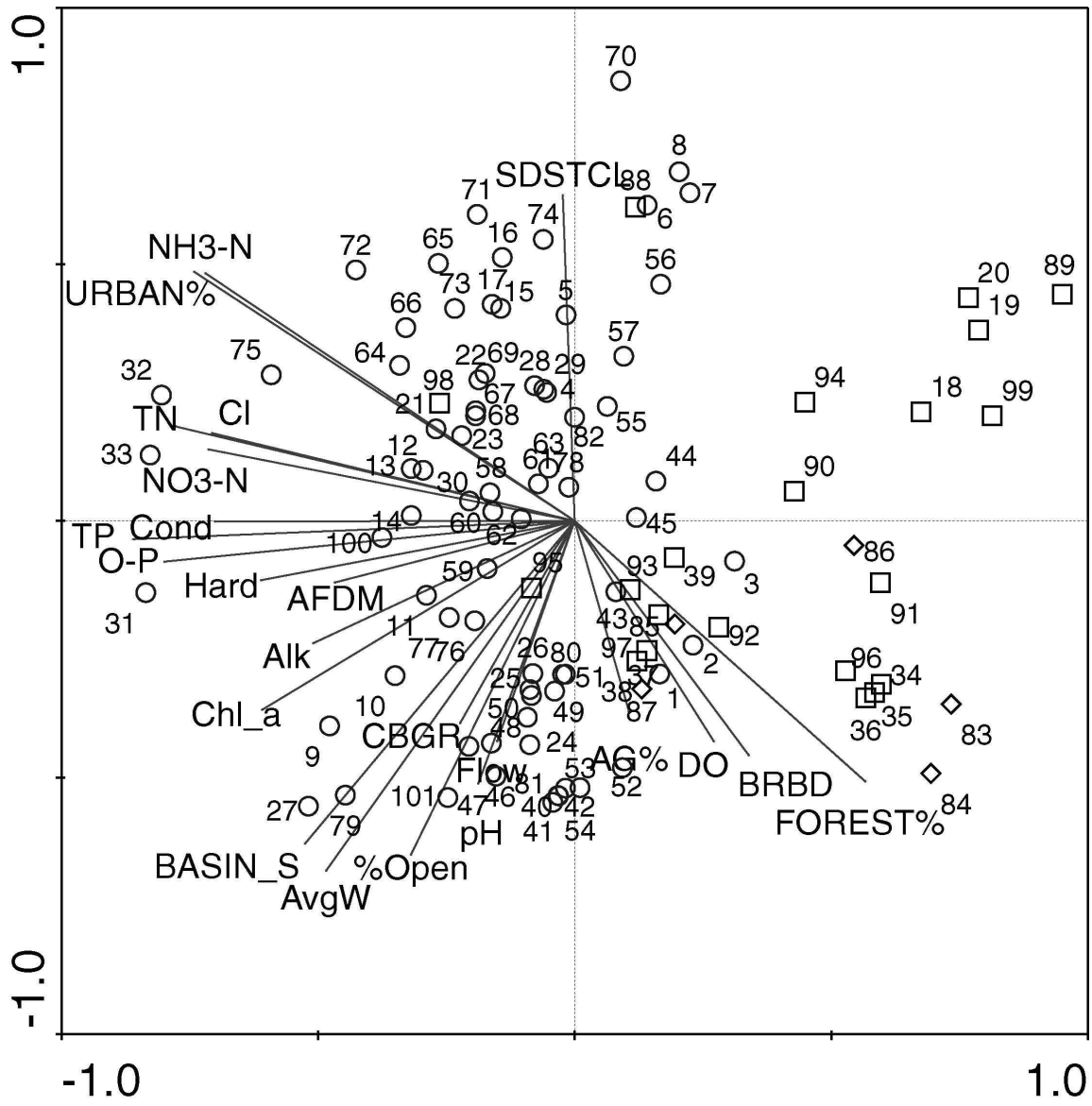

FIG. 2. Principal components analysis (PCA) biplot showing environmental variables and sites. Horizontal axis = PCA axis 1; vertical axis = PCA axis 2; Ecoregions in which sites are located are indicated by specific symbols. Circles: Northern Piedmont; squares: Highlands; diamonds: Valley and Ridge. Sample ID's can be found in Table 1. Abbreviations used for environmental variables can be found in Table 2 and 3.

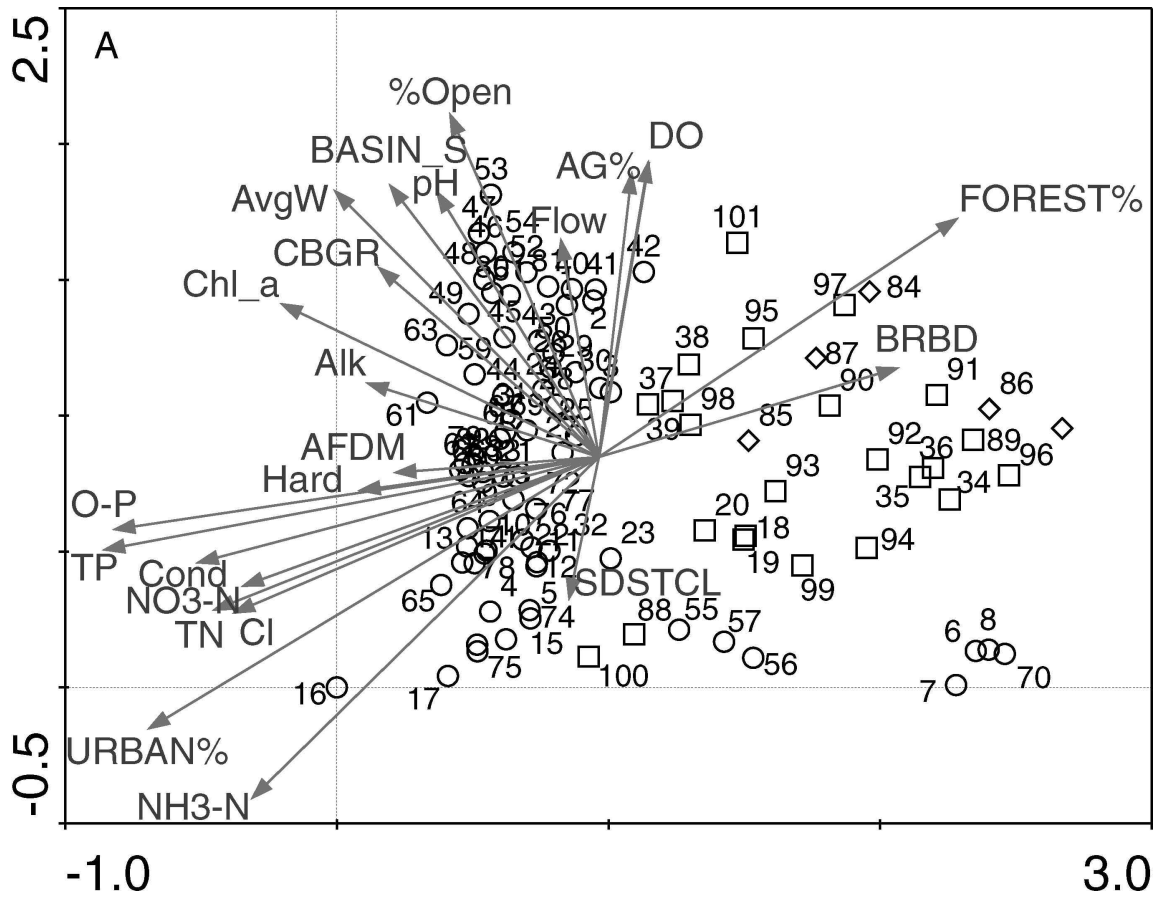

FIG. 3A.Detrended correspondence analysis (DCA) biplot showing samples and passive environmental variables. Horizontal axis = DCA axis 1; vertical axis = DCA axis 2. Ecoregions in which sites are located are indicated by specific symbols. Circles: Northern Piedmont; squares: Highlands; diamonds: Valley and Ridge. Sample ID's can be found in Table 1. Abbreviations used for environmental variables can be found in Table 2 and 3. Species ID's for species with highest number of occurences can be found in Table 4.

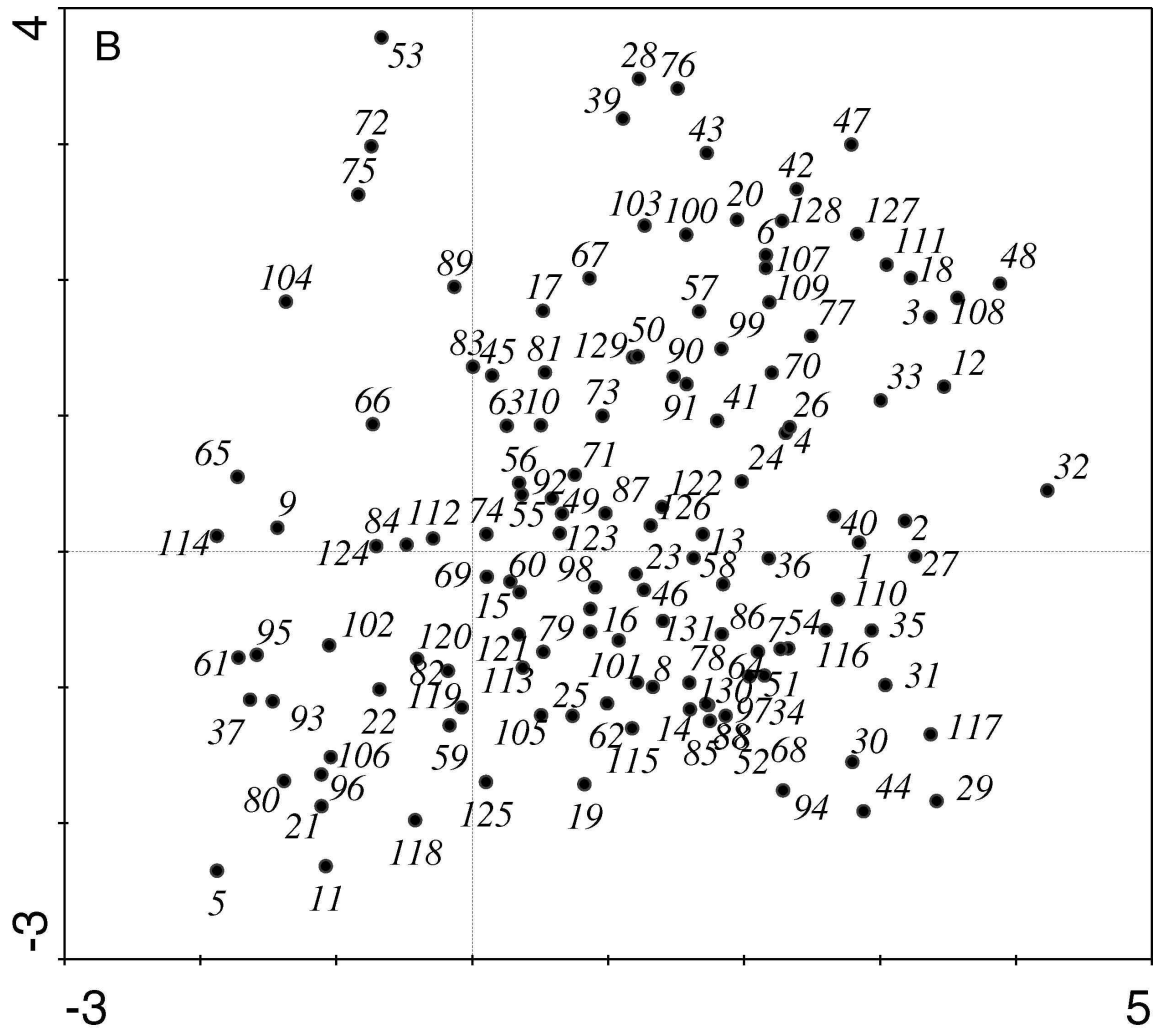

FIG. 3B. Detrended correspondence analysis (DCA) biplot showing diatom taxa. Horizontal axis = DCA axis 1; vertical axis = DCA axis 2. Species ID's for species with highest number of occurences can be found in Table 4.

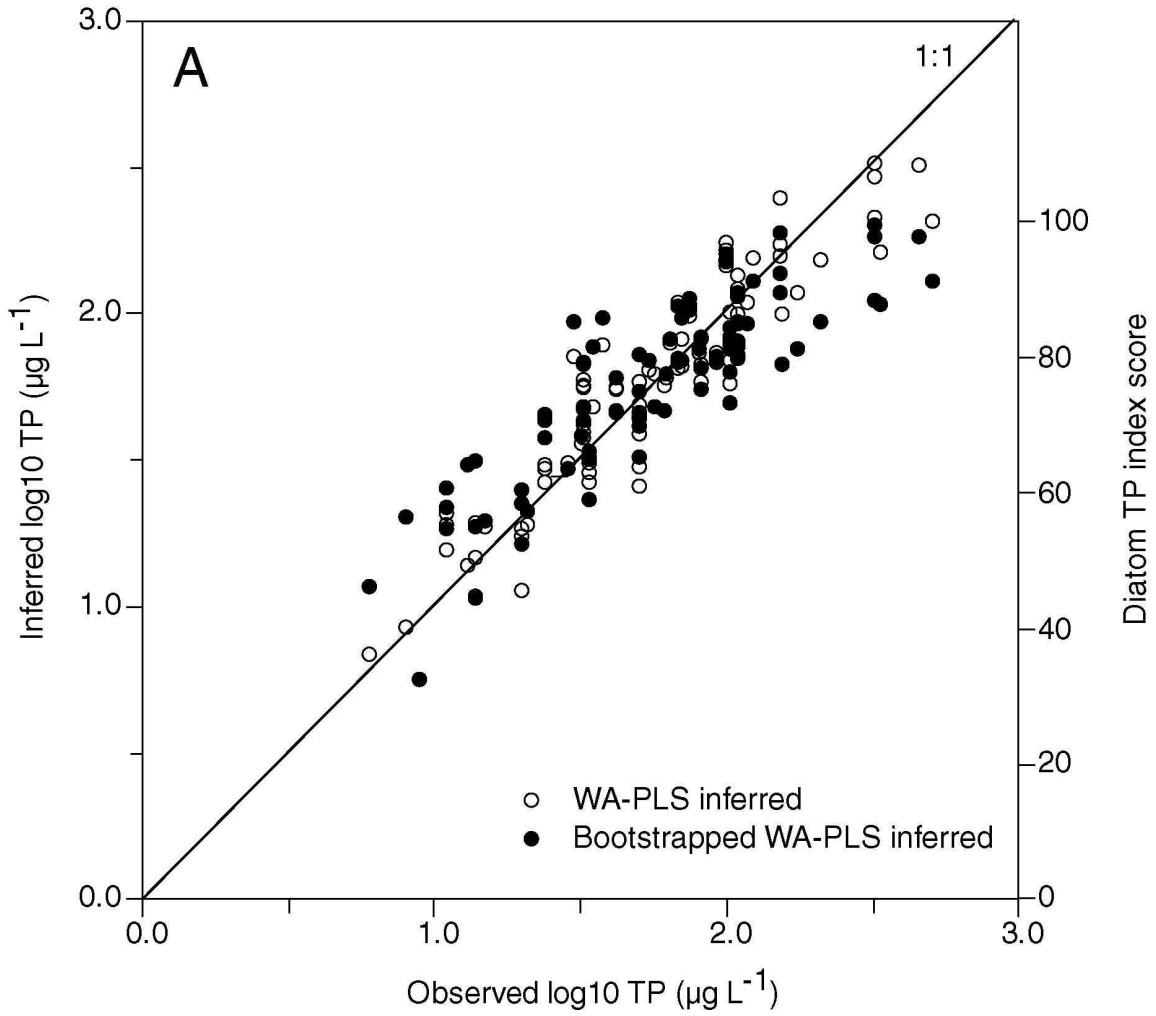

FIG. 4A. Plot of WA-PLS inference model showing diatom-inferred log 10 TP values versus observed log 10 TP values. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45). The diatom TP index score is shown for comparison.

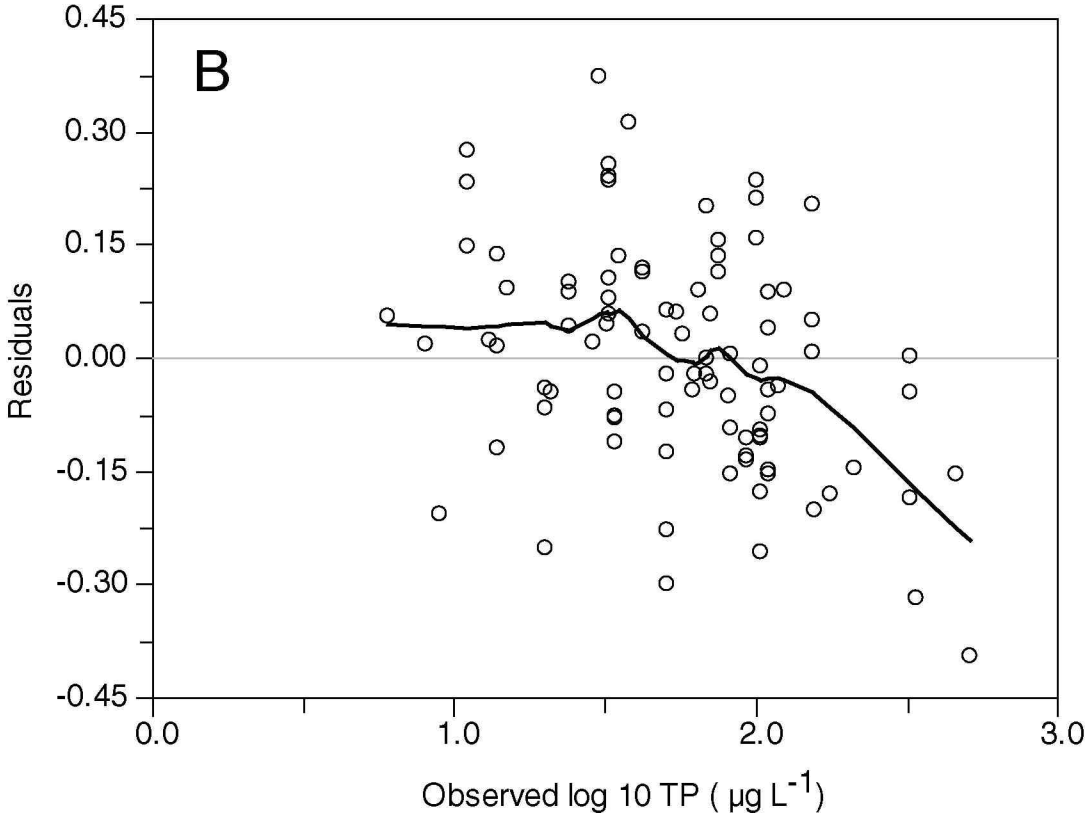

FIG. 4B. Plot of WA-PLS inference model showing residuals versus observed log 10 TP values. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45).

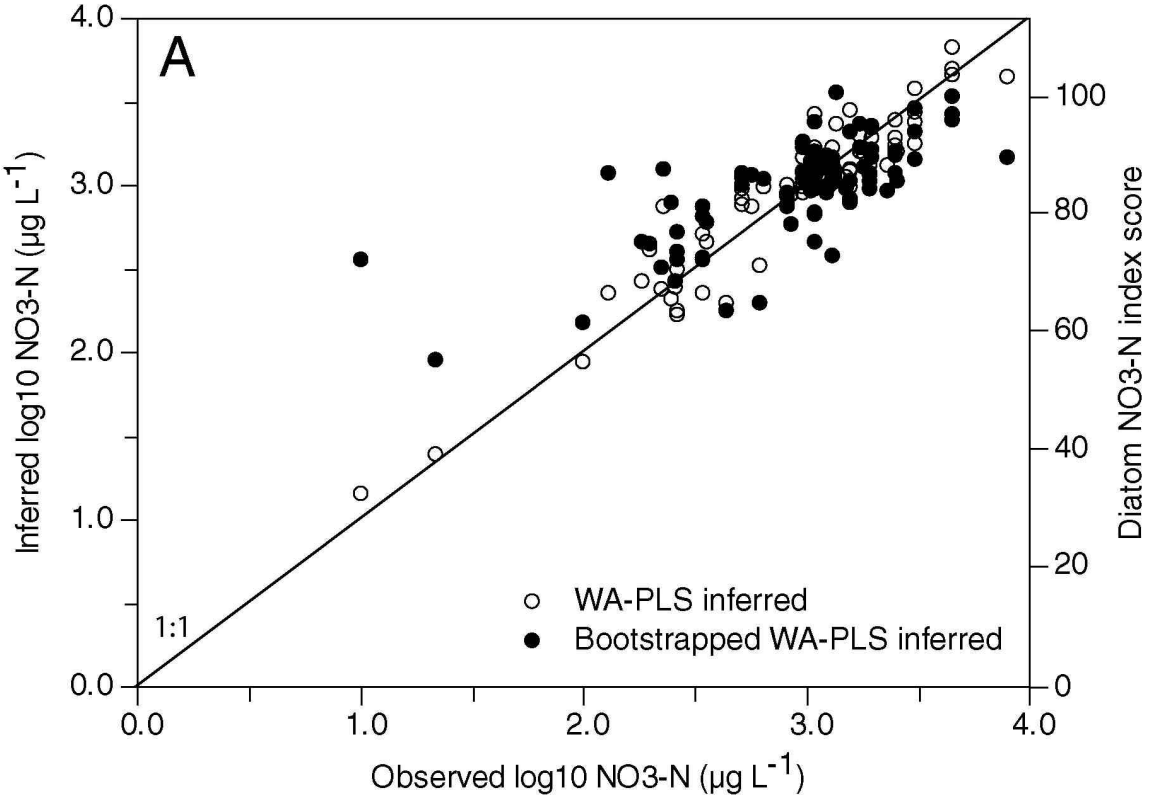

FIG 5A. Plot of WA-PLS inference model showing diatom-inferred log 10 NO3-N values versus observed log 10 NO3-N values. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45). The diatom NO3-N index score is shown for comparison.

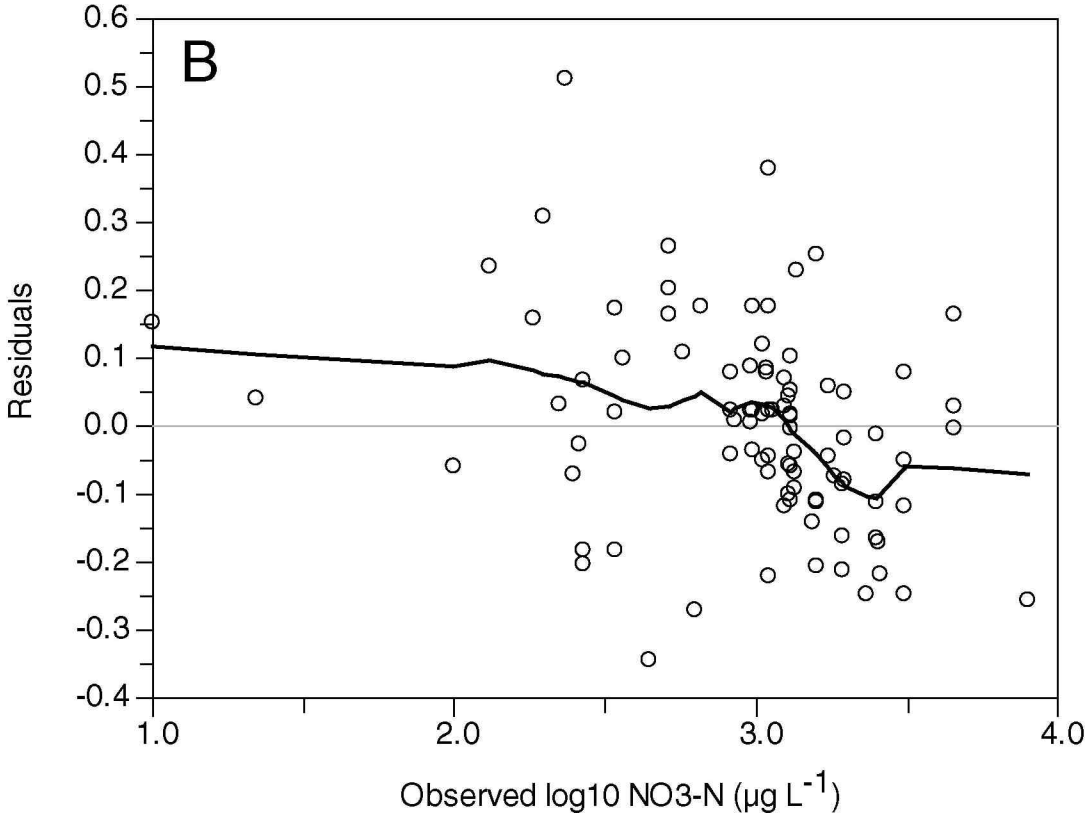

FIG. 5B. Plots of WA-PLS inference model showing residuals versus observed log 10 NO3-N values. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45).

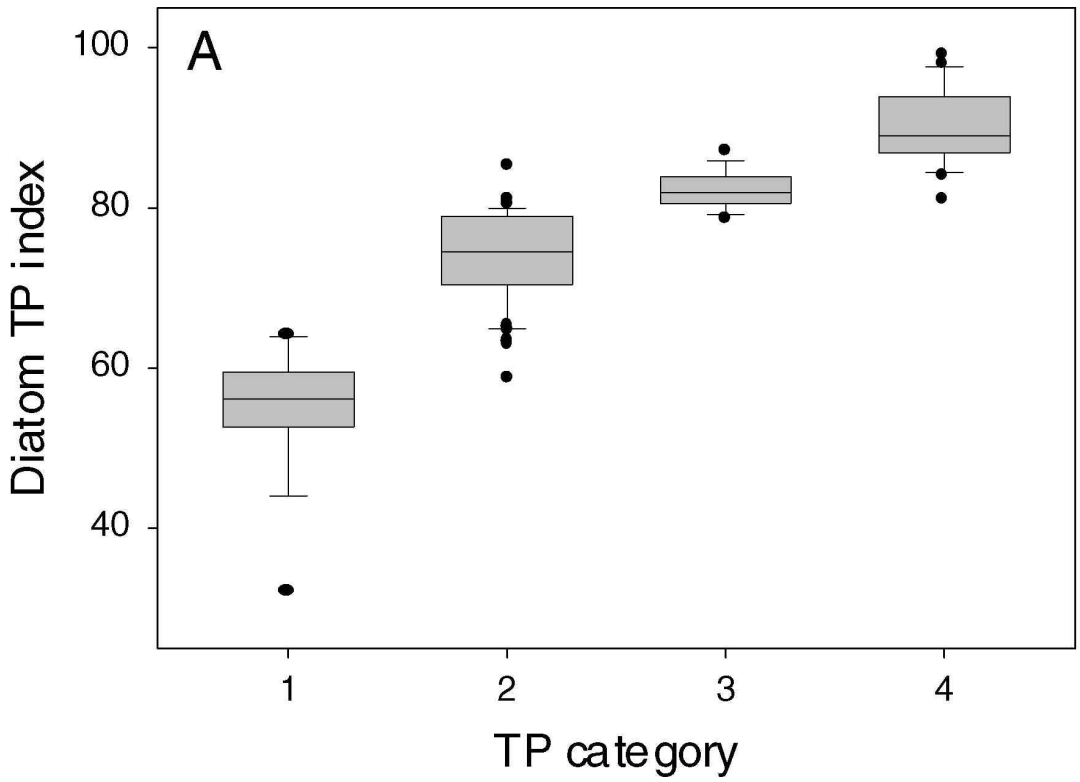

FIG. 6A. Box plots of the Diatom TP Index based on bootstrapped inference values. The index scores are grouped according to the water chemistry categories established for inferred TP. The upper and lower edges of the box indicate the 75th and the 25th percentile. The solid line is the median. Upper and lower whisker caps show the 90th and the 10th percentile. Filled circles represent individual values beyond the 90th and the 10th percentile. TP categories are: 1 (<0.025), 2 (0.025- 0.075), 3 (0.075- 0.1), 4 (>0.1) mg L^{-1} TP or 1 (<1.4), 2 (1.4-1.9), 3 (1.9- 2.0), 4 (>2.0) log 10 TP, corresponding to the index scores of 1) 0-60, 2) 60-81, 3) 81-86 and 4) above 86.

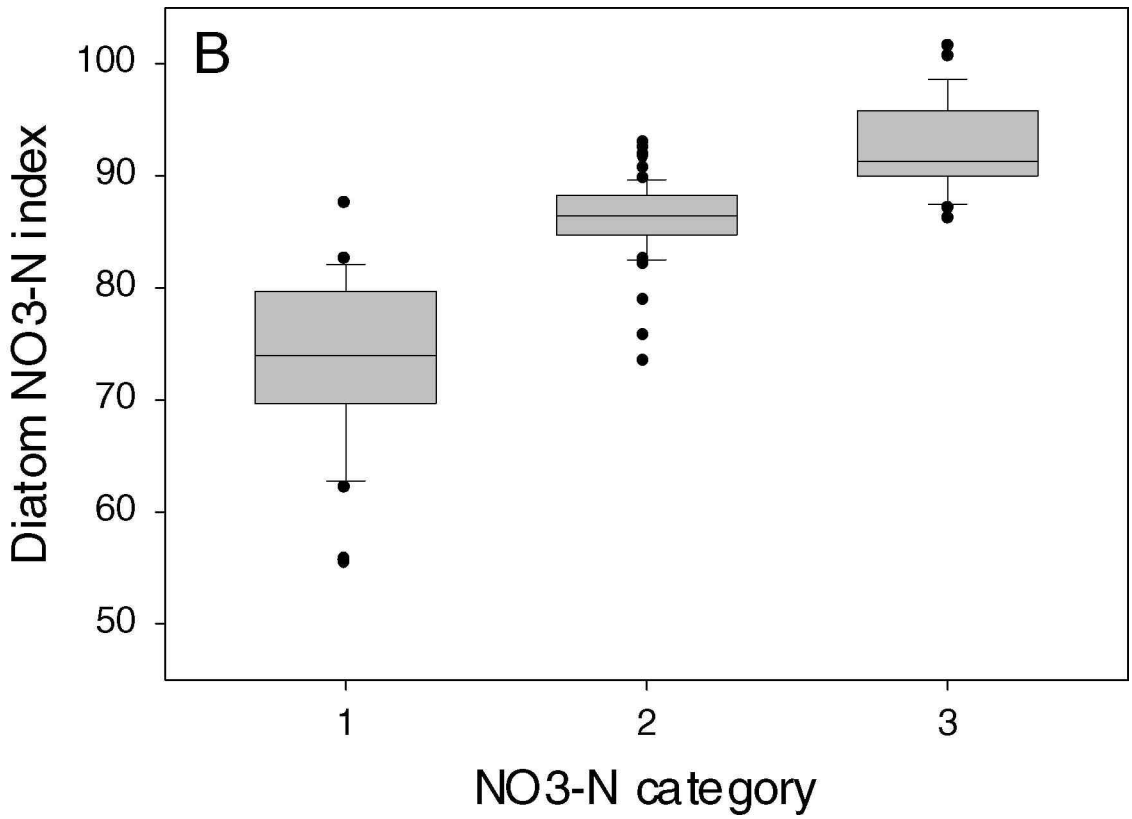

FIG. 6B. Box plots of the Diatom NO3-N Index based on bootstrapped inference values. The index scores are grouped according to the water chemistry categories established for inferred NO3-N. The upper and lower edges of the box indicate the 75th and the 25th percentile. The solid line is the median. Upper and lower whisker caps show the 90th and the 10th percentile. Filled circles represent individual values beyond the 90th and the 10th percentile. NO3-N categories are: 1 (< 0.7), 2 (0.7-1.5), 3 (>1.5) mg L^{-1} NO3-N, or 1 (<2.8), 2 (2.8-3.2), 3 (>3.2) log 10 NO3-N, corresponding to the index scores of 1) less than 81, 2) 81- 91 and 3) above 91.