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Diatom-based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams

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Abstract

We evaluated the potential for using diatoms to assess and monitor nutrient enrichment in New Jersey streams and rivers, and propose inference models and indices for regulatory purposes. We assessed the relationship between benthic diatom and water chemistry samples ($n = 101$) collected from 45 sites in 3 ecoregions: Northern Piedmont, Northeastern Highlands and Ridge and Valley. Diatom assemblages were dominated by pollution-tolerant taxa. Multivariate analysis showed that nutrient concentrations explained significant proportions of the variation in diatom species composition. Weighted-averaging partial least square (WA-PLS) total phosphorus (TP) and total nitrogen (TN) inference models ($n = 91$) showed good predictive ability (TP model: $r^2_{\text{apparent}} = 0.87$; $r^2_{\text{boot}} = 0.72$; $\text{RMSE}_{\text{boot}} = 0.23\ \log_{10}\ \mu\text{g L}^{-1}$ TP; TN model: $r^2_{\text{apparent}} = 0.88$; $r^2_{\text{boot}} = 0.58$; $\text{RMSE}_{\text{boot}} = 0.23\ \log_{10}\ \mu\text{g L}^{-1}$ TN). Diatom TP and TN indices were created to simplify presentation of results for the general public by rescaling the inferred TP and TN values from 0 to 100. The obtained index scores were assigned to nutrient impairment categories for regulatory assessment purposes.

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Keywords: Diatoms; Nutrients; Inference model; Index; Streams; USA

1. Introduction

New Jersey (NJ) is the most densely populated state in the US, with 2937 persons km^{-2} (US Census Bureau, 2002). Rivers and streams receive high nutrient loadings coming from a variety of urban, residential and agricultural sources (USGS, 2001). Almost half of the 2179 river miles recently assessed

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by the NJ Department of Environmental Protection (NJDEP) did not meet standards of less than 0.1 mg L^{-1} TP (NJDEP, 2003). These nutrient loadings and resulting algal growth can render waters unsuitable for NJs' designated uses, such as potable water supply, recreational use, fisheries, and aquatic life (NJDEP, 2001).

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). Benthic diatom species composition responds directly to nutrients (Pan and Lowe, 1994; Pan et al., 1996; Stevenson and Pan, 1999), and can be a more stable indicator of trophic state than measurements of nutrient concentrations or algal biomass (USEPA, 2000b).

The goal of this study was to develop robust diatom-based tools to monitor and assess nitrogen and phosphorus concentrations and periphyton responses in NJ streams and rivers. It was therefore important that these tools: (a) accurately characterize nutrient concentrations, (b) be consistent with NJ nutrient criteria categories, (c) be practical for routine monitoring, and (d) could be used in conjunction with other NJ bioindicators.

We chose weighted averaging (WA) inference modeling (ter Braak and Juggins, 1993) as our approach because it is the most accurate method for quantifying species response to nutrients (Hall and Smol, 1992; Dixit and Smol, 1994; Reavie et al., 1995; Pan et al., 1996; Winter and Duthie, 2000; Bennion et al., 2001; Bradshaw and Anderson, 2001; Soininen and Niemelä, 2002). We used the nutrient concentrations inferred from the models in two ways. First, we used the inferred values as estimates of the nutrient concentrations prevailing at the site during the time the algal assemblages were developing. Second, we rescaled the inferred concentrations from 0 to 100 to create diatom TP and TN indices to provide a management tool that is easily interpretable and partitioned into categories of protection for critical designated uses under the Clean Water Act (U.S. Code, 2002), and that are similar to existing 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).

2. Methods

2.1. Study sites

This study was conducted in northern and central NJ, USA, in the Northern Piedmont, the Northeastern Highlands and the Ridge and Valley ecoregions (Omernik, 1987, 1995) (Fig. 1). Land-use in the Piedmont is primarily urban and agriculture, whereas in the Highlands and the Ridge and Valley it is predominantly forest and agriculture (USEPA, 2000d).

Because of the approach we used to develop algal indicators of anthropogenic nutrient enrichment, 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 NJDEP and US Geological Survey (USGS) monitoring networks. All selected sites were part of an Ambient Biomonitoring Network (AMNET) (NJDEP, 2000), and reflected a range of known biological impairments based on macroinvertebrate data collected between 1992 and 1999 (NJDEP, 1993b, 1999). All sites selected were first to sixth order wadeable rivers and streams. We sampled 45 AMNET sites: 12 in the Highlands, 5 in the Ridge and Valley and 28 in the Piedmont (Fig. 1). Physical characteristics, water chemistry and other site information are summarized in Table 1. Detailed site information, including site name and location, and physical and water chemistry data are available at http://www.diatom.acnatsci.org/autecology/.

2.2. Field and laboratory procedures

Samples of epilithic diatoms were collected from August through October 2000–2002. The sampling area at each site was divided into three sections, so that within-site variability could be assessed. 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. One quantitative composite biomass sample was collected from three rocks per

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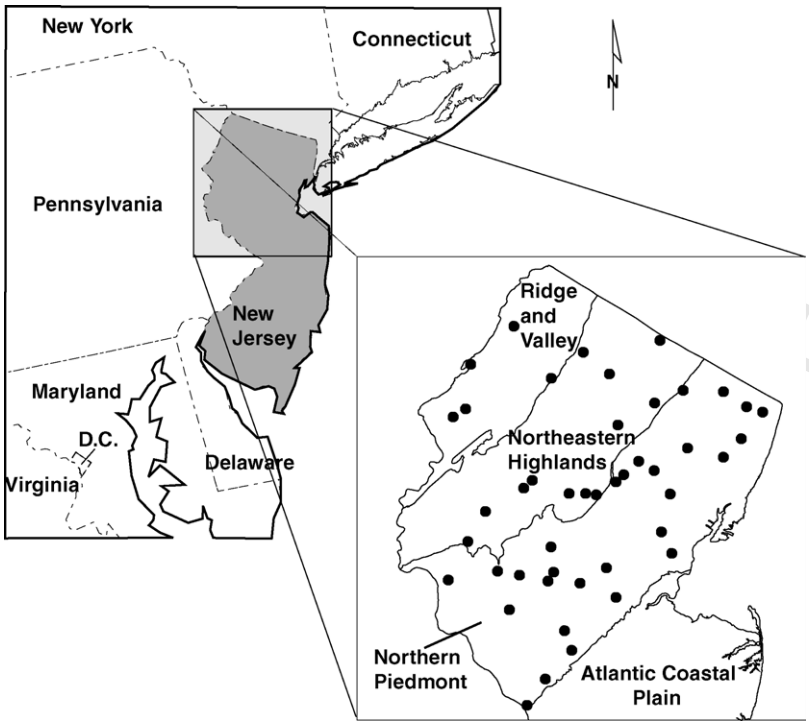

Fig. 1. Geographic location of study sites and ecoregions.

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 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 using a 0.7 μm Whatman glass microfiber filter. In addition, one unfiltered 500 mL 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 Patrick Center for Environmental Research (PCER) Geochemistry Section under the direction of Dr. D. Velinsky following USGS methods (Fishman, 1993). Total nitrogen (TN) was calculated from a combination of TKN and $NO_3 + NO_2$. During 2000 and 2001, samples for TKN analysis were collected by NJDEP and USGS. During 2003, TKN samples were collected at the time of algal sampling and analyzed by PCER. Chl *a* and AFDM samples were analyzed by PCER using standard methods (APHA et al., 1995) and USEPA method 445 (USEPA, 1992). Additional water chemistry data for water quality monitoring stations were provided by NJDEP and USGS staff. In 2002, we 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 NJDEP. We used water chemistry data for samples that were collected 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 USEPA (Barbour et al., 1999). A composite diatom sample was created by randomly selecting four to five rocks of ca. 5 cm diameter from mid-stream. Diatoms were removed from the rocks by scraping and brushing. Diatoms were cleaned, permanently mounted on microscope slides, and counted following USGS NAWQA protocols

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Table 1
Water chemistry parameters and physical site characteristics of all 45 sampling sites by ecoregion

Parameter	Northern Piedmont				Highlands				Ridge and Valley				All ecoregions combined			
	Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum
NO_3-N ($\mu g\ L^{-1}$)	1719	1307	232	5525	1013	441	22	7992	178	198	10	361	1524	1155	10	7992
TN ($\mu g\ L^{-1}$)	2241	1856	362	6200	1272	818	247	8547	416	538	170	577	1987	1653	170	8547
O-P ($\mu g\ L^{-1}$)	98	60	4	663	28	13	2	146	5	4	2	10	81	41	2	663
TP ($\mu g\ L^{-1}$)	127	75	12	731	41	32	6	177	15	14	8	29	107	62	6	731
NH_3-N ($\mu g\ L^{-1}$)	47	24	3	177	12	5	2	52	4	4	2	7	39	23	2	177
Chl *a* ($mg\ m^{-2}$)	124	85	3	1115	60	70	7	132	73	42	15	248	111	81	3	1115
AFDM ($g\ m^{-2}$)	25	17	4	153	10	9	4	17	14	7	5	39	22	13	4	153
Hard ($mg\ L^{-1}$)	102	90	45	280	100	107	25	240	157	136	46	306	105	90	25	306
Alk ($mg\ L^{-1}$)	69	64	20	186	66	64	7	182	109	90	30	236	70	64	7	236
DO ($mg\ L^{-1}$)	7.5	8.0	2.8	11.3	8.5	9.0	4.9	11.3	7.4	8.8	2.8	10.0	7.7	8.2	2.8	11.3
pH	7.6	7.6	6.5	9.0	7.6	7.7	6.8	8.2	7.9	7.8	7.7	8.1	7.6	7.6	6.5	9.0
Cond ($\mu S\ cm^{-1}$)	341	285	93	983	253	244	51	663	369	291	121	741	327	282	51	983
Cl^- ($mg\ L^{-1}$)	50.0	42.7	2.8	240.0	27.5	21.4	3.6	69.0	21.5	17.3	1.4	55.8	44.8	37.5	1.4	240.0
BASIN (km^2)	190	62	1	1962	45	31	5	190	139	76	10	417	163	56	1	1962
URB (%)	47	45	6	92	22	21	4	60	8	8	2	14	41	32	2	92
AG (%)	14	15	0	56	12	11	0	21	13	11	0	29	14	11	0	56
FOR (%)	29	23	3	93	51	51	20	76	64	70	30	86	34	33	3	93
Open (%)	48	31	1	100	27	20	1	77	53	53	29	75	45	31	1	100
BRBD (%)	9	5	0	51	28	13	0	72	36	30	10	60	13	7	0	72
CBGR (%)	60	65	0	95	48	50	28	70	46	50	30	60	57	60	0	95
SDSTCL (%)	31	27	0	100	23	27	0	55	18	20	10	30	29	26	0	100
AvgW (m)	14.1	9.0	2.8	50.0	6.8	8.0	2.0	10.0	6.8	8.0	2.0	12.5	12.5	8.5	2.0	50.0
SecL (m)	47.8	40.0	5.0	125.0	30.6	30.0	15.0	40.0	26.0	20.0	20.0	40.0	43.8	40.0	5.0	125.0
Flow (estimate)	1.9	2.0	1.0	3.0	1.8	2.0	1.0	2.5	1.7	1.5	1.5	2.0	1.9	2.0	1.0	3.0

NO_3-N: dissolved nitrate + nitrite; TN: total nitrogen; O–P: orthophosphate; TP: total phosphorus; NH_3-N: dissolved ammonia; Chl *a*: chlorophyll *a*; AFDM: ash free dry mass; Hard: total hardness; 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; SecL: section length; Flow: flow estimate, mean per section (1 = slow, 2 = medium, 3 = fast).

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(Charles et al., 2002). Per slide, 600 valves were identified to lowest taxonomic level and their relative abundance was recorded.

2.3. Numerical analysis

2.3.1. Dataset and data transformations

The complete dataset used in the analysis contained 101 samples from 45 sites: 45 samples were 'independent' samples, e.g. they were collected from section 1 of the respective sites; another 45 samples were 'multiple samples', e.g. samples collected from sections 2 and 3 of the respective sites. Eleven samples were 'repeat samples', e.g. samples from sites that had been sampled once in 2000, and which were revisited during the summers of 2001 and/or 2002 to provide a 2–3-year record of species composition for comparison. Multiple and repeat samples were collected to capture a bigger range of within-site as well as among-year variability. Of the total 101 samples, 5 were collected in the Ridge and Valley, 15 were collected in the Highlands and 81 were collected in the Piedmont physiographic provinces. The sites for which multiple and repeat samples were taken were distributed evenly along the measured TP and TN gradients and therefore the inclusion of these additional samples did not skew the dataset to either end of the nutrient range. Diatom taxa were included in the analysis if their abundance was $\geq$0.5% in at least two samples. All relative abundance data were square-root transformed. The water chemistry and physical parameters included in the ordinations are listed in Table 1. Three substrate categories were formed by combining the percentages of two or three substrate types: % bedrock and boulder (BRBD), % cobble and gravel (CBGR), and % sand silt and clay (SDSTCL). Environmental variables were transformed to reduce skewed distributions: all water chemistry variables, except pH, were $\log_{10}$-transformed; all data expressed in percentages (land-use, substrate, and open canopy cover) were square-root transformed.

2.3.2. Ordinations

Principal components analysis (PCA) was performed to detect major gradients and principal patterns of variation within the environmental variables (ter Braak and Prentice, 1988). 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 26 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 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 correspondence analyses (CA) and canonical correspondence analyses (CCAs) with down-weighting of rare taxa was performed, in order to determine the variables that independently explained a significant amount of variation in diatom species composition (ter Braak, 1995). First, a CA was run with passive environmental variables 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 (VIFs). Variables with VIFs > 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 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 vari-

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ables, we ran CCAs constrained to one variable at a time. A high ratio between the first (constrained) eigenvalue and the second (unconstrained) eigenvalue (e.g. $\lambda_1/\lambda_2 > 0.5$) indicated strong influence of these variables on diatom species composition, and justified development of inference models (Bigler and Hall, 2002; Winter and Duthie, 2000). All ordinations were produced using Canoco for Windows, version 4.5 (ter Braak and Šmilauer, 2002).

2.3.3. 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 TN. The models were developed using the program C^2, version 1.3 (Juggins, 2003). Several models were constructed, based on WA inverse and classical deshrinking (Birks et al., 1990) and on weighted-averaging partial least square regression (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 (e.g. the 'minimal adequate model' sensu Birks, 1998), 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).

2.3.4. Index development

We created diatom TP and TN indices based on values inferred for each diatom sample using the two- and three-component WA-PLS models ($n = 91$). Index values were calculated by multiplying the inferred nutrient values ($\log_{10}$) obtained for each sample by a constant that converted them to a scale from 0 to 100 (TP index = 33.33 × inferred $\log_{10}$ TP); TN index = 50 × (inferred $\log_{10}$ TN-2). The 0–100 scales correspond to $\log_{10}$ TP from 1 to 1000 μg L^{-1} and $\log_{10}$ TN from 100 to 10,000 μg L^{-1}. The ranges for both parameters were set slightly above the maximum and below the minimum values in our dataset.

We divided the 0–100 scale into trophic state categories ranging from 1 (low) to 3 (high) for TN, and from 1 (low) to 4 (very high) for TP, 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). The four TP categories established were (1) less than 0.025 mg L^{-1} TP, (2) 0.025–0.075 mg L^{-1} TP, (3) 0.075–0.1 mg L^{-1} TP, 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) 0–47, (2) 47–63, (3) 63–67, and (4) above 67. The lower boundary of category 4 (0.1 mg L^{-1} TP) is also the nutrient criteria for NJ. Because the TN gradient was shorter, we established only three categories for TN, from 1 (low) to 3 (high). The final TN categories established were (1) less than 0.7 mg L^{-1} TN, (2) 0.7–1.5 mg L^{-1} TN, and (3) above 1.5 mg L^{-1} TN. These correspond to the following $\log_{10}$ TN 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 42, (2) 42–59, and (3) above 59.

3. Results

3.1. Diatom species composition

In all, 399 diatom taxa were identified. Of these, 131 taxa were included in the data analysis. The 10 most abundant species, determined by high abundances and high effective numbers of occurrences based on Hill's N2 (Hill, 1973), had high TP and TN WA-optima (Table 2). Pollution-tolerant taxa dominated.

3.2. Environmental gradients and species distributions

We identified two major gradients in the environmental data, based on PCA. The first PCA axis (30.6% variance explained) reflected a chemical gradient driven by all inorganic nutrients measured, % urban land-use, conductivity and chloride. The second axis (16.7% variance explained) revealed a physical gradient influenced by average river width and basin size, % open canopy cover, and % sand, silt, and clay substrates. In the DCA of diatom data, the eigenvalues

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Table 2
Species apparent optima and tolerances estimated by WA

Taxon name	Hill's N2	TP ($\mu g\ L^{-1}$)		TN ($\mu g\ L^{-1}$)	
		Optima	Tolerance	Optima	Tolerance
Achnanthidium rivulare Potapova and Ponader	27	25.26	2.62	956.27	2.72
Navicula antonii Lange-Bertalot	15	30.19	2.12	919.77	1.75
Cocconeis placentula var. *euglypta* (Ehrenberg) Cleve	14	30.51	2.49	834.31	2.41
Nitzschia fonticola Grunow	16	30.86	2.64	915.29	2.28
A. minutissimum (Kützing) Czarnecki	48	32.54	2.62	1016.46	2.47
Encyonema minutum (Hilse) Mann	25	35.05	2.33	1040.66	2.13
N. cryptocephala Kützing	21	37.25	2.52	1241.02	2.65
N. cryptotenella Lange-Bertalot	44	38.84	2.35	1155.82	2.16
Cymbella tumida (Brébisson ex Kützing) Van Heurck	13	39.79	2.60	890.14	2.13
C. pediculus Ehrenberg	26	40.30	2.72	1097.97	2.67
N. decussis Østrup	18	42.34	2.23	1073.77	2.21
Diatoma vulgaris Bory	23	43.49	2.17	1216.35	1.85
Nitzschia archibaldii Lange-Bertalot	16	43.53	2.91	1152.74	2.16
Nitzschia linearis (Agardh ex W. Smith) W. Smith	11	43.53	2.25	926.96	2.25
Amphora inariensis Krammer	11	45.14	2.41	1255.19	2.22
N. tripunctata (O.F. Müller) Bory	27	45.84	2.50	1266.25	2.09
Reimeria sinuata (Gregory) Kociolek and Stoermer	49	47.31	2.24	1279.38	2.04
Gomphonema minutum (Agardh) Agardh	24	47.99	2.39	1322.18	1.98
Synedra ulna Ehrenberg	27	48.13	2.13	1263.80	1.73
N. capitatoradiata Germain	25	50.34	2.17	1274.71	1.91
Nitzschia liebethruthii Rabenhorst	35	51.73	2.50	1498.79	2.12
Nitzschia recta Hantz. ex Rabenhorst	17	51.97	2.62	1980.48	2.22
Planothidium lanceolatum (Brébisson ex Kützing) L.-B.	49	52.22	2.14	1472.86	2.09
G. parvulum (Kützing) Kützing	53	52.68	2.32	1331.37	2.04
Amphora pediculus (Kützing) Grunow	42	54.09	2.08	1448.77	1.80
Achnanthes subhudsonis var. *kraeuselii* Cholnoky	34	55.43	3.14	1472.55	2.28
N. perminuta Grunow	27	56.02	2.11	1639.34	1.71
Melosira varians Agardh	51	58.49	2.25	1538.05	1.83
Caloneis bacillum (Grunow) Cleve	40	59.36	2.53	1554.71	2.12
N. germainii Wallace	42	60.50	2.06	1488.02	1.68
Mayamaea atomus (Kützing) Lange-Bertalot	22	60.67	1.66	1601.95	1.46
Nitzschia dissipata (Kützing) Grunow	31	62.56	2.42	1606.46	1.85
C. placentula var. *lineata* (Ehrenberg) Van Heurck	48	63.29	2.24	1602.54	1.78
P. frequentissimum (Lange-Bertalot) Lange-Bertalot	56	63.92	2.25	1641.01	2.03
Staurosirella pinnata (Ehrenberg) Williams et Round	18	64.25	3.47	1323.58	2.57
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	70	64.46	2.36	1569.24	1.96
Achnanthes conspicua Mayer	35	67.18	1.77	1842.85	1.64
N. erifuga Lange-Bertalot	10	68.02	2.22	1527.64	1.78
Frustulia vulgaris (Thwaites) De Toni	11	68.15	2.09	1438.53	1.65
N. canalis Patrick	21	69.13	2.50	2094.02	1.87
Nitzschia palea (Kützing) W. Smith	53	69.92	2.34	1720.68	1.81
N. rostellata Kützing	27	71.52	1.82	1779.18	1.56
N. minima Grunow	67	71.86	2.21	1626.86	1.86
N. symmetrica Patrick	46	72.39	2.21	1613.84	1.74
Gyrosigma acuminatum (Kützing) Rabenhorst	13	72.67	2.39	1404.91	1.76
Cyclotella meneghiniana Kützing	33	73.71	2.74	1491.94	1.89
N. gregaria Donkin	58	74.07	2.23	1764.09	1.78
N. subminuscula (Manguin)	33	74.07	1.98	1621.92	1.54
N. lanceolata (Agardh) Ehrenberg	45	74.94	2.27	1934.33	1.71
N. capitata Ehrenberg	11	75.12	2.22	1560.52	1.70
Nitzschia amphibia Grunow	60	75.42	2.06	1841.20	1.80
Fragilaria vaucheriae (Kützing) Petersen	17	75.56	3.02	1704.39	2.05

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Table 2 (*Continued*)

Taxon name	Hill's N2	TP ($\mu g\ L^{-1}$)		TN ($\mu g\ L^{-1}$)	
		Optima	Tolerance	Optima	Tolerance
Nitzschia inconspicua Grunow	56	75.84	1.87	1830.04	1.60
Nitzschia capitellata Hustedt	20	76.30	1.89	2194.22	1.62
A. exiguum (Grunow) Czarnecki	17	76.53	2.18	1883.52	1.80
Bacillaria paradoxa Gmelin	18	77.08	2.30	2457.93	1.75
N. ruttnerii var. *capitata* Hustedt	23	78.85	1.89	1901.91	1.63
N. agrestis Hustedt	13	81.02	2.15	1866.16	1.69
Staurosira construens (Ehrenberg) Williams et Round	11	82.34	2.93	1586.97	2.66
Cyclotella pseudostelligera Hustedt	21	85.75	2.50	2077.26	1.76
Sellaphora seminulum (Grunow) Mann	55	88.40	2.11	1835.86	1.72
S. pupula (Kützing) Meresckowsky	24	88.91	2.21	1941.51	1.63
G. kobayasii Kociolek and Kingston	39	90.67	2.58	1785.54	1.80
N. ingenua Hustedt	12	91.75	1.78	1773.62	1.30
N. recens Lange-Bertalot	21	101.10	2.28	1742.53	1.56
Aulacoseira granulata (Ehrenberg) Simonsen	10	124.04	3.13	2628.27	1.80
Cyclotella atomus Hustedt	11	155.60	2.18	2070.28	1.51
Luticola goeppertiana (Bleisch) Mann	13	163.02	2.15	2564.25	1.40

Only species with effective numbers of occurrence (Hill's N2) > 10 are shown. Species are sorted by increasing TP optima.

of the first two axes were $\lambda_1 = 0.3$ and $\lambda_2 = 0.17$, accounting for 20.1% of the variance in the species data (Fig. 2). The gradient lengths of the first two DCA axes of 2.7 and 1.8 standard deviation (S.D.) units indicated that either linear or unimodal species response models would be effective (ter Braak and Prentice, 1988). As the gradient length of the first DCA axis was above two S.D., we chose to use techniques based on assumptions of unimodal species distribution (ter Braak, 1995).

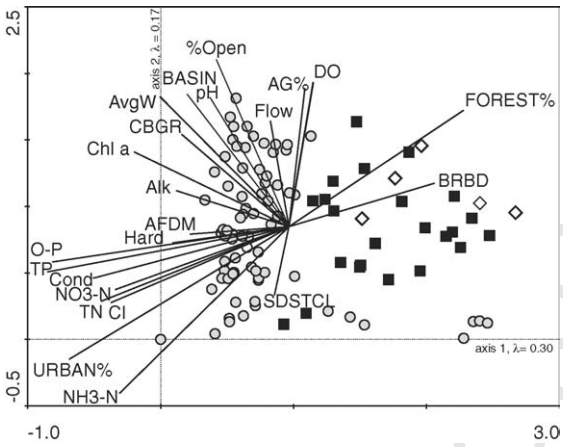

Fig. 2. Detrended correspondence analysis (DCA) biplot showing samples and passive environmental variables. Horizontal axis = DCA axis 1; vertical axis = DCA axis 2. Ecoregions in which sites are located are indicated by specific symbols. Circles: Northern Piedmont; squares: Highlands; diamonds: Valley and Ridge. Abbreviations used for environmental variables can be found in Table 1.

3.3. 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. All three sites had high conductivity values (595–983 $\mu S\ cm^{-1}$) and a high percentage of sand, silt, and clay sediments. The nutrient values for two of these sites were high (TP: 0.69 and 0.73 $mg\ L^{-1}$; TN: 5.4 and 6.1 $mg\ L^{-1}$). Although it was important to keep samples with high nutrient concentrations 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 six sites were identified as outliers because of their high sample score means on the DCA axes (Fig. 2). Because all six 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 the five samples from the two sites with the lowest pHs (6.5 and 6.7) in the dataset. In addition, one site had a high % of sand substrate. Despite the fact that both sites represented the low end of the phosphorus gradient, we decided to

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exclude them from further model development to avoid the influence of a strong pH and alkalinity gradient. Data analyses were performed using the reduced dataset of 91 samples, that excluded the 10 outlier samples.

3.4. Relationships between diatom assemblages and environmental variables

We used CA to determine the environmental variables explaining most of the variation in diatom species composition, and especially the importance of nutrients as compared with other environmental characteristics. In a CA including all 24 environmental variables as passive variables and diatom data for 91 sites, 21.8% of the total variance of the diatom data was explained by CA axis 1 ($\lambda_1 = 0.27$) and CA axis 2 ($\lambda_2 = 0.19$). The species-environment correlations were high for both, axis 1 ($r = 0.90$) and axis 2 ($r = 0.91$), accounting for 35.1% 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 CA. Nevertheless, strong weighted correlations and relatively high variance inflation factors (VIFs > 5) indicated strong co-linearity among several environmental variables. Consequently, we omitted the following 11 variables from further analysis: *O*-P, NO_3-N, NH_3-N, average river width, reach length, % urban land-use, % open canopy cover, % cobble and gravel, AFDM, hardness, and conductivity. Because TN and NO_3-N, as well as TP and *O*-P, were highly correlated, only one of the two variables, respectively, could be selected. Because TP and TN are more important with respect to management purposes, we chose to eliminate the variables *O*-P and NO_3-N. 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, TN, basin size, % forested land-use, and % bedrock and boulder. The first two axes 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 72.4% of the variance in the species–environment relationships. The correlations between TP and TN and the first ordination axis were strong ($r = -0.72$ and -0.61). In comparison with the CA 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.

CCAs constrained to one variable at a time were run on the remaining five variables to assess the strength of their relationship with diatom species composition, and to determine which variables had a sufficiently strong relationship with diatom assemblages to justify development of inference models. Of the five variables, TP had the highest λ_1/λ_2 ratio (0.78), capturing 7.2% of the variation in the species data. TN had a λ_1/λ_2 ratio of 0.54, capturing 5.6% of the variation in species data. This confirmed that both variables had significant influence on the diatom assemblages and that development of inference models for both variables was justified.

3.5. Species WA-optima and tolerances

TP and TN 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) > 10 (Table 2). Species apparent WA TP optima ranged from 13 to 163 $\mu g\ L^{-1}$. Weighted-average TN optima ranged from 362 to 4412 $\mu g\ L^{-1}$. Taxa with high and low TP optima had values comparable to those calculated in other studies conducted in eastern North America (Winter and Duthie, 2000; Potapova et al., 2004).

3.6. Inference models

Models to infer TP and TN ($\log_{10} \mu g\ L^{-1}$) were developed and tested using WA and WA-PLS. The models were run on the $n = 91$ calibration set, which included 131 taxa. In order to test if the inclusion of multiple and repeat samples biased the model performance, we developed additional TP and TN WA-PLS models using the dataset with independent samples only ($n = 40$). This dataset was obtained after deleting 5 of the same outliers as in the models above, reducing the set of 45 'independent' samples to 40 samples, including only 128 taxa.

Table 3 shows performance measures for all inference models. The best TP model was a two-component WA-PLS model using the $n = 91$ dataset,

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Table 3
Performance of weighted-averaging (WA) and weighted-averaging partial least squares (WA-PLS) regression and calibration models developed for the $n = 91$ sample datasets including multiple and repeat samples and the $n = 40$ sample datasets including independent samples only

n	Method	Variable	r^2 (r^2_{boot})	RMSE ($RMSEP_{boot}$)	Mean $bias_{boot}$	Max $bias_{boot}$
91	WA-inv.desh.	TP	0.70 (0.61)	0.22 (0.26)	−0.009	0.57
91	WA-inv.desh.	TN	0.63 (0.47)	0.19 (0.24)	−0.010	0.66
91	WA-class.desh.	TP	0.70 (0.61)	0.26 (0.28)	−0.012	0.47
91	WA-class.desh.	TN	0.63 (0.48)	0.24 (0.26)	−0.014	0.64
91	**WA-PLS-2 components**	**TP**	**0.87 (0.72)**	**0.15 (0.23)**	**−0.010**	**0.45**
91	**WA-PLS-3 components**	**TN**	**0.88 (0.58)**	**0.11 (0.23)**	**−0.015**	**0.66**
40	WA-PLS-3 components	TP	0.96 (0.69)	0.08 (0.28)	−0.030	0.65
40	WA-PLS-1 component	TN	0.71 (0.50)	0.19 (0.27)	−0.015	0.81

All TP and TN 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. Models with best performance are highlighted in bold.

showing the highest r^2_{boot} (0.72) and the lowest $RMSE_{boot}$ (0.23 $\log_{10}$ μg L^{-1} TP). For TN, a three-component model using the $n = 91$ dataset showed best performance, with an r^2_{boot} of 0.58 and an $RMSE_{boot}$ of 0.23 $\log_{10}$ μg L^{-1} TN. The TP gradient included in this model was 6–458 μg L^{-1} and the TN range was 170–8547 μg L^{-1}. 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 TN (0.88–0.58) (Table 3 and Figs. 3A and 4A). The residuals plot (Fig. 3B) shows that the TP model using WA-PLS ($n = 91$) tends to underestimate TP values above 100 μg L^{-1}. A similar trend is shown in the TN model using WA-PLS ($n = 91$), where the difference between bootstrapped versus inferred values is higher above 3000 μg L^{-1} TN (Fig. 4A and B). Generally, Figs. 3 and 4 show that the TN data have a less even distribution along the nutrient gradient than the TP data. The performance of the best TP and TN WA-PLS models using the independent samples only ($n = 40$) are slightly lower (TP model: $r^2_{boot} = 0.69$; $RMSE_{boot} = 0.28$ $\log_{10}$ μg L^{-1} TP; TN model: $r^2_{boot} = 0.50$; $RMSE_{boot} = 0.27$ $\log_{10}$ μg L^{-1} TN) than compared with the best models ($n = 91$) that include multiple and replicate samples (Table 3). Nevertheless, because the model performance does not significantly decrease when excluding the multiple and replicate samples, we believe that inclusion of these samples in the dataset provides models that are more robust. It includes spatial and temporal variability of the diatom species composition at these sites, which increases the reliability of the inferred value, when the model is applied to similar sites.

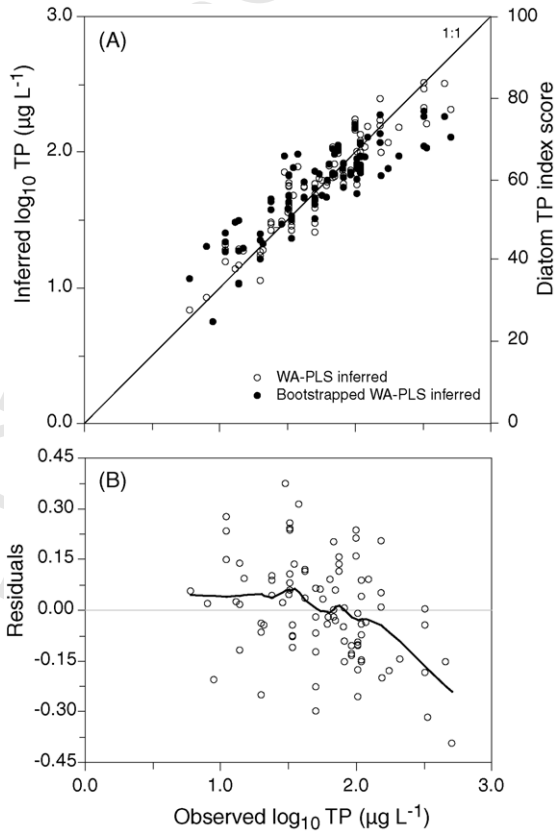

Fig. 3. Plots of WA-PLS inference model showing (A) diatom-inferred $\log_{10}$ TP values vs. observed $\log_{10}$ TP values, and (B) residuals vs. observed $\log_{10}$ TP values. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45). The diatom TP index score is shown for comparison.

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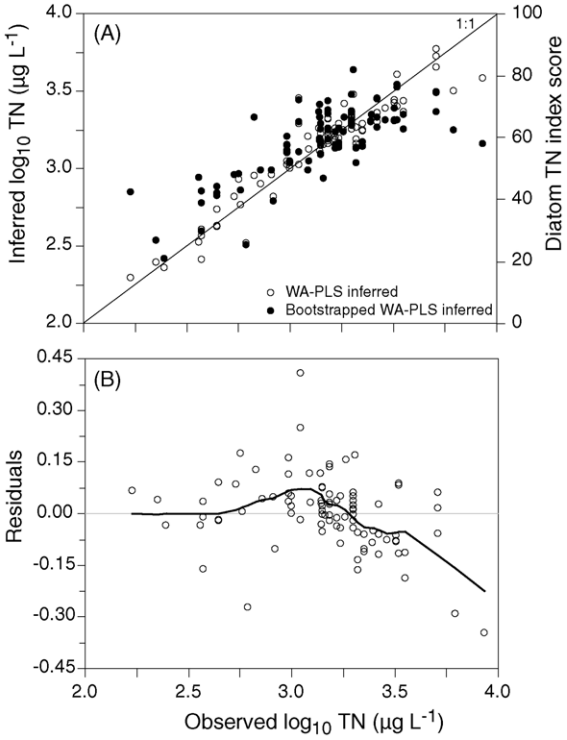

Fig. 4. Plots of WA-PLS inference model showing (A) diatom-inferred $\log_{10}$ TN values vs. observed $\log_{10}$ TN values, and (B) residuals vs. observed $\log_{10}$ TN values. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45). The diatom TN index score is shown for comparison.

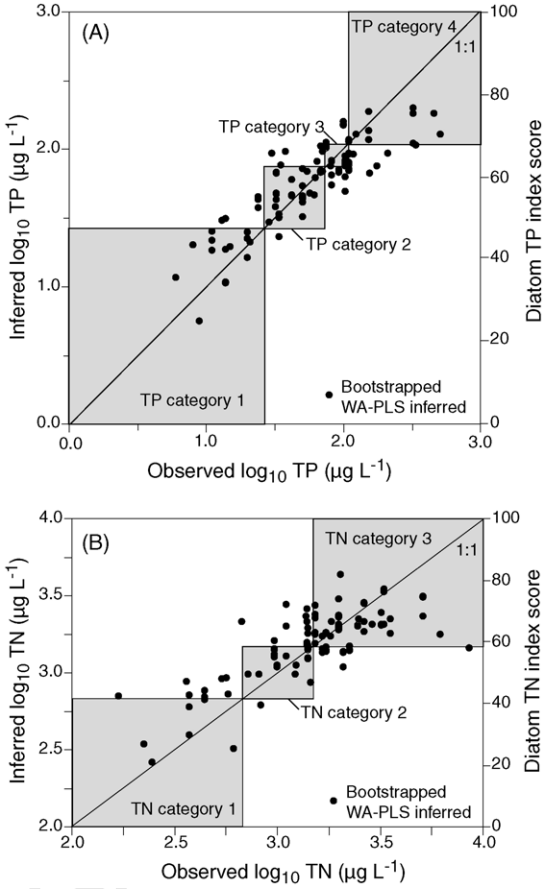

Fig. 5. Plots of inferred bootstrapped versus observed TP and TN and the assignment of the two diatom index scores: (A) the diatom TP index and (B) the diatom TN index to the corresponding nutrient categories. Shaded rectangles correspond to the water chemistry categories established for TP and TN. TP categories are: (1) <0.025 mg L^{-1} TP, (2) 0.025–0.075 mg L^{-1} TP, (3) 0.075–0.1 mg L^{-1} TP, (4) >0.1 mg L^{-1} TP; TN categories are: (1) <0.7 mg L^{-1} TN, (2) 0.7–1.5 mg L^{-1} TN, (3) >1.5 mg L^{-1} TN.

3.7. Diatom TP and TN indices

We evaluated how accurately the index values, calculated using bootstrapped inferred nutrient concentrations, could be assigned to the nutrient categories based on the TP and TN values measured at each site (Fig. 5A and B). The Diatom TP Index correctly assigned the majority (63%) of the samples. The TP categories 1 and 3 had the highest proportion of samples correctly assigned, whereas categories 2 and 4 had the highest proportion of samples assigned to a neighboring category. The Diatom TN Index correctly assigned the majority (68%) of the samples to the correct categories, and placed 32% of the samples into neighboring categories. The Diatom TN index correctly assigned the highest proportion of samples to TN category 1. The indices showed least accuracy placing samples in the TP categories 2 and 4 and in the TN categories 2 and 3, which are the categories with the highest number of samples.

4. Discussion

4.1. Environmental factors influencing diatom assemblages

Multivariate analyses showed that nutrient concentrations (TP and TN) are important in explaining

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variation in diatom assemblage composition, more so than in other regional studies (Pan et al., 1996; Winter and Duthie, 2000). Our analysis shows that TP and TN were strongly correlated with the first CCA axis ($r = -0.72$ and -0.61). The results of CCAs constrained to TN and TP, respectively, showed a strong relationship between TN, TP, and diatom assemblages with high λ_1/λ_2 ratios of 0.78 for TP and 0.54 for TN. Other regional studies present lower λ_1/λ_2 ratios for TP (0.21) and TN (0.32) (Winter and Duthie, 2000), ratios comparable to the ones presented in this study are recorded for TP ($\lambda_1/\lambda_2 = 0.81$) from Finland (Soininen and Niemelä, 2002). This high ratio reflects the relatively even distribution of nutrient concentrations measured in the streams in this study, and the narrow range in other factors usually influencing diatom distributions such as pH and conductivity.

4.2. Diatoms as nutrient indicators-inference model performance

Compared to similar studies conducted using one-time chemistry measurements to develop benthic diatom nutrient indicators for streams in eastern North America (Pan et al., 1996; Winter and Duthie, 2000; Potapova et al., 2004), the TP and TN WA-PLS models presented here ($n = 91$) show high apparent and bootstrapped r^2 and low apparent and bootstrapped RMSE.

We took two steps to maximize the performance of our models. First, site selection was restricted to three ecoregions in New Jersey with a rather limited range in water chemistry and geology. Sites were selected based on knowledge of their chemistry to avoid wide ranges of pH, alkalinity and conductivity that might complicate development of nutrient inference models. Second, we tried to capture a wide range of concentration along the nutrient gradients. This dataset included a TP range of 6–458 $\mu g\ L^{-1}$ ($n = 91$), and the range in TN was 170–8547 $\mu g\ L^{-1}$. The nutrient gradients included in all models were relatively large, and the model error is low when compared to other studies based on comparable or shorter nutrient gradients (Pan et al., 1996; Potapova et al., 2004). The dataset presented in Winter and Duthie (2000) included a shorter TP gradient (5–215 $\mu g\ L^{-1}$) and a wide TN gradient, with a range of relatively higher values (1000–16,290 $\mu g\ L^{-1}$), but the models show higher RMSEs when using one-time chemistry measurements.

There are other factors that might affect the performances of our models. These include the influence of temporal variability in nutrient concentrations in streams (Cattaneo and Prairie, 1995; Pan et al., 1996) and the indirect influence of nutrients on diatom species composition through increasing competition with non-diatom species (e.g. *Cladophora* sp.) (Winter and Duthie, 2000).

Despite the relatively wide ranges in TP and TN captured in our models, model performance could certainly be improved by adding samples to the dataset. Fig. 4A and B shows that fewer numbers of samples at the low and the high total phosphorus range cause the TP model to be less reliable towards both ends of the gradient. This "edge-effect" (Hall and Smol, 1999) occurs with models developed on samples with an uneven distribution along the TP gradient (Reavie et al., 1995; Soininen and Niemelä, 2002), 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 fell below concentrations of 100 $\mu g\ L^{-1}$ TP. This might explain why the model is less reliable at concentrations of $>100\ \mu g\ L^{-1}$. Another explanation may be that above higher concentrations of TP (e.g. concentrations of $>100\ \mu g\ L^{-1}$) smaller 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). Addition of more samples toward the ends of the gradients might help improve the TP model performance, although significant improvement is more likely at the lower nutrient range of the model; our results show that diatom assemblages respond less strongly to TP values over 100 $\mu g\ L^{-1}$.

A similar trend is shown for the TN model, where model performance decreases at the higher end of the gradient (Fig. 4A and B). The average TN in the Piedmont (2241 $\mu g\ L^{-1}$) and the combined dataset (1987 $\mu g\ L^{-1}$) (Table 1) is low compared to the average TN (4940 $\mu g\ L^{-1}$) in the EPA Northern Piedmont aggregate nutrient ecoregion (USEPA, 2000c), 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

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underrepresents sites with low and high nitrogen. This explains the decrease of the TN model performance, especially at the higher end of the gradient. To improve the TN model performance, it would be important to add samples to both ends of the nitrogen gradient, but especially to the higher end.

In general, our TP and TN inference models have relatively 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 1).

4.3. Use of diatom inference models and trophic indices for management purposes

Our results show that diatom WA-PLS models can reliably infer late summer nutrient concentrations in NJ rivers. 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-equations provide the best predictions when used to model regional diatom–nutrient responses (Potapova et al., 2004).

The diatom TP and TN indices, which are rescaled inferred nutrient values, are meant to provide a management tool for regulators. Because of their presentation on a scale from 1 to 100, 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 (NJDEP, 1993a, 2000). They are similar to commonly used diatom indices, such as trophic diatom indices (TDIs), with the main difference that the indices presented here are based on WA-PLS inference models. When tested, the diatom TP and TN indices assigned the samples to the correct water chemistry category in the majority of cases. Because the boundaries between the categories assigned are based on correlations between nutrients and high algal biomass (Dodds et al., 1998; Wetzel, 2001), the categories not only reflect nutrient concentrations but at the same time are estimates of impairment associated with high biomass (i.e. rendering the waters unsuitable for designated uses). The assignment of samples to nutrient categories may therefore provide NJDEP with a practical tool for regulatory assessments of eutrphication.

In addition to the categories, there are other useful reference points for evaluating inferred nutrient concentrations, which 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). We showed previously that TP values above 0.05 mg L^{-1} and NO_3-N values above 0.2 mg L^{-1} can lead to nuisance algal biomass (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. Therefore, both the inference models and the diatom TP and TN 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 both ends of the nutrient gradients.

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USEPA (1998, 2000a).

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