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Quantifying species indicator values for trophic diatom indices: a comparison of approaches

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Abstract

This study compares two approaches for constructing diatom-based indices for monitoring river eutrophication. The first approach is based on weighted averaging of species indicator values with the underlying assumption that species have symmetrical unimodal distributions along the nutrient gradient, and their distributions are sufficiently described by a single indicator value per species. The second approach uses multiple indicator values for individual taxa and is based on the possibility that species have complex asymmetrical response curves. Multiple indicator values represent relative probabilities that a species would be found within certain ranges of nutrient concentration. We used 155 benthic diatom samples collected from rivers in the Northern Piedmont ecoregion (Northeastern U.S.A.) to construct two datasets: one used for developing models and indices, and another for testing them. To characterize the shape of species response curves we analyzed changes in the relative abundance of 118 diatom taxa common in this dataset along the total phosphorus (TP) gradient by fitting parametric and non-parametric regression models. We found that only 34 diatoms had symmetrical unimodal response to TP. Among several indices that use a single indicator value for each species, the best was the weighted averaging partial least square (WA-PLS) inference model. The correlation coefficient between observed and inferred TP in the test dataset was 0.67. The best index that employed multiple indicator values for each species had approximately the same predictive power as the WA-PLS based index, but in addition, this index provided a sample-specific measure of uncertainty for the TP estimation.

Introduction

Diatoms are widely used in bioassessments, and a substantial number of 'diatom indices' have been developed for estimation of water quality in various geographic areas. Despite their apparent diversity, most of the contemporary 'diatom indices', e.g., the 'Trophic Diatom Indices' of Hofmann (1994) and Kelly & Whitton (1995), the saprobic index of Sladeček (1973), the 'Pollution Index' of Descy (1979), and the 'Generic Diatom Index' (Coste & Aypassorho, 1991), are in essence numerical models that use species indicator values (related to species optima) as predictors of water quality parameters. In this respect they are identical to the weighted averaging (WA) inference models widely used to infer environmental parameters from biological assemblages (Pan et al., 1996; Hall & Smol, 1999). The difference between a WA inference model and an index is essentially the numerical scale on which species indicator values are expressed and estimations are made. In inference models, species indicator values (termed 'optima') are absolute values of the parameter that is estimated, while in the 'index,' species indicator values are usually expressed on a convenient scale, for example ranging in value from 0 to 5 or from 0 to 10. The algorithm to calculate the value of an index is usually a weighted averaging equation (Sladeček, 1973; Kelly & Whitton, 1995). The underlying assumption of inference models or indices based on weighted averaging of species indicator values is that the shapes of species response curves along the environmental gradient are unimodal and symmetrical. The notable exception to the use of a single indicator value per species is the saprobic index proposed by Zelinka & Marvan (1961). They did not assign aquatic organisms to a single category, i.e. did not specify an optimum or a single indicator value for each taxon. Instead, Zelinka & Marvan (1961) assigned the relative probabilities of occurrence of each taxon in each of five categories of saprobity. By providing these probabilities, they effectively described species distributions along a saprobity gradient. Distributions of most taxa as described by these multiple indicator values were asymmetrical. The resulting index was not a single value, but rather a set of probabilities that the sample belonged to each of five categories of organic pollution.

Authors of a number of other diatom-based systems of water quality assessment have also recognized that diatom species do not necessarily follow unimodal or symmetrical environmental distribution patterns (Sladeček, 1973; Rott et al., 1997) and have estimated the relative probability of species occurrence across several saprobic zones. Nevertheless, for the sake of simplicity of calculation and representation of results, they based their saprobic indices on a single indicator value for each species.

Recent advancements in statistical methods, such as Generalized Linear Models (GLM) and non-parametric (smoothed) regression techniques, have greatly improved our ability to investigate species responses to the environment. These methods have also revealed that species often have complex response curves, and not linear or symmetrical bell-shaped responses (Bio et al., 1998; Austin, 2002). The aim of this study was to test the idea that more accurate characterization of species response curves could lead to improved methods for water quality assessment. We reasoned that if non-unimodal and asymmetrical distributions are rare, it is acceptable to use indices based on weighted averaging of species indicator values calculated with an assumption of symmetrical bell-shaped response curves, i.e. single indicator value per species (SI). On the other hand, if they are common, it may be more effective to use indices based on multiple indicator values for each species (MI), which would better describe species distributions along environmental gradients.

We sought to develop trophic diatom indices by studying diatom species response to total phosphorus (TP) concentration in the rivers of the Northern Piedmont Ecoregion, USA (sensu Omernik, 1995). This region is among the most densely populated and urbanized in North America, and there is an urgent need to develop algae-based methods to monitor eutrophication in its rivers. TP was chosen as a measure of trophic status because phosphorus is thought to be the most important nutrient responsible for eutrophication in these rivers (Litke, 1999; NJCRP, 2003), and because orthophosphate measurements in this dataset were insufficiently precise.

We modeled species responses to TP by means of parametric and non-parametric regression. Single and multiple species indicator values were obtained from parameters of species response curves and from the rescaled and updated weighted averages calculated in weighted averaging (WA) and weighted averaging partial least squares (WA-PLS) inference models. The best-performing index based on single indicator value per species (SI index) was then compared to the best-performing index based on multiple indicator values (MI index).

Methods

Study sites and sampling

The Northern Piedmont dataset consisted of diatom counts for 155 samples collected from rocks at 118 river sites in 1994–2001 (Fig. 1). The samples were collected as part of four water-quality assessment projects: (1) the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program, (2) a study designed to develop algal indicators of river eutrophication in the state of New Jersey, and (3) two studies on the influence of riparian reforestation on streams in suburban watersheds in Pennsylvania. Algal samples were collected in June–September during periods of relatively stable flow. NAWQA algal samples were collected following the protocols described by Porter et al. (1993). Samples from other three projects were collected similarly to NAWQA samples, each sample representing a mixture of algae scraped from several randomly chosen rocks representing typical algal cover for the sampling reach. Most of the sampling sites were rather shallow wadeable streams, and rocks were taken across the whole river channel. Only a few sites in the NAWQA dataset were non-wadeable rivers, where rocks were collected from the areas close to banks. Water chemistry samples were collected either simultaneously with algal sample

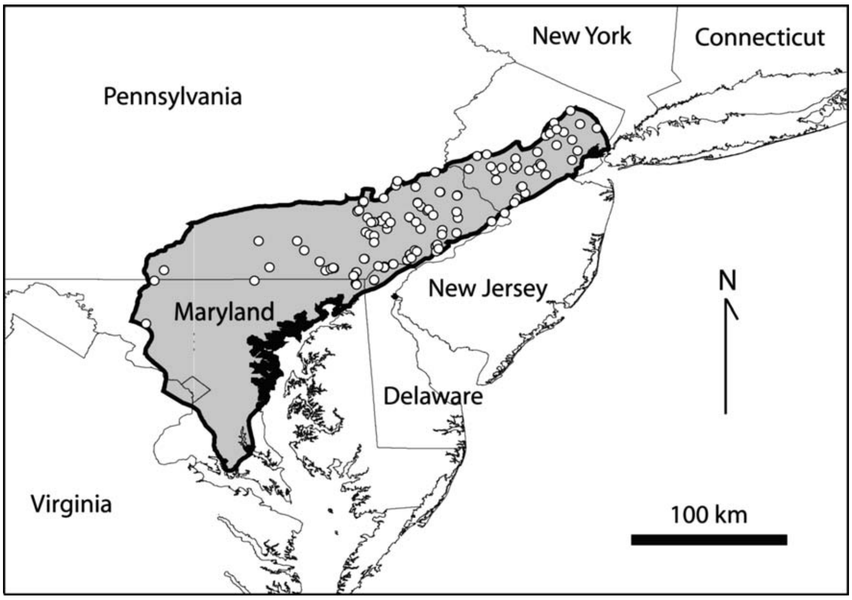

Figure 1. Location of the sampling sites in the Northern Piedmont Ecoregion, USA.

Table 1. Selected characteristics of 118 sampling sites in the Northern Piedmont Ecoregion.

Parameter	Minimum	1st quartile	Median	3rd quartile	Maximum
Conductivity (μS cm^{-1})	115	206	265	368	1339
pH	5.7	7.3	7.6	8.0	8.6
Total phosphorus (μg l^{-1})	6	25	46	92	761
Total nitrogen (μg l^{-1})	360	1250	1900	3126	11136
Watershed area (km^2)	0.8	12	24	79	2121

collection or within one month preceding or following algal sampling.

The purpose of selecting sites within a single ecoregion was to eliminate extreme variation in physical and natural geochemical river characteristics. Most sampled rivers had circumneutral or slightly alkaline water of moderate salt content but varied substantially in their nutrient status (Table 1). Nutrient concentrations did not correlate strongly with other environmental parameters known to influence diatom assemblages. The highest correlations were observed between TP and conductivity (0.40) and TP and watershed area (0.31).

Chemical analyses were performed at the USGS National Water Quality Laboratory, Lakewood, CO (Fishman, 1993) and in the Patrick Center Biogeochemistry Section (ANSP) under the direction of Dr D. Velinsky, following methods described in APHA (1992).

Preparation of permanent diatom slides and diatom counts followed protocols established in the Patrick Center Phycology Section, ANSP (Charles et al., 2002). Light microscope images of the identified diatom taxa are available at http://diatom.acnatsci.org/AlgaeImage.

Datasets

We split the initial dataset of 155 diatom samples into a calibration and a test dataset. The calibration dataset included 120 samples, with some of the samples being collected from the same sites twice, during two different years. The test dataset included 35 samples, all collected from independent sites. To select test samples, we sorted all available samples by TP within each of the four project subsets, and then chose every fourth sample from each list. In this manner we ensured that the test dataset was representative of the whole dataset. The values of total phosphorus ex-

Table 2. TP indicator values of diatoms in the Northern Piedmont dataset. Optima estimated from quadratic regression are in parentheses if non-significant. Slope of the linear regression is '−' if species abundance is decreasing, and '+' if it is increasing with TP.

Species	Weighted average (μg l^{-1})	WA-PLS 'updated' optima (μg l^{-1})	Quadratic regression optima (μg l^{-1})	Linear regr. slope	Multiple indicator values in 5 TP (μg l^{-1}) categories					N
					<25	25–63	63–158	158–398	>398	
Encyonema silesiacum (Bleish) Mann	13.49	17.46	4.71	−*	97	3	0	0	0	8
Gomphoneis herculeana (Ehr.) Cl.	17.14	0.22		−	91	7	0	0	2	11
Achnanthidium sp. 10 NAWQA	21.83	1.03		−	78	16	0	0	6	57
Synedra parasitica (W. Sm.) Hust.	23.71	0.03	4.57	−*	69	26	5	1	0	17
Navicula longicephala Hust.	31.19	0.07	(19.95)		50	33	16	2	0	9
Navicula cryptocephala Kütz.	31.26	0.30	(6.46)	−	49	32	16	4	0	47
Gomphonema sphaerophorum Ehr.	31.33	0.44	(2.88)		51	31	15	3	0	13
Encyonema minutum (Hilse) Mann	32.06	34.35		−	52	11	6	16	16	53
Achnanthes subhudsonis var. *kraeuselii* (Cholnoky) Cholnoky	32.14	11.08	9.91*	−	56	31	7	3	4	58
Achnanthidium minutissimum (Kütz.) Czarnecki	33.27	2.49		−	62	22	7	5	3	94
Navicula reichardtiana Lange-Bertalot	34.28	0.05	(30.90)		40	42	18	0	0	15
Frustulia vulgaris (Thwaites) De Toni	34.36	2.23			(43	24	14	9	10)	19
Achnanthidium affine (Grun.) Czarnecki	34.91	20.45			(30	13	34	23	0)	10
Geissleria decussis (Hust.) Lange-Bertalot & Metzeltin	35.08	0.49	(5.89)		(39	29	20	10	1)	23
Nitzschia dissipata (Kütz.) Grun.	35.48	0.28		−	50	25	11	8	7	66
Fragilaria capucina Desmazières	35.97	3.21		−	47	22	14	12	6	42
Synedra acus Kütz.	36.22	11.48			42	17	13	14	14	7
Nitzschia sigmoidea (Nitzsch) W. Sm.	36.48	0.91	(33.11)		(33	38	23	6	0)	17
Navicula antonii Lange-Bertalot	37.07	19.93	(5.75)		(34	31	4	4	27)	12
Surirella angusta Kütz.	37.76	4.88	(0.35)		(41	28	21	9	0)	25
Cymbella tumida (Bréb.) V. H.	38.37	3379.09		+	48	6	2	13	31	26
Eunotia implicata Nörpel et al.	38.55	46.89	(22.91)		(39	33	21	6	0)	11
Navicula tenelloides Hust.	39.45	0.02	(28.18)		36	34	25	4	0	14
Achnanthidium exiguum (Grun.) Czarnecki	40.18	0.55	(33.88)		(29	42	24	4	0)	28
Nitzschia paleacea Grun.	40.83	21.46	(39.81)		26	48	25	0	0	7
Nitzschia fonticola Grun.	41.02	1.59		−	30	18	7	13	33	50
Nitzschia archibaldii Lange-Bertalot	41.21	2.50		−	32	16	8	13	31	40
Karayevia clevei (Grun.) Round & Bukt.	42.76	0.23	(12.59)		(30	26	17	14	12)	15
Surirella minuta Bréb.	43.95	7.39	(35.48)		(27	32	26	14	0)	23
Hippodonta capitata (Ehr.) Lange-Bertalot et al.	44.06	3.10	(5.37E-5)		(32	24	24	18	2)	17
Gomphonema olivaceum (Lyngb.) Kütz.	44.57	3.26			(30	11	2	12	44)	12

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Table 2. Continued.

Species	Weighted average ($\mu g\, l^{-1}$)	WA-PLS 'updated' optima ($\mu g\, l^{-1}$)	Quadratic regression optima ($\mu g\, l^{-1}$)	Linear regr. slope	Multiple indicator values in 5 TP ($\mu g\, l^{-1}$) categories					N
					<25	25–63	63–158	158–398	>398	
Meridion circulare (Grev.) Ag.	44.77	7.74	(46.77)		(25	30	30	16	0)	12
Cymbella affinis Kütz.	46.77	0.35	(60.26)		(17	31	36	15	0)	9
Geissleria acceptata (Hust.) Lange-Bertalot & Metzeltin	46.88	2.42	54.95		(17	40	36	7	0)	21
Thalassiosira weissflogii (Grun.) Fryxell & Hasle	46.99	0.10	(33.11)		(26	34	27	11	1)	16
Nitzschia linearis (Ag.) W. Sm.	47.10	22.66	(53.70)		22	33	32	12	0	19
Mayamaea agrestis (Hust.) Lange-Bertalot	47.53	31.61			(32	23	17	14	13)	8
Meridion circulare var. *constrictum* (Ralfs) V. H.	47.75	5.72	(48.98)		17	49	34	0	0	7
Navicula peregrina (Ehr.) Kütz.	48.08	71.85	(43.65)		18	56	25	0	0	8
Gomphonema parvulum (Kütz.) Kütz.	49.66	55.13			(25	17	15	20	24)	105
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	50.23	8.80	40.74		24	28	25	12	11	56
Amphora veneta Kütz.	50.35	0.56	(36.31)		(24	38	13	4	21)	7
Navicula canalis Patr.	50.58	15.07	(50.12)		(23	29	21	14	13)	20
Pseudostaurosira brevistriata (Grun.) Williams & Round	50.58	3.38	(58.88)		(19	20	42	19	0)	9
Amphora montana Krasske	50.70	6.05	(51.29)		(18	41	33	7	0)	10
Reimeria sinuata (Greg.) Koc. & Stoermer	50.82	13.71	(10.47)	–	31	27	18	14	11	79
Navicula rostellata Kütz.	51.64	20.26	(5.89E-5)		(25	23	17	16	19)	48
Navicula seminuloides Hust.	52.97	2.32	56.23		5	51	43	1	0	8
Caloneis bacillum (Grun.) Cl.	53.21	15.24	72.44		15	29	25	15	16	50
Nitzschia sociabilis Hust.	53.33	2.93			26	10	25	26	12	9
Navicula cryptotenella Lange-Bertalot	53.58	78.23			(20	15	16	23	25)	57
Nitzschia recta Hantzsch	53.95	43.99	(56.23)		19	36	31	14	1	18
Navicula germainii Wallace	54.33	29.55	(81.28)		16	24	20	18	21	71
Amphora inariensis Krammer	54.45	213.60	69.18		18	27	27	22	6	13
Navicula trivialis Lange-Bertalot	55.08	1.51E+05	(63.10)		8	39	43	9	0	10
Navicula gregaria Donkin	55.46	40.55	(83.18)		(20	20	24	21	15)	95
Bacillaria paradoxa Gmelin	55.98	195.30	100		11	22	37	24	6	41
Cocconeis placentula var. *lineata* (Ehr.) V.H.	55.98	42.76	120.23*	+	13	22	23	20	22	109
Navicula capitatoradiata Germain	56.62	13.22	(416.87)		(16	17	24	26	16)	49
Nitzschia capitellata Hust.	57.15	35.25	61.66		11	41	38	11	0	28
Planothidium lanceolatum (Bréb. ex Kütz.) Lange-Bertalot	57.54	25.57	69.18		13	34	34	16	4	86
Nitzschia palea (Kütz.) W. Sm.	57.81	105.34	138.04*	+	12	21	21	25	21	87
Navicula veneta Kütz.	58.21	4068.18			(19	20	12	13	36)	14

Continued on p. 30

Table 2. Continued.

Species	Weighted average (μg l^{-1})	WA-PLS 'updated' optima (μg l^{-1})	Quadratic regression optima (μg l^{-1})	Linear regr. slope	Multiple indicator values in 5 TP (μg l^{-1}) categories					N
					<25	25–63	63–158	158–398	>398	
Mayamaea atomus (Kütz.) Lange-Bertalot	58.61	28.14	(63.10)		15	35	32	15	3	26
Navicula symmetrica Patr.	59.02	39.61	102.33		11	24	27	23	16	55
Gyrosigma acuminatum (Kütz.) Rab.	59.29	0.76	(52.48)		(12	51	34	3	0)	7
Synedra ulna (Nitz.) Ehr.	59.29	27.97	93.33		14	24	27	24	11	61
Encyonema prostratum (Berkeley) Kütz.	59.43	52.02	(199.53)		(9	14	30	35	12)	8
Melosira varians Ag.	60.12	125.92	(363.08)	+	13	17	23	26	21	84
Gomphonema minutum (Ag.) Ag.	60.39	146.69	(165.96)		(14	21	19	22	24)	41
Luticola mutica (Kütz.) Mann	61.09	538.64	(60.26)		17	32	17	18	16	11
Nitzschia amphibia Grun.	62.52	80.00	83.18*	+	16	24	25	19	15	98
Rhoicosphenia abbreviata (Ag.) Lange-Bertalot	62.81	66.80	(158.49)	+	12	19	24	25	20	105
Surirella brebissonii Kr. & Lange-Bertalot	63.53	40.94	(79.43)		(18	20	19	17	26)	9
Navicula minima Grunow	65.01	112.56	112.20*	+	9	23	28	22	18	100
Navicula rhynchocephala Kütz.	65.16	1140.51	(316.23)	+	4	4	26	44	21	12
Nitzschia liebetruthii Rab.	65.31	0.32	(69.18)		7	39	40	14	0	9
Fragilaria vaucheriae (Kütz.) Petersen	65.77	1297.18			(19	19	16	22	24)	31
Gomphonema angustatum (Kütz.) Rab.	65.77	318.79	(95.50)		(13	23	31	28	5)	9
Nitzschia acicularis (Kütz.) W. Sm.	65.92	309.60			(17	20	16	17	29)	12
Navicula lanceolata (Ag.) Ehr.	66.37	739.09	(707.95)	+	10	13	22	31	25	76
Achnanthes conspicua Mayer	66.53	19.98	67.61*	+	3	41	44	13	0	42
Amphora pediculus (Kütz.) Grun.	67.45	36.50	97.72*	+	12	23	30	23	13	79
Diatoma vulgaris Bory	67.45	698.39			13	7	8	21	52	28
Staurosira construens var. *venter* (Ehr.) Hamilton	67.76	505.48		+	11	11	7	19	52	22
Nitzschia inconspicua Grun.	68.08	356.53	891.25*	+	8	16	20	22	35	106
Sellaphora pupula (Kütz.) Mereschk.	68.87	556.16	72.44		8	36	37	15	3	41
Nitzschia frustulum (Kütz.) Grun.	71.12	17.80	81.28		11	29	28	21	11	27
Sellaphora seminulum (Grun.) Mann	71.12	263.21	194.98*	+	4	15	28	27	26	78
Navicula ruttneri var. *capitata* Hust.	71.61	261.04	89.13		7	27	39	23	4	18
Navicula tripunctata (Müll.) Bory	71.61	187.63	(275.42)	+	8	13	27	32	20	37

Continued on p. 31

Table 2. Continued.

Species	Weighted average (μg l^{-1})	WA-PLS 'updated' optima (μg l^{-1})	Quadratic regression optima (μg l^{-1})	Linear regr. slope	Multiple indicator values in 5 TP (μg l^{-1}) categories					N
					<25	25–63	63–158	158–398	>398	
Cocconeis pediculus Ehr.	74.30	218.83	134.90*	+	5	18	32	32	13	47
Cyclotella stelligera Cl. & Grun.	74.30	1706.87			11	8	8	25	47	7
Frustulia rhomboides (Ehr.) De Toni	77.27	55.46	(112.20)		(3	23	37	28	8)	7
Pinnularia microstauron (Ehr.) Cl.	79.07	9990.79	(630.96)		(8	10	27	34	20)	12
Navicula subminuscula Manguin	79.62	728.28	199.53*	+	5	16	23	29	28	57
Tryblionella apiculata Gregory	79.62	267.30	(165.96)		(7	14	30	35	13)	14
Navicula biconica Patr.	81.47	211.40	(2454.71)		(5	7	20	37	30)	9
Navicula shroeteri var. *escambia* Patr.	83.18	10553.58	(660.69)	+	4	7	20	37	33	13
Navicula ingenua Hust.	84.14	127.03	(95.50)		3	27	39	22	8	10
Cyclotella meneghiniana Kütz.	84.33	1717.12	218.78*	+	1	9	31	35	24	63
Amphora copulata (Kütz.) Sch. & Arch.	84.92	17959.74			(8	12	18	26	35)	18
Navicula perminuta Grun.	85.70	3686.38	(3.24E+6)	+	3	13	14	18	52	17
Gyrosigma spencerii (Quekett) Griffith & Henfrey	86.50	13851.60	(112.20)		2	23	35	28	11	7
Navicula erifuga Lange-Bertalot	86.50	18672.39	(302.00)		(5	20	16	18	41)	19
Cyclotella pseudostelligera Hust.	88.92	13283.12	(831.76)		(6	17	16	23	38)	19
Aulacoseira granulata (Ehr.) Simonsen	92.47	46291.37	(478.63)		(5	14	19	27	36)	13
Staurosira construens (Ehr.) Williams & Round	98.63	19098.53	(8.71E+6)		(4	5	18	33	40)	10
Diadesmis confervacea Kütz.	99.77	1.94E+05	165.96*	+	0	9	34	42	15	13
Staurosirella pinnata (Ehr.) Williams & Round	103.28	38097.81	169.82*	+	0	11	33	36	20	28
Luticola goeppertiana (Bleisch) Mann	105.68	3.87E+06	870.96	+*	3	3	21	38	35	19
Cocconeis placentula var. *euglypta* Ehr.	109.14	1.30E+05	239.88*	+	0	5	26	42	27	15
Gomphonema kobayasii Koc. & Kingston	114.82	10468.87	524.81*	+	1	4	19	34	42	45
Achnanthes stewartii Patr.	117.76	2.18E+06	218.78*	+	0	3	30	46	22	7
Gomphonema gracile Ehr.	123.31	7.52E+05	(1.78E+21)		3	7	13	28	49	7
Cyclotella atomus Hust.	128.53	6.50E+06		+	3	3	10	29	55	14
Stephanodiscus hantzschii Grun.	131.52	9.11E+06		+	3	2	6	30	59	7
Navicula recens Lange-Bertalot	141.91	4.19E+05	22387.21	+*	1	4	12	30	54	11

*Indicates the most parsimonious parametric model if both linear and quadratic regression were significant. Multiple indicator values are in parentheses if they were not used in the final MI index. N = Number of species occurrences in the 120 sample calibration dataset.

pressed in μg l^{-1} were log-transformed prior to the analyses to approximate normal distribution of this parameter.

Evaluation of species response curves

Species responses to TP were first studied by fitting parametric regression models using a Generalized Linear Modeling (GLM) approach. A significant fit to a quadratic (second-order) model implied a symmetric bell-shaped (Gaussian) response curve. A statistically significant linear (first-order) model indicated monotonically decreasing or increasing species abundance along TP gradient. 'Generalized linear model' does not imply a straight-line response. It is linear in that it consists of a linear combination of explanatory variables and can describe non-linear relationships between response and explanatory variables. The procedure GENMOD in SAS statistical software (SAS Institute, version 8.0, Cary, North Carolina) was used to fit a model by maximum likelihood estimation of the regression parameters. We created models with Poisson error distribution and log link function that are appropriate for count data, and tested for significance of parameter estimates. When both linear and quadratic models were significant, we selected the most parsimonious model by estimating the drop in residual deviance, which occurred after adding the quadratic term. If this drop was larger than 3.84, which equals $\chi_{0.05}$ at one degree of freedom, then the quadratic model was considered as providing significant improvement over the linear model in explaining species distribution and was selected as the best (ter Braak & Looman, 1995).

To better characterize species responses to TP, especially for those taxa not having a symmetrical response, we also fit non-parametric (smoothed) models. Smoothing techniques are increasingly used to study species distributions along environmental gradients (Bio et al., 1998). Non-parametric modeling is helpful when species response is expected to have a complex form, or there is no reason to suggest a particular model. To fit non-parametric models we used the TPSPLINE procedure in SAS 8.0, which employs the penalized least squares method. TPSPLINE computes thin-plate smoothing splines (Wahba & Wendelberger, 1980) to approximate smooth multivariate functions. Being a smoothing technique, TPSPLINE does not assume any particular shape of the response curve and makes it possible to reveal asymmetrical and complex species responses. The amount of smoothing can be selected either subjectively by setting the degree of freedom (DF) option to some chosen value, or by using a generalized cross validation (GCV) function, which selects the best (most parsimonious) model combining lowest complexity and highest predictive power. We used two approaches. In the first, we created a set of models by subjectively setting the DF option to 4 for all the species. In the second, we produced another set of models by allowing the program to select the best model by GCV function, but limiting the highest possible DF to 4. This is because more complex curves resulting from models with higher DF most likely model noise and have little biological meaning. Although there is no established limit for the complexity of the biological response curve, the complexity of 3 or 4 DF has also been chosen in other studies (Bio et al., 1998).

Species indicator values

We used three main methods to obtain a single indicator value for each diatom species. First, we calculated species optima from the parameters of quadratic regression as described in ter Braak & Looman (1995). Second, we obtained a single indicator value for each species by calculating an average TP value using data for all the sites where the species was present, weighted by species relative abundance (WA indicator value, or 'apparent' WA optimum). This method does not assume any particular shape of species response curve, but in cases where distributions are unimodal, the weighted average is expected to approximate the regression-estimated species optimum (ter Braak & Looman, 1995). Third, we used optima derived from WA inference models. Apparent WA optima (described above) are usually not used directly in inference models, but undergo transformations, such as rescaling in WA models, or 'updating' as in WA-PLS modeling (ter Braak & Juggins, 1993). These transformations produce indicator values that provide the best predictions when used in WA equations. We used the 'inverse deshrinking' option to calculate 'rescaled' optima, and the WA-PLS option to quantify 'updated' optima or 'beta' in the CALIBRATE program (Juggins & ter Braak, 1993).

To derive multiple indicator values for each taxon, we used the TPSPLINE smoothed response curves to calculate relative probability of species occurrence in five categories of TP concentration. Most of the samples in our dataset had corresponding log TP value between 1 and 3. We established five TP categories

Table 3. Performance of two-component weighted-averaging partial least squares regression models (WA-PLS) for inferring log TP, μg l^{-1}. *R* – correlation coefficient between inferred and observed TP values, RMSEP - root mean squared error of prediction. 'Cross-val' – statistics derived from cross-validation (jack-knifing) of the calibration dataset, 'test' – statistics derived from applying the models to the 35 sample test dataset. 9 outlier samples were those with a residual distance in the initial model greater than 0.4 log TP, μg l^{-1}.

Calibration dataset	*R* cross-val	RMSEP cross-val	*R* test	RMSEP test
120 Sample dataset	0.68	0.33	0.67	0.33
111 Sample dataset: 9 outlier samples excluded	0.77	0.25	0.68	0.33

by dividing this range by 5, and expanding the lowest category to zero and the highest to indefinitely high TP concentration. The resulting categories had log TP values (a) less than 1.4, (b) 1.4–1.8, (c) 1.8–2.2, (d) 2.2–2.6, and (e) higher than 2.6, or respectively (a) less than 25, (b) 25–63, (c) 63–158, (d) 158–398, and (e) higher than 398 μg l^{-1} TP. Each species received five indicator values, which were percent probabilities of species occurrence in each TP category, calculated as area under the smoothed curve in between vertical lines drawn at the TP values separating those five TP categories.

Development of inference models and indices

Canonical correspondence analysis (CCA) was used to determine the significance of TP in accounting for differences in composition of the diatom assemblages in the dataset. Detrended correspondence analysis (DCA) was employed to identify possible outliers, or samples with unusual species composition (Birks et al., 1990). CCA and DCA were carried out in the CANOCO 4.5 program (ter Braak & Šmilauer, 2002).

To select the best SI index we compared indices based on indicator values quantified as (1) modes of bell-shaped response curves calculated from parameters of quadratic regression, (2) 'rescaled' WA optima of WA inference model with inverse deshrinking, (3) 'updated' WA optima, or 'beta' of WA-PLS inference model. The value of each SI index was calculated by averaging species indicator values weighted by species relative abundance.

Multiple indicator values derived from smoothed species response curves were used as predictors to estimate the probability that a sample belongs to each of the established TP categories. This MI index provides probabilistic estimation in the same way as the saprobic index of Zelinka & Marvan (1961), although without using any measure of tolerance or indicator weight. The value of the MI index for each TP category is calculated by summing up products of each species' relative abundance by its indicator value. We compared performance of the MI index using multiple indicator values derived from smoothed curves (1) with equal degree of complexity, when DF option was set to 4, and (2) with varying degrees of complexity chosen by GVC function, but not higher than DF = 4. We also evaluated how the MI index performed when certain species lacking definitive response to TP were eliminated from the calculation.

Predictive abilities of the SI and MI indices were evaluated using the number of samples from the test dataset that were correctly assigned to their TP category and by rank correlation between the observed and predicted TP categories.

Results

Composition of diatom assemblages

The complete dataset included 368 diatom taxa, but only the 118 diatoms that occurred in at least 7 samples in the 120 sample calibration dataset were retained for analyses (Table 2). The most common taxa were *Cocconeis placentula* var. *lineata, Nitzschia inconspicua, Gomphonema parvulum, Rhoicosphenia abbreviata, Navicula minima, Nitzschia amphibia*, and *Navicula gregaria*. These diatoms are known as cosmopolitan, widely distributed in inland waters, and considered as indicators of eutrophic conditions (Van Dam et al., 1994). Only six of the 118 species would be classified as oligo- and mesotraphentic species in Van Dam's system, while the vast majority would be categorized as eutraphentic diatoms.

Species response curves

Only 34 of the 118 diatom taxa had a symmetrical unimodal response to TP, displaying significant ($P < 0.05$) fit to the quadratic regression (Table 2). Some examples of species with a significant fit to a symmetrical unimodal curve are shown in Fig. 2.

Forty-two diatoms had a monotonic response to TP based on their significant fit to the linear GLM models. When graphed, these responses appear as exponential curves because of the use of log-link function (Fig. 3). Twenty-one of those species also had a significant fit to a quadratic model. The quadratic model was significantly better for 17 of those taxa, while for the other 4 the drop in residual deviance was not high enough (less than 3.84), so the preferred parametric model was still linear (Table 2).

Some diatoms (e.g., *Navicula subminuscula*, *N. minima*, and *Gomphonema kobayasii*) had strongly asymmetrical complex response curves (Fig. 4), as revealed by thin-plate spline smoothing. Several species (e.g., *Sellaphora seminulum, Navicula tenelloides*) had smoothed response curves that suggest a threshold type of response to TP (Fig. 4). Most of the species with significant linear response in GLM, such as *Achnanthidium minutissimum*, *Fragilaria capucina*, and *Synedra parasitica* had smoothed response curves close to linear or exponential (Fig. 4). A number of species (*Mayamaea atomus*, *Navicula ingenua*, *N. seminuloides*, *Nitzschia capitellata*, *Gyrosigma acuminatum*, *Achnanthes conspicua*, *A. stewartii*, *Planothidium lanceolatum*, *Meridion circulare*) had smoothed responses similar to bell-shaped curves, although the curve in most cases was at least slightly skewed. Complex non-parametric models based on high DF invariably provided better fit (lower residual deviance) than parametric linear and quadratic regression models.

Relative abundance of 43 species did not follow any of the expected standard patterns of ecological response to TP (e.g., unimodal or strongly asymmetrical). For these species, both first- and second-order parametric models were statistically insignificant and the GCV function selected the non-parametric model with highest possible degree of smoothing ($DF = 2$), thus failing to reveal any response pattern different from a straight line. Several species had a bell-shaped response curve with a minimum instead of maximum (inverted bell shape), which is difficult to interpret. This group of 43 species included both diatoms with relatively low number of occurrences (*Amphora veneta, Frustulia rhomboides, Gyrosigma acuminatum*), and some of the most common species (*Gomphonema parvulum*, *G. minutum*, *Navicula gregaria*, *N. capitatoradiata*, *N. rostellata*, and *N. veneta*). For the common species, lack of an explicit response to changes in phosphorus concentration obviously cannot be explained by their poor representation in the dataset.

Species indicator values

Optima estimated from bell-shaped curves for the 34 species with significant fit to a quadratic model varied from 10 $\mu g\ l^{-1}$ TP for *Achnnathes subhudsonis* var. *kraeuselii* to 891 $\mu g\ l^{-1}$ TP for *Nitzschia inconspicua* (Table 2). Optima were also estimated from the parameters of quadratic regression for an additional 57 species with non-significant fit to a quadratic model. These optima often lay outside the range of measured TP values and varied from negligibly small ($<0.1\ \mu g\ l^{-1}$ TP for *Navicula capitata* and *N. rostellata*) to meaninglessly high ($2 \times 10^{21}\ \mu g\ l^{-1}$ TP for *Gomphonema gracile*). The optima for the remaining 27 species could not be estimated from the parameters of quadratic regression either because the curve had a minimum instead of a maximum, or because the maximum likelihood algorithm failed to converge.

Species indicator values calculated as weighted averages ranged from 13 $\mu g\ l^{-1}$ TP for *Encyonema silesiacum* to 142 $\mu g\ l^{-1}$ TP for *Navicula recens*. The relative abundance of most species with relatively low weighted averages, such as *Achnanthidium* sp. 10 NAWQA and *Navicula cryptocephala* decreased exponentially with increasing phosphorus concentration (Fig. 3). Taxa with high weighted averages often increased exponentially in relative abundance along the TP gradient (*Navicula recens*, *N. tripunctata*, *N. lanceolata*, *Luticola goeppertiana*, Fig. 4). Weighted averages of common diatoms (those occurring in at least one third of all samples) that did not display any meaningful response to TP were all in the middle range of 50–60 $\mu g\ l^{-1}$ TP.

Multiple indicator values derived from smoothed response curves are represented as probabilities of species occurrence at sites within five TP categories (Table 2, Fig. 4).

Inference models and indices based on single indicator values per species

A Monte-Carlo permutation test in CCA with TP as the only constraining variable proved that TP was sig-

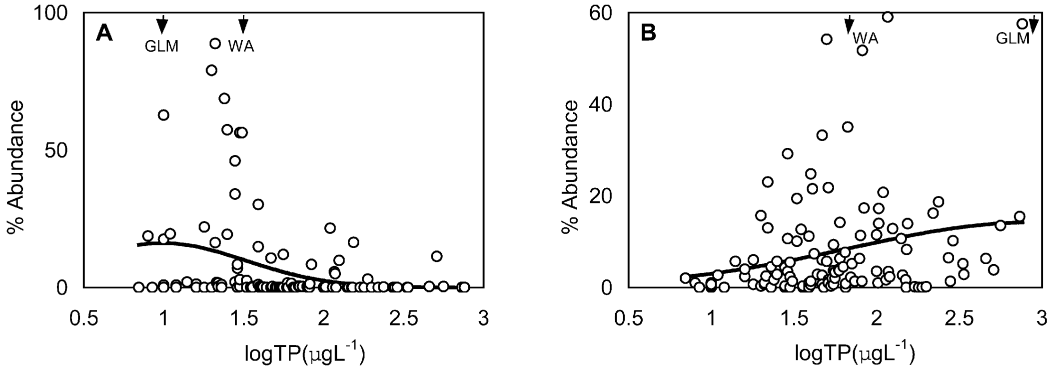

Figure 2. Relative abundance and modeled unimodal response of *Achnanthes subhudsonis* var. *kraeuselii* (A) and *Nitzschia inconspicua* (B) to TP. Arrows show species indicator values calculated as weighted averages (WA) and estimates from parameters of quadratic regression (GLM).

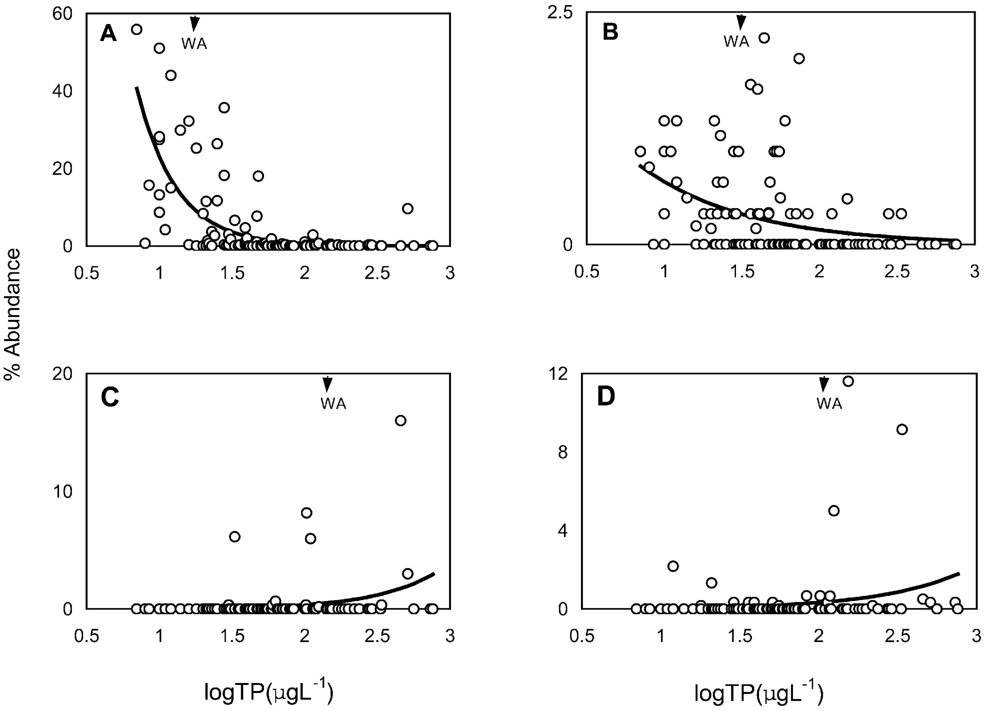

Figure 3. Relative abundance and modeled linear response of *Achnanthidium* sp. 10 NAWQA (A), *Navicula cryptocephala* (B), *Navicula recens* (C), and *Luticola goeppertiana* (D) to TP. Arrows show species indicator values calculated as weighted averages (WA).

nificant ($P < 0.05$) in explaining variability among the diatom assemblages, thus indicating that it is possible to develop a TP inference model from our dataset.

Two component WA-PLS inference models performed better than simple WA models and WA models with tolerance downweighting. Only WA-PLS model results are reported here. The WA-PLS model had moderate predictive power when all 120 calibration samples were used in the calibration dataset (Table 3, Fig. 5). It is common practice to improve inference models by removing outlier samples that are either associated with unusual environmental conditions or that have unique species composition. We identified outliers using criteria suggested by Birks et al. (1990), and removed samples with a residual distance in the initial model greater than 0.4 log TP (μg l^{-1}). Nine samples were taken out based on this last criterion. No sample was found to be an extreme outlier on structural DCA axes, or to have extreme residual distance from the TP axis in CCA with TP as the only environmental variable. The removed samples, there-

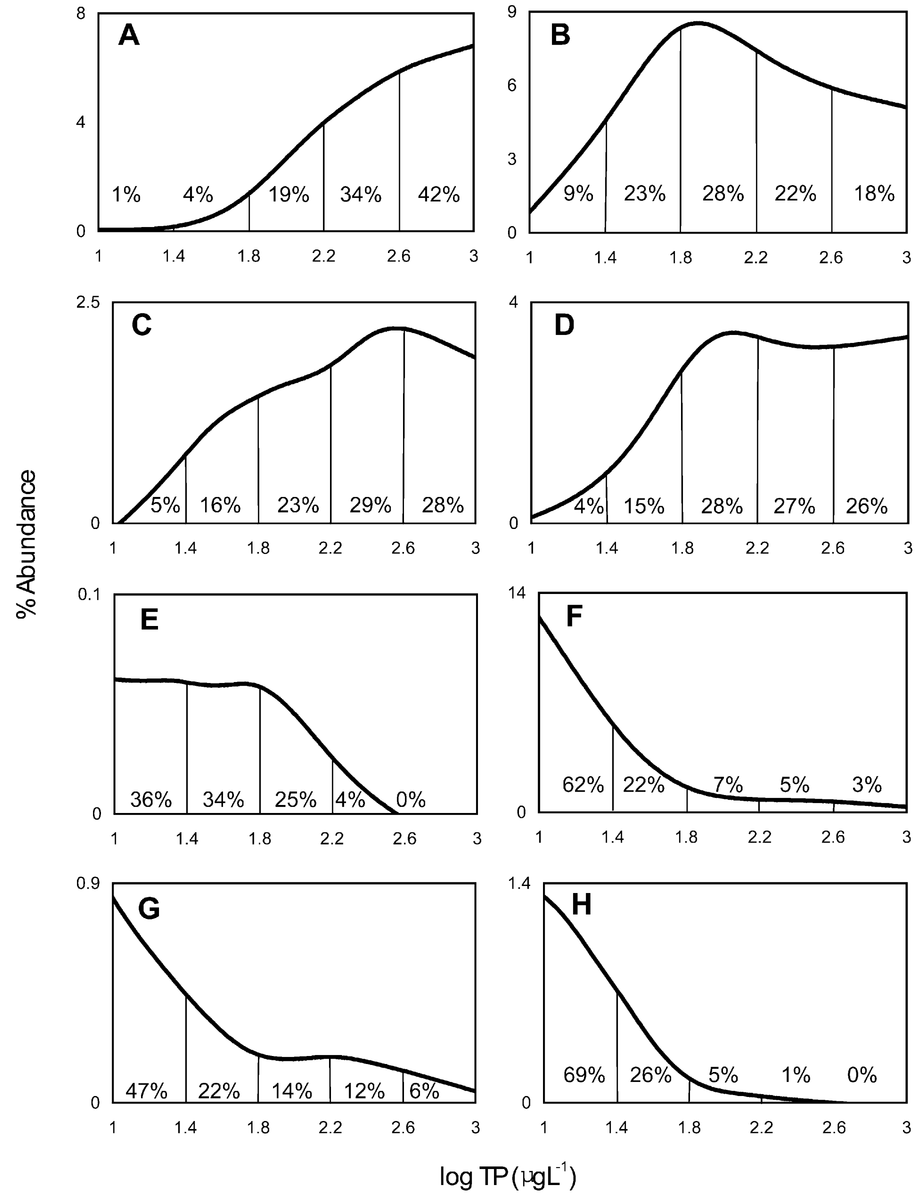

Figure 4. Smoothed responses of *Gomphonema kobayasii* (A), *Navicula minima* (B), *N. subminuscula* (C), *Sellaphora seminulum* (D), *Navicula tenelloides* (E), *Achnanthidium minutissimum* (F), *Fragilaria capucina* (G), and *Synedra parasitica* (H) to TP, and probabilities of their occurrence in five TP categories.

fore, differed for the other samples of the dataset not so much in their species composition, but in the assemblage response to TP, perhaps due to the influence of other environmental factors. The predictive power of the resulting model, judged by correlation between observed and inferred TP values in the test dataset, improved only marginally (Table 3).

To represent the results of the WA-PLS model based on the 120 sample calibration dataset in the form of a simpler ‘index’, we converted the inferred value of log TP to an ordinal scale of the above mentioned arbitrary five TP categories. This SI index correctly assigned 17 out of 35 test samples to their observed TP categories, and placed 15 samples in the neighboring category (Fig. 5). The rank correlation coefficient between observed and diatom-estimated TP category was 0.55.

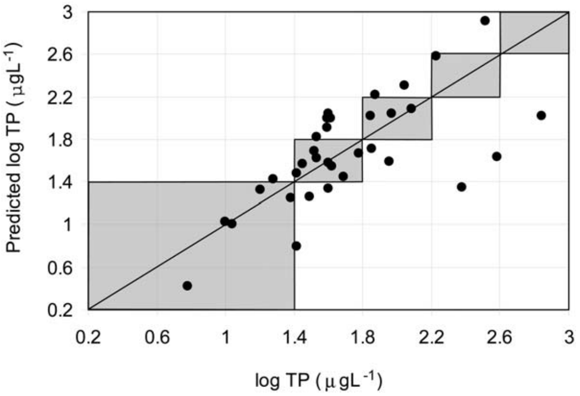

Figure 5. Plot of predicted versus observed TP values for two component WA-PLS regression and calibration model applied to 35 independent test samples. Shaded rectangles correspond to the five arbitrarily established TP categories. Circles within shaded rectangles represent samples correctly assigned to their TP categories.

A SI index based on weighted averaging of 91 species optima estimated from quadratic regression (34 species with significant plus 57 species with nonsignificant quadratic fit) had lower quality ($R_{\text{test}} = 0.36$, RMSEP = 0.56) compared with the inference models/indices based on WA optima estimates (Table 3). This is evidently the result of using optima estimated from response models that were not statistically significant. If only the taxa with significant optima estimates were used in the weighted averaging equation, the correlation coefficient between the observed and inferred TP in the test dataset (Rtest) increased to 0.42. This correlation is still relatively low because the model is based on 34 species only, and a number of good indicator species that had linear or more complex responses were not included in the model.

Indices based on multiple indicator values for each species

TP was inferred in 35 test samples by using multiple indicator values. These values were derived from nonparametric regression and expressed as the probability of species occurrence in samples in each of the five TP categories. The probability that a sample belongs to one of the five TP categories is calculated by multiplying the indicator value for each species by its proportion in that sample and summing up those products. Thus, for every sample we obtain five numbers, each being the relative probability that a given sample belongs to one of the five TP categories. For purposes of comparing results of this index with others, the category with the highest probability is considered to be the inferred TP.

The number of correct placements of the test samples in their respective TP categories (the category of the highest predicted probability corresponds to the observed TP value) was highest (14 out of 35 samples) in the case when indicator values were derived from smoothing curves based on DF = 4 (Table 2), and when only 75 species with meaningful response curves were considered as indicators (Fig. 6). Indicator values for the 43 species lacking obvious response to TP, and not included in the calculation of the MI index, are in parentheses in Table 2. The MI index placed eighteen test samples in the category adjacent to the observed TP category. The highest proportion of the correct placements occurred in the categories <25 μg l^{-1} TP and 63–158 μg l^{-1} TP. The rank correlation coefficient between observed and diatom-estimated TP category was 0.60, which is slightly higher than in the case of best SI index.

Discussion

Responses of diatoms to nutrients

In this study we analyzed ‘ecological’ species responses to TP, that is, species response in the presence of other species. Biological species are commonly assumed to have predominantly symmetrical unimodal distribution patterns along environmental gradients (Whittaker, 1967), at least those that are not resource gradients. Ter Braak & van Dam (1989) found that 16 out of 26 common diatom species in their Western European lake dataset had significant fit to a symmetric unimodal pH model. Juggins (1992) fitted unimodal curves to the distributions of 58 out of 92 diatom taxa along the salinity gradient of the Thames estuary. There is no proof, however, that this type of response is also common for resource (e.g., nutrient) gradients. Although physiological responses (i.e. responses in the absence of competition with other species) of algal species to nutrients were shown to have the shape of a rectangular hyperbola (Borchardt, 1996), it is unclear what the shape of ‘ecological’ nutrient response curves might be. Our results suggest that at least some diatom species that are abundant in river benthic assemblages, and often considered important as water quality indicators, may have strongly asymmetrical nutrient response curves. Exponential curves, fitted by linear regression in GLM, are common for diatoms that are

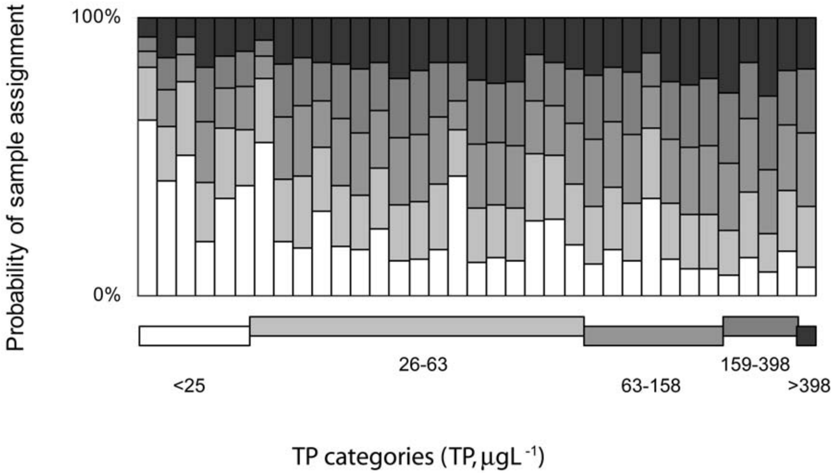

Figure 6. Percent probability for 35 test samples to be placed in five TP categories by using the diatom index based on multiple indicator values. Vertical columns correspond to 35 test samples, ordered by observed TP value, and grouped into five TP categories (horizontal bars).

the best TP indicators in our dataset; for example, *Gomphoneis herculeana* and *Achnanthidium* sp. 10 NAWQA which indicate low TP, or *Luticola goeppertiana* and *Navicula recens*, which indicate high TP. Because ecological species response is partly the result of competition, the shapes of response curves will depend to some extent on the species composition of the calibration dataset, and consequently on the particular environmental and geographical settings of the study sites. Further investigation is needed to study how the shape of response curves for individual species might vary among different datasets, and what factors are most important in determining competitive ability of diatoms.

Diatom inference models developed to reconstruct water pH and salinity (e.g., Birks et al., 1990; Fritz et al., 1999) often have extremely high predictive power. However, nutrient inference models for lakes (review in Hall & Smol, 1999) and rivers (e.g., Pan et al., 2000; Winter & Duthie, 2000) in general have lower predictive power than pH and salinity models. In cases where a model yields high correlation ($r > 0.85$) between observed and inferred nutrient values (as judged by applying the model to test samples or by using cross-validation), a significant collinearity in species responses to nutrients and other factors is usually observed. For instance, such a collinearity between nutrients and ionic content and/or pH is obvious in the ordination results presented by Leland & Porter (2000) and Soininen & Niemelä (2002). The apparently high predictive power of these models might be due to strong response of the diatoms to ionic content and pH, which were correlated with nutrient concentrations. The TP inference model (WA-PLS) developed in our study had moderate power, and could not be improved significantly by deleting outlier samples. Our study suggests that violation of the assumption of the predominantly unimodal species distributions is one of the possible reasons for relatively low nutrient model quality compared with models developed to infer direct environmental factors, although other reasons cannot be excluded. A possible cause of relatively low predictive power of nutrient models is variability of nutrient concentration, which is usually not taken into account because one-time nutrient measurements are used in most studies. In this study, collection of diatom and water chemistry samples was sometimes separated by a significant time, up to one month. Concurrent sampling of diatoms and chemistry would not, however, guarantee that measured nutrient concentration remained the same during the whole period of algal assemblage development. There is a definite need to investigate whether better characterization of nutrient conditions during the growth of diatoms might improve prediction power of models. One more reason for limited performance of nutrient inference models is the simultaneous influence of several environmental parameters on diatom assemblages, with some of the factors (e.g., salinity, pH, flow regime) having stronger effect than nutrients. A possible solution to minimize the effect of these factors is to create separate models for relatively homogeneous sample subsets, but this would undesirably limit applicability of the models.

Non-parametric regression methods provide powerful tools for studying species responses to the environment (Bio et al., 1998) because there is no restriction on the particular mathematical model that should be fit to the data. Although the TPSPLINE procedure used in this study might not prove to be the best technique to model species responses, it was sufficient to demonstrate the high occurrence of asymmetrical responses in our dataset.

A number of diatoms known as good indicators of high nutrient concentration did not show meaningful response to TP in this study, even though their occurrence in the dataset was relatively high. Examples include *Navicula veneta, Gomphonema parvulum*, and *G. minutum,* all of which had deep minima in their response curves. In other words, these species were abundant at sites with lowest and highest TP. If indices or models that use these species as indicators are applied to our dataset, the inferences are likely to be incorrect, or at least the predictive power of these models will be lowered. It is beyond the scope of this study to investigate why species responses to nutrients vary among different studies. Perhaps species physiological response depends on other environmental factors. Species competitive abilities might also vary among communities of different composition. In addition, we cannot exclude the possibility that some taxa include one or more ecotypes, or cryptic species, that are identical or very similar morphologically, but that differ in their physiological requirements. For practical purposes of water quality assessment it is important to note that observed differences in species responses justify the need to develop regional models and indices.

Which index is the best?

Both SI and MI indices provided similar quality of TP estimation for 35 test samples from the Northern Piedmont ecoregion. The MI index has an advantage the SI index does not, however, in that it also provides sample-specific information about the reliability of each estimate. For example, if the probability that a sample belongs to each of the 5 TP categories is 20%, this sample could not be regarded as a good source of information on the phosphorus concentration. In contrast, the error estimates (e.g., RMSE) associated with SI indices (WA inference models) depend only on the quality of the model, and are the same for each sample; they would not indicate large potential error for a sample with a high proportion of taxa that are poor indicators. The method used in paleoreconstructions to assess reliability of estimation for individual fossil or test samples uses analogue measures, i.e. the estimate is considered reliable if the test sample is similar in its composition to the samples in a calibration dataset (Birks et al., 1990). But even this method does not account for the indicator quality of individual taxa.

Estimation of species optima from fitted symmetrical unimodal response curves is sounder theoretically than weighted averaging because both species occurrences as well as absences are taken into consideration (ter Braak & Looman, 1995). However, in practice, only a relatively small proportion of species from our dataset (34 from 118) had a significant fit to such curves. Indices based on these optima could not provide satisfactory TP estimation. The best single indicator values were found to be weighted averages ‘updated’ by the WA-PLS algorithm. These numbers are often far distant from species optima calculated as modes of the bell-shaped curves or simple weighted averages (Table 2), but provide the best TP predictions in the real-life situation when species responses vary in shape and several environmental gradients determine community composition.

The results of this study show that although many species have non-unimodal asymmetrical responses to nutrients, it is possible to regard them as symmetrical and use a WA equation with a single indicator value when the goal is to construct a regional diatom-based index without an exhaustive investigation of these responses. On the other hand, the additional information on species responses provided by MI values could be indispensable for estimating reliability of estimations and for future improvements of the water quality assessment methods. The two types of indices thus might be complementary and used simultaneously to maximize information on the nutrient status of rivers.

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