

## **TROPHIC DIATOM INDICES (TDI) AND THE DEVELOPMENT OF SITE-SPECIFIC NUTRIENT CRITERIA**

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### **ABSTRACT**

In 2004 a four year study performed for NJDEP by the Patrick Center for Environmental Research showed that trophic diatom inference models could be used effectively to assess late-summer nutrient concentrations and benthic algal responses specific to different physiographic provinces in the state. Diatoms are widely recognized and used as indicators of river and stream water quality because benthic diatom species composition responds directly to nutrients and can be a more stable indicator of trophic state than measurements of nutrient concentrations or algal biomass (e.g., chlorophyll a). Objectives of the study were to: 1) develop New Jersey specific field and lab protocols for characterizing eutrophication (nutrient concentrations) in streams using attached periphyton algae, 2) assess the relationships between stressors (i.e., total phosphorus/nitrogen) and overt signs of eutrophication (e.g., algae), and 3) develop biological metrics as potential biocriteria (e.g., diatom community structure and trophic diatom indices - TDI). We chose weighted average inference modeling as our approach because it incorporated the most accurate method for quantifying species response to nutrients. We used nutrient concentrations inferred from the models in two ways: 1) directly, by using the inferred diatom values as estimates of the nutrient concentrations prevailing at the site during the time the algal assemblages were developing, and 2) indirectly, by rescaling the inferred concentrations from 0-100 to create trophic diatom indices (TDI) more easily interpretable by non-specialists. In the current study in 2004 we performed a pilot study to test the TDIs above and below two Sewage Treatment Plant (STP) facilities in New Jersey to evaluate their usefulness as a stressor-response model necessary to generate site-specific biocriteria for nutrients. We conclude that the TP and TN diatom inference models and indices are promising tools to monitor and infer nutrient conditions but that further study is necessary to adequately evaluate their effectiveness for routine use as part of a regulatory program. Diatom community composition differed among sites, and the differences can be explained by variation in nutrient concentrations. Diatom indices indicated relatively high nutrient conditions at all sampling sites, and between-site differences were consistent with measured values. Both the diatom indicators of enrichment and the measured nutrient concentrations showed a marked increase below one STP, but not below the other. This may be because the discharge from one plant was much greater than the other, one-time sampling did not

represent longer-term nutrient conditions, or there are other important, unaccounted-for factors influencing nutrient concentrations.

**KEYWORDS:** Water-quality, criteria, trophic, diatoms, inference models, designated uses, phosphorus, nitrogen, nutrients, algae, periphyton.

## INTRODUCTION

The New Jersey Surface Water Quality Standards (SWQS) include both numeric and narrative (N.J.A.C. 7:9B-1.14(c)) water quality criteria for total phosphorus (TP). The numeric criteria require that stream water TP shall not exceed 0.1 mg/L unless it can be demonstrated that TP is not a limiting nutrient and will not otherwise render the waters unsuitable for their designated uses. Specific examples of how waters may be rendered unsuitable is given in narrative SWQS polices at N.J.A.C. 7:9B-1.5(g)2, which states that “except as due to natural conditions, nutrients shall not be allowed in concentrations that cause objectionable algal densities, nuisance aquatic vegetation, abnormal diurnal fluctuations in dissolved oxygen or pH, changes to the composition of aquatic ecosystems, or otherwise render the waters unsuitable for the designated uses.”

A challenge faced in bridging these quantitative criteria with the narrative nutrient policies has been the development of a set of water quality indicators that may effectively address how, and to what degree, water has been rendered unsuitable (e.g., objectionable algal densities), or reciprocally how much TP reduction would be necessary to return a eutrophic waterway to a healthy ecological condition. This SWQS dilemma must also be evaluated from a point-source permitting perspective because if a NJPDES discharge goes to a waterway segment already listed as water quality impaired for TP (i.e., 303(d) listed under the Clean Water Act), then the NJPDES permit must require a 0.1 mg/L phosphorus limit at the end-of-pipe. This has ramifications for municipal utilities (the major point source dischargers of TP) because phosphorus stripping equipment can be costly and difficult to install and operate.

Recently a NJDEP funded study carried out by the Patrick Center for Environmental Research (PCER) evaluated whether biological measures of eutrophication (i.e., diatom algae) could be used to assess potential nutrient impacts on receiving waters (Ponader and Charles, 2004). An important conclusion was that the relationship between algal biomass (i.e., chlorophyll a) and nutrient concentration as a measure of eutrophication (as recommended by U.S. EPA) could be confounded by such co-factors as upstream basin size, river width, light penetration conditions, and nitrogen (NO<sub>3</sub>-N), which were all highly correlated with TP. PCER recommended that NJDEP develop NJ specific eco-regional diatom inference models (e.g., Trophic Diatom Index - TDI) as a more defensible means to link nutrient levels (i.e., TP and TN) with measurable changes in algal biodiversity.

Diatoms are widely recognized and used as indicators of river and stream water quality, including trophic state conditions (Stevenson and Pan, 1999). Benthic diatom species composition responds directly to nutrients (Pan and Lowe, 1994, Pan et al., 1996), and

can be a more stable indicator of trophic state than measurements of nutrient concentrations or algal biomass (U.S. Environmental Protection Agency, 2000). The U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) and the U.S. Geological Survey (USGS) National Water Quality Assessment Program (NAWQA), the two largest national surface water monitoring programs, both use diatom and other algal indicators, as do several state agencies.

This present study was designed to address the status of eutrophic conditions on localized stream segments of two waterways in northern New Jersey; the Musconetcong and the Pequest Rivers, where Municipal Utility Authorities (MUAs) have expressed interest in performing phosphorus evaluation studies possibly resulting in a site-specific water quality based effluent limit (WQBEL) for TP (Ponader and Charles, 2004). The immediate objective of this study was to investigate whether diatoms and specifically the TDIs developed for northern NJ could be used to give more detailed information concerning the biotic integrity of streams above and below the outfalls of the STPs and to compare and contrast diatom community structure at both ends of the stream segment's receiving waters.

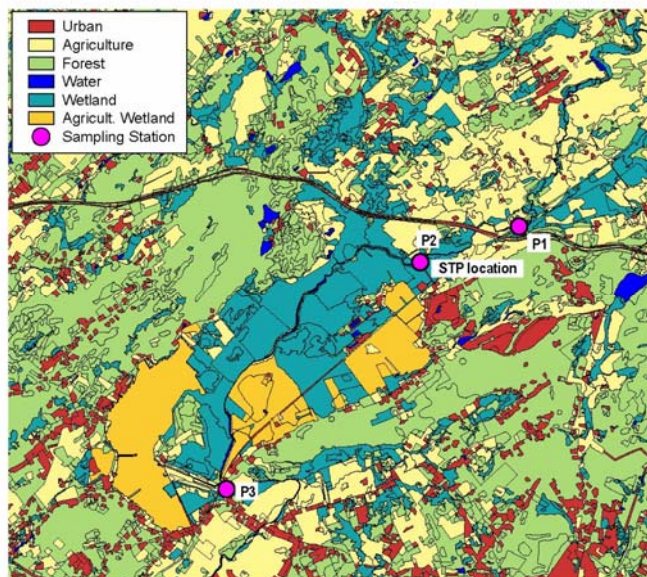
In this study, we tested diatom TP and TN models and indices developed for the Piedmont, Highlands and the Ridge and Valley physiographic provinces of New Jersey (Ponader and Charles, 2003, 2004) on reaches above, below and at the discharge of two selected STPs on the Pequest and Musconetcong Rivers.

## STUDY AREA AND METHODS

Three wadeable locations were selected and studied on both the Pequest and Musconetcong Rivers: a) 1-2 miles upstream of the STP, b) directly downstream of the STP, and c) 1-5 miles downstream of the STP (Figure 1 and 2; Table 1). The STP on the Pequest is a 0.6 MGD facility discharging to an intermittent stream which leads to the mainstem river, which is classified as a Freshwater 2, non-trout (FW-NT) waterway but is also listed as impaired by the NJDEP for "aquatic life designated uses" due to high historical phosphorus levels. The Musconetcong STP is a 4.31 MGD facility and discharges to a Freshwater 2, trout maintenance (FW2-TM) classified waterway and is also listed as impaired due to high historical phosphorus levels.

**Figure 1 - Pequest River sample site locations**

The Pequest River is located in the Ridge and Valley physiographic province of New Jersey and the Musconetcong River in the Highlands physiographic province. Both provinces are characterized by long river valleys draining from the northeast into the Delaware



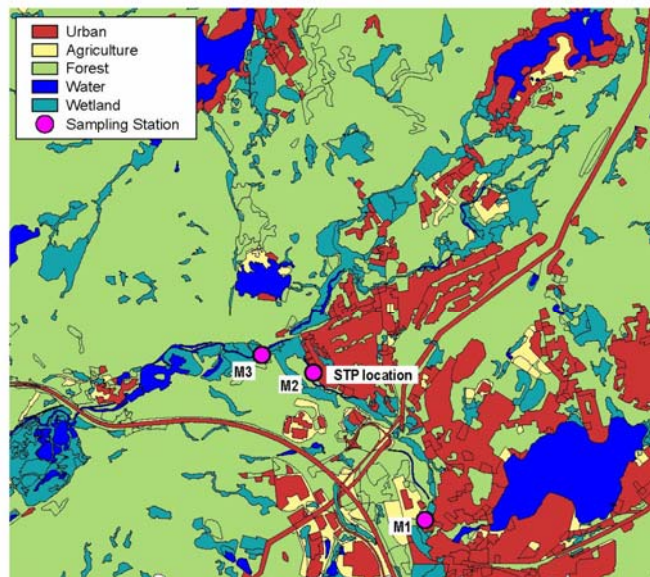
River valley with land use tending to forest and barren rock on the ridges, and agriculture and suburban/urban communities concentrated primarily in the river valleys along the riparian corridors. In addition to point sources, such as the STPs, nutrients can come from non-point sources such as agricultural fertilizer, storm water run off, and suburban lawn maintenance. In this present context, due to the large number of lawn turf farms, row crops and suburban communities, all occurring in a narrow riparian corridor, it may be problematic as to whether the influences of point versus non-point sources could be separated with just a one time sampling of algal community structure. More prolonged seasonal sampling may be necessary to distinguish between anthropogenic sources or from natural background conditions.

**Table 1 - Locations of sample sites**

Site ID	AMNET #	Latitude	Longitude	Site Location Name
Pequest River				
P1	AN0039	40 55.284'N	74 50.431'W	Pequest River at Rt 615, Long Bridge Rd
P2		40 54.888'N	74 51.811'W	Pequest River at Freeborn Lane extension
P3		40 52.629'N	74 54.285'W	Alphano Rd
Musconetcong River				
M1	AN0063	40 54.134'N	74 42.808'W	Musconetcong River, Waterloo Rd
M2		40 54.877'N	74 43.327'W	50 ft downstream outfall
M3		40 55.156'N	74 44.137'W	Musconetcong River, Continental Drive, Off Rt 604

**Figure 2 - Musconetcong River sample site locations**

Land-use in the Musconetcong watershed upstream from our sampling sites was more urban (15-31% vs. 12-14%) and slightly more forested (45-68% vs. 45-49%) than in the Pequest drainage, which had higher % agriculture (22-26% vs. 0.0-0.9%) (Figures 1 and 2; Table 2). Almost all of the land above P1 is “cropland and pasture,” whereas about 50% of the land-use below P2 and down to P3 is “agricultural wetlands” (much of it in the riparian corridor and including turf farms). Lawn turf farms may have an over-riding affect on receiving water quality due to their land management strategies such as pulse spraying of fertilizer to green up grass before harvesting, followed by extraction of the turf with underlying soil layers resulting in a



significant amount of erosion and sediment buildup in downstream shoals. The rest of the riparian corridor tends to be wooded/shrub wetlands. Note that there are also turf farms above P1.

**Table 2 - Physical habitat characteristics** Avg. width: Average river width at reach, %Open: percent open canopy cover, Flow: flow estimate (1=slow; 2=moderate; 3=fast) %Bo: % boulder, %Cob: % cobble, %Gra:% gravel, %San: % sand, %Si: % silt, %Urb: % urban land-use, %For: % forested land-use, %Ag: % agriculture land-use, %Wet: % wetland, Area (km<sup>2</sup>): watershed size.

Site ID	Avg width (m)	Open (%)	Flo w	Bo (%)	Cob (%)	Gra (%)	San (%)	Si (%)	Urb (%)	%For (%)	Ag (%)	Wet (%)	Area (km <sup>2</sup> )
P1	8.0	93.8	2.5	5	75	0	20	0	13.9	49.2	22.4	11.4	124.0
P2	3.5	10.4	1.5	0	0	0	90	10	13.2	47.1	24.1	13.0	178.4
P3	6.0	86.5	2.5	0	10	20	70	0	12.5	45.0	25.8	14.6	232.7
M1	3.5	21.9	2.5	15	60	15	10	0	31.6	45.5	0.0	7.6	76.7
M2	6.0	42.7	2.5	10	65	10	15	0	23.4	57.0	0.4	9.1	69.6
M3	7.0	54.2	2.5	20	70	5	5	0	15.2	68.6	0.9	10.6	62.5

Two Academy of Natural Sciences of Philadelphia (ANSP) field staff sampled all six study sites on September 16, 2005. The sampling date was chosen to avoid sampling within 14 days of a scouring event as per NAWQA protocols (Moulton et al., 2002). Prevailing high flow conditions during late summer 2005 with three hurricanes moving across New Jersey made this difficult. Periphyton samples were collected in accordance with ANSP Procedure P-13-64 “Field sampling procedures for the New Jersey Algae Indicators Project” (Charles et al., 2000). These sampling procedures are consistent with those used in the USGS NAWQA program (Moulton et al., 2002). Rocks were sampled at all sites, except at site P2. This site was dominated by sandy substrate and samples were collected from branches, cobble, mollusk shell, and other miscellaneous hard substrates; no sand/silt substrate was sampled. Water chemistry samples were collected at each site for nutrients (SRP, TP, NH<sub>3</sub>-N, NO<sub>3</sub>-N, TKN and TN), alkalinity, hardness and chloride following Patrick Center water chemistry sampling procedures (Velinsky, 2000). Samples for analysis of alkalinity, Cl<sup>-</sup>, and hardness were collected at each upstream site only. Water temperature, pH and specific conductivity were measured in the field using a handheld OAKTON pH/conductivity meter (Model No. WD-35630-60).

At the Academy, samples for water chemistry and algal community analysis were processed and analyzed employing standard protocols developed for the New Jersey Department of Environmental Protection (NJDEP) following standard analytical procedures (PCER, 2000). Diatom community metrics were calculated using the Phycological Indicators and Data Exploration Program (Phyco-AIDE) version 1 (Sprouffs et al., 2004). Diatom metrics calculated were: Centrales /Pennales ratio (C/P), Diatom taxa (species) richness (#Taxa), Percent dominant diatom taxon (% Dom), Percent of *Achnanthes minutissimum* (%AM), Shannon-Wiener diatom diversity index (Dvsty\_SW), Siltation index (SiltIdx; = Percent motile taxa). Ordinations were produced using Canoco 4.5 for Windows (ter Braak and Šmilauer, 2002). Weighted averaging (WA) calibration techniques were used to test diatom

inference models for TP and TN. These were run using the program C<sup>2</sup>, (version 1.3, University of Newcastle, Newcastle upon Tyne) (Juggins, 2003). We applied the data from the six sites to the best inference models that were developed for TP and TN in previous years (Ponader and Charles, 2004), based on weighted-averaging partial least square regressions (WA-PLS) (ter Braak and Juggins, 1993). These WA-PLS models were developed using diatom data from 91 samples and showed good predictive ability for total phosphorus (TP) and total nitrogen (TN) (TP model:  $r^2_{\text{apparent}} = 0.87$ ;  $r^2_{\text{boot}} = 0.72$ ;  $\text{RMSE}_{\text{boot}} = 0.23 \log_{10} \mu\text{g} / \text{L TP}$ ; TN model:  $r^2_{\text{apparent}} = 0.88$ ;  $r^2_{\text{boot}} = 0.58$ ;  $\text{RMSE}_{\text{boot}} = 0.23 \log_{10} \mu\text{g} / \text{L TN}$ ). Model error estimation was performed by bootstrapping with 1000 cycles (Birks, 1995). Diatom TP and TN indices were created by rescaling the inference model results from 0-100.

## RESULTS

### Environmental Variables and Diatom Assemblage Composition

Generally, both rivers are characterized by high pH, and relatively high alkalinity, hardness, and conductivity compared with most streams in the Ridge & Valley and Highlands physiographic provinces, and moderate to high nutrient concentrations (Table 3).

**Table 3 - Water quality characteristics**

Site ID	Temp °C	pH	Cond µS/ cm	Cl- mg/l	Alk mg/l	Hard mg/l	NO3 +2 mg/l	NH3- N mg/l	TKN mg/l	TN mg/l	SRP mg/l	TP mg/l
P1	19.0	8.4	577	45.0	215	253	0.94	0.014	0.32	1.26	0.002	0.018
P2	18.9	8.3	584	45.0	215	253	1.00	0.013	0.34	1.34	0.002	0.021
P3	18.8	8.2	562	45.0	215	253	1.07	0.020	0.46	1.53	0.017	0.057
M1	21.4	7.9	430	88.5	53	94	0.11	0.016	0.41	0.52	0.004	0.020
M2	20.3	8.2	610	88.5	53	94	2.57	0.006	0.48	3.05	0.026	0.061
M3	20.4	8.3	598	88.5	53	94	2.28	0.008	0.43	2.71	0.025	0.055
max	8.4	8.4	610	88.5	215	253	2.57	0.020	0.48	3.05	0.026	0.061
min	8.0	7.9	430	45.0	53	94	0.11	0.006	0.32	0.52	0.002	0.018
mean	8.2	8.2	560	66.8	134	174	1.33	0.013	0.41	1.73	0.013	0.039
median	8.3	8.3	581	66.8	134	174	1.04	0.014	0.42	1.44	0.011	0.038

Based on conditions at the time of 2004 field work, the concentrations of TP and TN (Table 3) were moderately elevated, sufficient to support substantial algal growth (Stevenson et al., 1996), but not exceeding State standards, even though historical sampling of nutrients have shown consistently elevated levels of TP for both waterways sufficient to have them both listed as impaired for the State 303d list under the Clean Water Act. In the Pequest, concentrations below the STP do not differ much from the upstream site, suggesting either that the influence of the STP on nutrient concentrations is minimal, or that concentrations above the STP are also elevated, but due to another source. The nutrient concentrations at P3 are higher than P1 and P2 (especially for phosphorus) suggesting the possibility of yet additional nutrient sources between P2 and P3, possibly from the turf farms. In the Musconetcong, nutrient concentrations are

considerably higher at the two stations below the STP (M2 and M3) than the one above (M1), consistent with a substantial increase being caused by the STP. Because there are nutrient measurements in this study for only one point in time, it is not possible to assess how accurately they represent long-term concentrations in either of the rivers.

The diatom flora at the Pequest River sites is composed of 45-72 (mean = 59) taxa and of 32-50 (mean = 35) taxa at the Musconetcong River sites (Tables 4 and 5). The flora from both rivers is generally dominated by pollution-tolerant species.

The Pequest River samples were dominated by *Amphora pediculus* (mean relative abundance: 17.6%) and *Rhoicosphenia abbreviata* (9.7%), accompanied by *Nitzschia dissipata* (8.0%), *Navicula gregaria* (4.5%), *Achnantheidium minutissimum* (3.9%) and *Cocconeis placentula* var. *lineata* (3.9%). All other species had average abundances of <4.0%.

In the Musconetcong sites, the flora was dominated by *Achnanthes subhudsonis* var. *kraeuselii* (mean relative abundance: 33%), *Rhoicosphenia abbreviata* (17%) and *Cocconeis placentula* var. *lineata* (12%), accompanied by *Achnantheidium minutissimum* (6.6%), *Gomphonema kobayasii* (4.3) and *Navicula cryptotenella* (4.0%). All other species had average abundances of <4.0%.

WA-optima calculated for all dominant species ranged from 0.5-0.9 mg/l TP and from 1.45-1.79 mg/l TN, with the exception of *Achnantheidium minutissimum* and *Navicula cryptotenella* which both had lower calculated WA-optima of ~ 0.3mg/L TP and 1.1 mg/L TN (Ponader and Charles, 2003).

Diatom assemblages from both rivers have moderate taxa richness and Shannon-Wiener species diversity, with the Pequest River samples showing relatively higher average diversity (4.0) than the Musconetcong River samples (3.1) (Table 5). The higher diversity for site P2 may be influenced by the larger number of substrate types from which the diatom sample was collected. The Siltation Index, a measure of the proportion of motile diatoms is higher in the Pequest, probably reflecting the higher proportion of sand and silt in the Pequest compared with the Musconetcong (Table 2).

**Table 4 - Diatom taxa occurring in abundance >4% in at least one sample, sorted in order of increasing TP optima** Also, taxon abundance in all samples, and autecological characteristics. NA = not available. opt. = optima, cat. = category. TP optima <sup>1</sup>(Potapova et al., 2004): 1) <25 microg/l, 2) 25-63 microg/l, 3) 63-158 microg/l, 4) 158-398 microg/l, 5) >398 microg/l. Saprobity index <sup>2</sup>(Van Dam, et al. 1994): 1) oligosaprobous, 2)  $\beta$ -mesosaprobous, 3)  $\alpha$ -mesosaprobous, 4)  $\alpha$ -meso/ polysaprobous, 5) polysaprobous. Trophic State index <sup>2</sup>(Van Dam, et al. 1994): 1) oligotraphentic, 2) oligo-mesotraphentic, 3) mesotraphentic, 4) meso-eutraphentic, 5) eutraphentic, 6) hypereutraphentic, 7) oligo- to eutraphentic. Cond. Opt. = Conductivity optima <sup>3</sup>(Potapova and Charles, 2003).

Taxon Code	Diatom Taxon Name	% Relative Abundance of Taxon at Site						TP opt. <sup>1</sup> (µg/L)	TP cat. <sup>1</sup>	Saprobity <sup>2</sup>	Trophic State <sup>2</sup>	Cond. opt. <sup>3</sup> (µS/cm)	Cl <sup>-</sup> opt. <sup>3</sup> (mg/L)
		P1	P2	P3	M1	M2	M3						
ACsubkra	<i>Achnanthes subhudsonis</i> var. <i>kraeuselii</i> Cholnoky	0.0	0.0	0.0	3.0	69.4	25.8	32	1	NA	NA	NA	NA
AHminuti	<i>Achnanthidium minutissimum</i> (Kützing) Czarnecki	3.8	4.5	3.7	10.1	2.0	8.0	33	1	2	7	229	11.0
NI dissip	<i>Nitzschia dissipata</i> (Kütz.) Grunow	9.4	4.7	10.0	0.2	0.5	0.8	35	1	2	4	361	12.4
RE sinuta	<i>Reimeria sinuata</i> (Greg.) Kociolek & Stoermer	0.7	0.7	0.0	7.3	0.2	0.5	51	1	2	3	251	11.7
NA cryten	<i>Navicula cryptotenella</i> L.B. in Krammer & Lange-Bertalot	0.0	0.5	0.0	0.2	0.3	0.0	54	5	2	7	371	15.2
NA gregar	<i>Navicula gregaria</i> Donk.	4.0	7.6	2.0	0.8	0.0	1.7	55	3	3	5	392	20.2
CC plalin	<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurck	3.8	5.8	2.2	20.2	6.2	10.6	56	3	2	5	270	11.0
ME varian	<i>Melosira varians</i> Agardh	4.6	2.3	0.5	0.8	0.0	0.7	60	4	3	5	309	12.8
NI amphib	<i>Nitzschia amphibia</i> Grunow	0.3	3.2	0.3	4.1	1.3	2.3	63	3	3	5	400	22.3
RS abbrevre	<i>Rhoicosphenia abbreviata</i> (Agardh) L-B. Lange-Bertalot	14.7	2.8	11.6	28.5	11.8	9.3	62	4	2	5	384	18.1
NA minima	<i>Navicula minima</i> Grunow	0.3	4.0	4.3	0.3	0.7	4.0	65	3	4	5	319	12.4
NA lanceol	<i>Navicula lanceolata</i> (Agardh) Ehrenberg	5.1	0.8	0.3	0.0	0.0	0.7	66	4	3	5	406	20.2
AM pedcls	<i>Amphora pediculus</i> (Kützing) Grunow	11.7	11.6	29.6	0.3	0.7	4.0	67	3	2	5	470	21.3
SF seminu	<i>Sellaphora seminulum</i> (Grun.) Mann	0.3	1.0	4.0	0.7	0.8	5.7	71	3	4	5	305	16.0
GO kobaya	<i>Gomphonema kobayashii</i> Kociolek & Kingston	4.1	2.0	2.7	7.8	1.7	3.5	1152	5	NA	NA	NA	NA
AM ovalis	<i>Amphora ovalis</i> (Kützing) Kützing	4.1	6.3	1.0	0.0	0.0	0.5	NA	NA	2	5	NA	NA



**Table 5 - Diatom diversity and other metrics** Centrales /Pennales ratio (C/P), Diatom taxa (species) richness (#Taxa), Percent dominant diatom taxon (% Dom), Percent of *Achnantheidium minutissimum* (%AM), Shannon-Wiener diatom diversity index (Dvsty\_SW), Siltation index (SiltIdx). Statistical abbreviations: Standard deviation (SD), coefficient of variation (CV).

Site ID	C_P	#Taxa	%Dom	%AM	Dvsty_SW	SiltIdx
Pequest River						
P1	0.05	59	14.7	3.8	4.9	39.4
P2	0.04	72	8.0	4.5	5.4	46.7
P3	0.01	45	15.3	3.7	4.4	42.6
Musconnetcong River						
M1	0.01	32	28.5	10.1	3.4	15.8
M2	0.00	22	69.4	2.0	1.8	7.2
M3	0.01	50	25.8	8.0	4.1	32.1

The percent similarities among samples (Table 6) show that there are substantial differences among samples, even though there are many species in common. The differences in similarity values generally correspond to distances between sample stations, and may reflect spatial factors as well as nutrients and other chemical and physical habitat characteristics.

**Table 6 - Percent similarity in diatom species composition between samples**

Between sample	% Similarity	% Dissimilarity
P1:P2	58.0	42.0
P2:P3	50.4	49.6
P3:P1	39.7	60.3
M1:M2	30.8	69.2
M2:M3	51.6	48.4
M3:M1	27.4	72.6

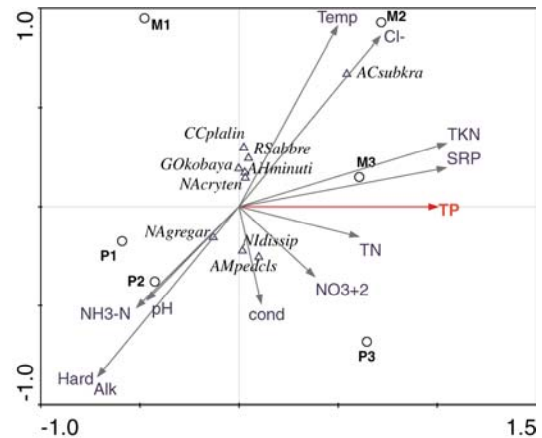
### **Diatom flora and composition changes related to the effects of discharges from the sewage treatment facilities**

The CCA ordinations of all sites (Figure 3) shows the relative differences in the diatom assemblages among the sites, the main species responsible for those differences, and relationships of both with water chemistry characteristics.

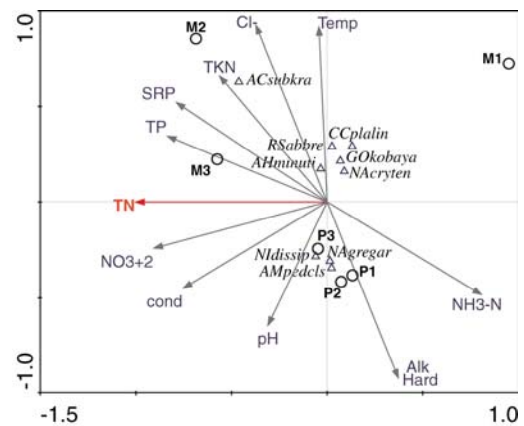
**Figures 3a and 3b. CCA triplots constrained to one variable at a time.** The constrained variables are indicated by a red arrow **a)** TP, **b)** TN. The black arrows indicate water chemistry characteristics entered as passive variables. Circles represent sites, triangles represent diatom species. For site abbreviations see Table 1. For species abbreviations see Table 4.

To investigate which nutrients correlated most strongly with diatom species composition, we ran six separate CCAs, each constrained to one of six nutrient variables: TN, TKN, TP, SRP, NO<sub>2</sub>+3 and NH<sub>3</sub>-N. All other variables were entered as passive variables. Of the six variables, TKN had the highest influence with a  $\lambda_1/\lambda_2$  ratio of 1.01, capturing 27% of the variation in the species data. SRP had a  $\lambda_1/\lambda_2$  ratio of 0.84, capturing 24% of the variation in species data. All other variables had lower  $\lambda_1/\lambda_2$  ratios: NH<sub>3</sub>-N (0.78) TP (0.65), TN (0.41), NO<sub>3</sub>-N (0.39). Results of the TP and TKN CCAs are presented in Figures 3a and 3b, respectively, and show the relationships among the water chemistry variables.

**Figure 3a**



**Figure 3b**



The CCA graphs (Figure 3) also show the locations of the diatom assemblages and the most common taxa, with respect to each other and the nutrient gradients. Distance between sample points indicates similarity in species composition. Locations of the diatom assemblage points show that for both rivers the most upstream stations (M1 and P1) fall along the low-concentration end of the phosphorus and nitrogen gradient (visualize by drawing a line from the sample point to the horizontal axis so it intersects at a right angle). The species composition data points do not indicate a significant change above and below the STP on the Pequest (P1 vs. P2), but there is a marked difference between the Musconetcong upstream site (M1) and the sample that was taken below the discharge location (M2). The diatom composition at the Pequest station P3 (most downstream) shows the strongest response to nutrients, compared to station P1 and P2. In contrast, diatom assemblages at the furthest downstream Musconetcong station (M3) indicate nutrient conditions that are relatively lower as compared to M2, indicating some recovery has occurred.

The CCA triplots (Figure 3), the differences in the diversity metrics (Table 5), and the Percent Similarities (Table 6) indicate that the change in diatom species composition between the upstream locations (P1, M1) and the samples taken below the discharge (P2, M2) is more pronounced at the Musconetcong River than at the Pequest River. The main reason for the high dissimilarity between species composition of sample M1 and M2 is a strong increase in the abundance of *Achnanthes subhudsonis* var. *kraeuselii* which dominates (69.3%) at sample location M2. This species has a WA-optimum of 0.5 mg/L

TP and 1.4 mg/L TN (Ponader and Charles, 2004) and indicates a shift to higher trophic conditions. The diatom assemblages at the downstream location on the Pequest River (P3) are most dissimilar with the upstream site (P1). This change is mainly caused by an increase of the relative abundance of *Amphora pediculus* (Kütz.) Grun , which is the most abundant species (30%) in this sample and indicates a shift towards increased trophic conditions; the calculated WA-optimum for *A. pediculus* is 0.5 mg/L TP and 1.5 mg/L TN (Ponader and Charles, 2004).

### TP and TN inference models and indices

The TP and TN Weighted Averaging inference models developed in an earlier study (Ponader and Charles, 2004) were applied to diatom samples from all six study sites using the program C<sup>2</sup> (Juggins 2003). The predictive characteristics of the TP model are:  $r^2_{\text{apparent}} = 0.87$ ;  $r^2_{\text{boot}} = 0.72$ ;  $\text{RMSE}_{\text{boot}} = 0.23 \log_{10} \mu\text{g} / \text{L TP}$ ; for the TN model they are:  $r^2_{\text{apparent}} = 0.88$ ;  $r^2_{\text{boot}} = 0.58$ ;  $\text{RMSE}_{\text{boot}} = 0.23 \log_{10} \mu\text{g} / \text{L TN}$ . We compared the model-inferred nutrient values for the six sites with the measured values by performing simple regressions (Figures 4a and 4b) (Birks et al., 1990). The inferred TP values correlated fairly well with measured TP values ( $r=0.71$ ;  $r^2= 0.50$ ) (Figure 4a). In contrast, agreement with the measured and inferred TN values was weak ( $r=0.22$ ;  $r^2= 0.05$ ) (Figure 4b).

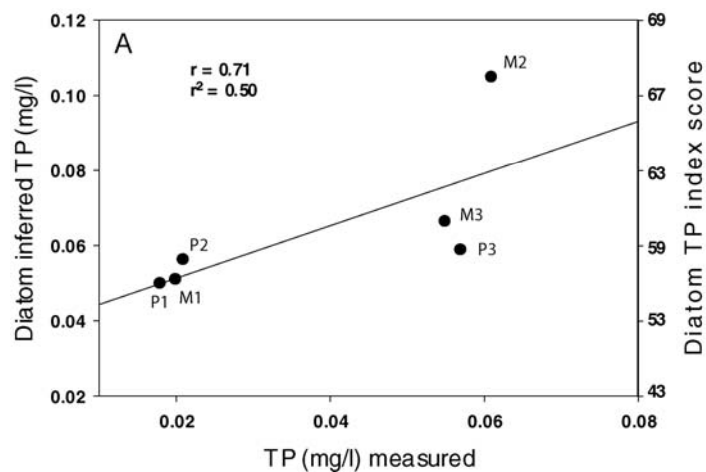
Diatom TP and TN indices were calculated by converting the  $\log_{10}$  of the inferred nutrient concentrations to a TDI scale of 1 – 100. This step creates trophic diatom indices (TDI) for TP and TN (Table 7) that represent species composition responses to nutrient conditions. Diatom TP index values are lowest for the upstream stations on each river (P1, M1), and increase downstream only slightly for the Pequest, but more markedly for the Musconetcong (Table 7, Figure 4).

**Table 7 - TP and TN diatom indices calculated based on diatom inferred TP and TN values**

Site ID	TP Index	TN Index
P1	56.6	42.5
P2	58.3	48.0
P3	58.9	55.1
<hr style="border-top: 1px dashed black;"/>		
M1	56.9	59.4
M2	67.3	55.9
M3	60.7	58.7

**Figures 4a and 4b** TP (A) and TN (B) inference model performance when tested on samples from the 6 sites in this study. Plots show inferred versus measured TP and TN and the corresponding diatom index scores, (A) the Diatom TP Index and (B) the Diatom TN Index.

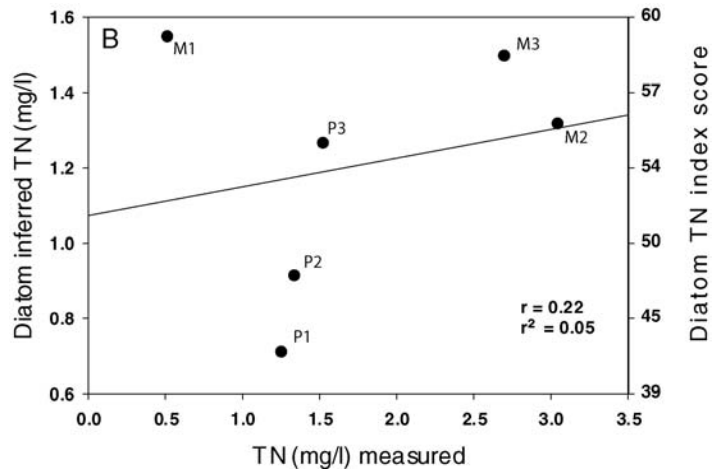
**Figure 4a.**



Another use of the diatom nutrient indices is that they can be related to trophic state conditions associated with impaired designated uses (nuisance algal blooms) for aquatic life, recreation and aesthetics. The 0-100 unitless scale can be divided into trophic state categories ranging from 1 (low) to 3 (high) or 4 (very high). The water chemistry categories established for these TP categories are: 1 (< 0.025), 2 (0.025-0.075), 3 (0.075--0.1), 4 (> 0.1) mg / L TP. These correspond to the TP index scores of 1) 0-47, 2) 47-63, 3) 63-67, and 4) above 67.

**Figure 4b.**

TN categories are: 1 (< 0.7), 2 (0.7-1.5), 3 (> 1.5) mg / L TN, corresponding to the TN index scores of 1) less than 42, 2) 42-59, and 3) above 59. The six sites in this study fall into TP categories 2 and 3 and TN category 2.



## DISCUSSION

The CCA ordinations showed that variation in diatom species composition at the six study sites is closely related to nutrient concentrations, and that nutrients can explain differences among samples better than other water chemistry and physical habitat characteristics. This relationship supports the rationale underlying use of diatoms as nutrient indicators and the development of metrics and indices.

The diatom species composition and TP indices did not differ greatly between stations above (P1) and below (P2) the STP on the Pequest River, but indicated greater enrichment at the site further downstream (P3). This may be because the STP discharge was not large (0.6 MGD compared with the Musconetcong STP discharge of 4.31 MGD), and may not have had a substantial influence on stream chemistry, possibly in part because river flow had been relatively high in the previous weeks. The fact that conductivity does not increase much below the STP, as often occurs elsewhere (including below the Musconetcong STP), supports this possibility. A possible reason species composition of P1 and P2 samples is more similar to each other than either is with P3 is that the sites are located much closer to each other. There may be considerable nutrient input from the turf farms below station P2 that could account for the greater enrichment indicated at P3.

The diatom species composition and TP indices indicated greater enrichment at both sites below the Musconetcong STP. The relatively short distance between M2 and M3 may

help explain the greater similarity of species composition at these sites, as well as the lack of evidence of recovery.

We did not examine the relative limitations of N and P to algal growth and how they might differ among the stations.

## CONCLUSIONS

This preliminary study shows that the TP and TN diatom inference models and indices developed for NJ are promising tools to monitor and infer nutrient conditions, but that further study is necessary to adequately evaluate their effectiveness for routine use as part of a regulatory program (i.e., NJPDES permitting decisions). Diatom assemblages provided useful information for assessing the biotic integrity of the streams with respect to nutrient conditions. Species composition differed among sites, and the differences can be explained by variation in nutrient concentrations. Diatom indices indicated relatively high nutrient conditions at all sampling sites, and between-site differences were consistent with measured values. Both the diatom indicators of enrichment and the measured nutrient concentrations showed a marked increase below the Musconetcong River STP, but not below the STP on the Pequest River. This may be because the volume of STP discharge into the Musconetcong is much greater than the Pequest, because there are important, unaccounted-for factors influencing nutrient concentrations (e.g., non-point source loads of nutrients from agriculture and/or storm water runoff), or because limited one-time sampling did not represent longer-term nutrient conditions. Additional study of these and other STP discharge sites would be necessary to provide information on the consistency of diatom indicators for use in future water quality permitting decisions.

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