

New Jersey Periphyton Bioassessment Development Projects

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

FINAL REPORTS (2000 – 2005)

**Submitted to
Thomas Belton, Project Manager**

**New Jersey Department of Environmental Protection
Division of Science, Research and Technology
401 East State Street, PO Box 409
Trenton New Jersey 08625**

**by
Karin Ponader and Donald Charles**

**Patrick Center for Environmental Research
The Academy of Natural Sciences
1900 Benjamin Franklin Parkway
Philadelphia, PA 19103-1195**

**Understanding the Relationship Between Natural Conditions And Loadings on Eutrophication:
Algal Indicators of Eutrophication for New Jersey Streams - Year 5**

Final Report Year 5

Submitted to the

**New Jersey Department of Environmental Protection
Division of Science, Research and Technology**

by Karin Ponader and Donald Charles

**Patrick Center for Environmental Research
The Academy of Natural Sciences
1900 Benjamin Franklin Parkway
Philadelphia, PA 19103-1195**

July 14, 2005

Contents

	Page
Introduction	1
General approach.....	1
Review of Year 5 Activities	2
Selection of study sites	2
Collection of samples	3
Status of sample preparation and analysis	4
Data analysis.....	5
Schedule.....	5
Status of progress in achieving the project goals	5
Deliverables.	7
Tasks left to do (year 5).....	7
References.	8
Appendix 1: List of pre-selected sites for field reconnaissance in Outer Coastal Plain.	10
Appendix 2: List of sites sampled in 2004.	11
Appendix 3: Trophic Diatom Indices (TDI) and the development of site-specific criteria. Belton, T.J., Ponader, K.C., and Charles D.F. 2005. Proceedings of the TMDL 2005 Speciality Conference, Water Environment Federation (WEF) and Pennsylvania Water Environment Association. June 26-29, Philadelphia, Pennsylvania. pp. 1042-1056.	12

Introduction

The New Jersey Department of Environmental Protection has a need for development of algal indicators of stream and river eutrophication. These indicators will be used to assess relationships between extant water quality criteria (e.g., phosphorus and nitrogen concentrations) and overt signs of eutrophication. They will be applied in a regulatory context as secondary criteria for identifying nutrient impairment. These indicators should be based on an understanding of algal dynamics in New Jersey streams, and be able to distinguish between situations where nutrient concentrations are high due to natural environmental conditions and those that result from anthropogenic influences. Protocols are needed that describe procedures for sample collection and processing, analysis and presentation of data, and interpretation of results.

This study was initiated in July 2000, and is currently in the fifteenth month of the fifth year. Years 1 and 2 of this project were limited to development of algae indicators in the Piedmont physiographic province in New Jersey. During the third year the study was expanded to include sites in the Highlands and the Ridge and Valley physiographic provinces. Data from sites studied during all three years were used to successfully develop and test indicator metrics for the northern part of the State (Ponader et al., submitted). The fourth year of the study extended the development of indicators to the Inner Coastal Plain. Data from sites studied during year four were used to develop and test additional indicator metrics (Ponader and Charles, 2004). During the current study year (fifth year), samples were taken in the Outer Coastal Plain to provide additional data that will allow development of a diatom index to be used in the Coastal Plain physiographic province of southern New Jersey.

General approach

The fourth and fifth years of the study extended the development of indicators to the Coastal Plain physiographic province, using the same general approach and specific methods as used in the first three years (Charles et al., 2000). Most specific procedures and quality assurance and quality control measures are described in the project QA/QC plan (PCER, 2000). Nevertheless, because of the different geomorphology of the rivers in the NJ Coastal Plains (sand and clay river bottoms), significant changes and adjustments in the sampling design and the methods for collection of algal samples were necessary during year 4, using diatometers as artificial substrates (Ponader et al., 2003). During the current study year (fifth year), the Outer Coastal Plain physiographic province was sampled with diatometers using the same protocols as last year. In addition, we sampled epipsammic (sand) and epipellic (silt) substrata, in order to explore differences in diatom assemblages and algal biomass collected from different substrates (e.g., artificial substrates (diatometers) versus natural substrates (sand/silt)). Therefore, development of a new sampling method and protocol for collection of sand and silt substrate was necessary for study year 5 (Ponader and Winter, 2004).

The main goals during year 5 are to a) prepare protocols and taxonomic documentation, b) to develop periphyton assessment indicators and methods as nutrient management tools for the Outer Coastal Plain, and c) to test existing models on individual sites to develop site specific criteria. To achieve goal a) we will prepare a methods manual for use by NJ DEP staff that describes all field and laboratory procedures. It will be based largely on our existing NJ protocols, as well as on NAWQA

protocols. In addition, we will prepare a diatom flora and additional taxonomic and ecological data to help facilitate analysis of diatom assemblages. The NJDEP will receive five hardcopies of the document as well as two electronic copies on compact discs. Specific tasks to achieve b) goals are to collect algal and water samples, to acquire additional chemistry and other habitat data from the NJ DEP, to analyze the algal and water samples, to examine relationships between algal assemblages and water quality and physical parameter data to be collected by DEP, and to develop algal metrics that indicate impairment due to high nutrient concentrations. Because all work in the new ecoregion will be done in one year, we do not plan to test metrics with a second year of data, and will do limited analysis of multiple, within-site samples (10 percent of the sites). Work tasks for goal c) are to test the diatom indices on a reach above, below and at the discharge of two selected sewage treatment plants, that are part of New Jersey's current Phosphorus Evaluation studies. ANSP staff collected diatom samples and water chemistry parameters (in particular TP and TN, as well as other variables). We will evaluate how well the models and metrics performed on a site-by-site basis, and report on how the results can be used to help develop site specific criteria. This task will require close cooperation and discussion with DEP staff. Toward the end of the fifth year, we will prepare a final report that will summarize all study results.

Review of Year 5 Activities

Selection of study sites

a) preselection of sites

We worked with NJ DEP staff, mainly Tom Belton, to carefully select a set of study sites. During study year five, three different types of sites were sampled; these are: diatometer sites (Outer Coastal Plain), re-sampling sites, e.g. sites sampled for the fifth consecutive year (Piedmont) and sewage treatment plant sites (Highlands and Valley & Ridge).

We used several criteria to select the Outer Coastal Plain sites. Because a goal was to develop indicators of anthropogenic nutrient increases, it was important to select a suite of sites with relatively similar natural environmental conditions, but with a wide range of nutrient concentrations. The sites were restricted to the Outer Coastal Plain physiographic province in southern New Jersey and have a limited range of hydrology, morphology and substrate type. We generally used the same selection criteria as in the previous years (Ponader and Charles 2001). As during project year four, we avoided sites in the tidal zone of the Delaware basin because taxonomic composition could potentially be influenced by higher salinity and by transport of algal specimens from up-/downstream location, and variation in water chemistry through tidal movement. We tried to select sites with a range from no-impairment to severe impairment, based on the AMNET classifications of 1992/93 and 1998/99. For all sites, chemistry data are available either from the NJ monitoring network program (SW sites, SS stations, or EWQ stations) or from USGS monitoring stations that have a gaging station at the site or at least up/downstream from the site. All sites are part of the NJ Ambient Monitoring Network. In the Outer Coastal Plain, the NJ monitoring network program only sampled few sites (SW sites, SS stations, or EWQ stations) for water chemistry in 2004. Therefore our pre-selection of sites was limited to 12 SW/SS and 7 EWQ stations that met our selection criteria (see Appendix 1).

In addition to the sites pre-selected in the Outer Coastal Plain, we decided to re-sample 3 stations that were sampled in 2000, 2001, 2002 and 2003 in the Piedmont province, to have a five year record we could use to compare variability at individual sites among years.

The sites selected for testing of the developed trophic diatom index at sewage treatment plants (STP's) were based on existing stations as established by the Phosphorus Evaluation Study QA work plans for the Musconetcong Sewerage Authority (February, 2004) and the Warren County Municipal Utilities Authority (April, 2004). At each river, samples were taken at three sampling points located at circa 1 mile upstream of the STP, directly downstream of the STP, and circa 1-5 miles downstream of the STP (see list of sites sampled in Appendix 1). When an established AMNET station was available up- or downstream of the established station, the AMNET station was sampled. This will make comparison between Macroinvertebrate and diatom ratings possible.

b) field reconnaissance

For several reasons, we had to reject 10 diatometer candidate sites based on field reconnaissance. The first reason for rejecting sites was the low pH of the rivers. Three sites (AN0153, AN0156 and AN0550) from different rivers (S. Br. Rancocas, S. Br. Burrs Mill Brook and Long Branch) were rejected because the pH was below 5 (3.8-4.4). The second reason was river depth. The rivers at two sites (AN0732, AN0751) were rather deep (chest-deep and above) and non-wadeable. The depth was due to the geomorphology typical of many rivers in the Outer Coastal Plain (channel-like), combined with the high rainfall during the summer of 2004, resulting in generally higher water levels in the sampling area. Other reasons for rejecting candidate sites were inaccessibility, such as sites located at Army Bases (AN0617, AN0119A), rivers too small in width (AN0593, AN0624), or the potential for vandalism of diatometers (active pastures with livestock, site AN0699).

Collection of samples

Algae samples were collected by D. Winter, E. Hagan and K. Ponader in late summer, from August 19th through September 16th, 2004, when the influence of water quality on algal assemblage composition is usually greatest. Water chemistry of samples collected during this time is also most directly comparable with sample data from other studies.

Diatometers were deployed from August 19th through September 24th, 2004 and were exposed for a period of between 21 to 24 days. They were removed in consecutive order from September 13 through 15. All diatometers were collected before Hurricane Ivan, which partly affected the sampling area during September 18, 2004. All algae samples were collected using techniques consistent with those used in the USGS NAWQA program (Moulton et al. 2002) and as documented in PCER protocols (Charles et al., 2000 and Ponader et al., 2003). Water chemistry was measured twice in the field: once at the time of diatometer deployment and another time when the diatometers were removed. Conductivity, pH and temperature were measured using an OKATON deluxe pH/conductivity meter. Samples were taken for laboratory analysis of soluble reactive phosphorus, total phosphorus, nitrate,

ammonia, total nitrogen, chloride, total alkalinity, total hardness and conductivity (Velinsky, 2000). Results of these analyses will supplement those collected by the NJ DEP. They may better represent conditions near the time that algae samples were collected, and will provide information of the nature and magnitude of variation in water chemistry. At each site, two reaches were established with 1 diatometer each (2 diatometers/site) for analysis of diatom species composition and algal biomass. In addition to the diatometer samples, surface sediment samples (sand/silt) were taken at each reach for analysis of diatoms and biomass.

The three re-sampling sites in the Piedmont were sampled for the fourth consecutive year on August 25, 2004. Natural rock substrates were sampled for analysis of diatom species composition and algal biomass. Samples were collected using techniques described in PCER protocols (Charles et al., 2000).

Two Academy of Natural Sciences (ANS) field staff spent one day visiting six wadeable sites on the Pequest River and the Musconetcong River, NJ. Sampling was done at selected stations, as established by the Phosphorus Evaluation Study QA work plans for the Musconetcong Sewerage Authority (February, 2004) and the Warren County Municipal Utilities Authority (April, 2004). Diatoms were sampled from rock substrates and water chemistry samples (TP and TN) were collected. In total, 6 STP samples were taken, 3 from the Musconetcong River and 3 from the Pequest River (see Appendix 2).

A total of eighteen sites were sampled. Nine were diatometer sites located in the Outer Coastal Plain, using 2 diatometers per site (total = 18 diatometers). Three were re-sampling sites in the Piedmont, sampled for the fourth consecutive year. Six sites were sampled at two selected sewage treatment plants on the Musconetcong (Highlands) and the Pequest (Ridge and Valley) Rivers, to test existing diatom indices (Ponader et al., submitted).

Status of sample preparation and analysis and status of data login

A total of 44 diatom samples and 39 biomass samples were prepared for analysis. Diatoms were permanently mounted on microscope slides. Per slide, 600 valves were identified to lowest taxonomic level and counted. Analysis of all 44 slides was completed in March 2005. Algal biomass for all 39 samples was analyzed using standard protocols (Velinsky and DeAlteris, 2000; Kiry et al., 1999). Twenty-seven water chemistry samples were analyzed for specific conductivity, chloride, total alkalinity, total hardness and conductivity. Nutrients (soluble reactive phosphorus, total phosphorus, nitrate, ammonia, total nitrogen) were measured for all 27 samples in the Patrick Center's Geochemistry Section using a Technicon Auto Analyzer (Velinsky, 2000).

All field data has been entered in the ANSP database, including substrate information, field measurements and photographs that were taken during fieldwork.

Data analysis

Data analysis will be performed using two different datasets: a) the Outer Coastal Plain dataset and b) the combined Inner- and Outer Coastal Plain dataset. We expect that the models and indices will show better performance when developed using the combined dataset, due to higher sample size (Ponader and Charles, 2004)

Data analysis and development of indicators will proceed in several steps. They will be consistent with the analysis of the first four years work on samples from northern NJ (Piedmont, Valley & Ridge and Highlands) and from the Inner Coastal Plain. The first step will be to explore the variation in algal assemblages among the sites and to determine which chemical and other environmental factors explain most of the variation among the assemblages. Second, we will quantify ecological characteristics of the algal taxa whose distributions correlate most closely with, and are most informative about, nutrient concentrations. The next step will be to use these ecological data on individual taxa to develop metrics that will distinguish the sites influenced by high anthropogenic nutrient inputs from those with natural levels, and various states in-between. In addition we will quantify relationships between nutrients and concentrations of chlorophyll *a*.

Schedule

The project started within 30 days of contract approval (April 1, 2004). The Quality Assurance Plan (QAP) developed for year 1 of the project has not been modified, but two new sampling protocols were added for the use of artificial substrate as well as for sampling of epipsammic/epipellic substrates (Ponader et al. 2003, Ponader & Winter 2004). Therefore the original QA plan used for year 1 through 4 of this project is still in effect, with additional protocols used in the Coastal Plain. Sampling was performed during the period from August 19th through September 16th, 2004. Analysis of diatoms was performed during winter 2004 and spring 2005. During summer 2005, chemistry and habitat data will be acquired from the NJ DEP. Data analysis and development and testing of indicators, will be done during late summer/fall of 2005. The Year 4 Final Report will be submitted in fall 2005 as a peer reviewed paper. Also, from winter 2004 through summer of 2005 we will work on preparing protocols and taxonomic documentation for delivery at the end of the project year (fall 2005).

Status of progress in achieving the project goals (see under “general approach” p.1)

The main goals during year 5 are to a) prepare protocols and taxonomic documentation, b) to develop periphyton assessment indicators and methods as nutrient management tools for the Outer Coastal Plain, and c) to test existing models on individual sites to develop site specific criteria.

The following lists our status in achieving these goals:

a) Progress was made towards providing protocols and taxonomic documentation. All existing field

protocols were compiled into one document and are currently being edited for release. All diatom pictures available were prepared to be arranged in plates (e.g. cropped and color adjusted). A diatom flora will be produced using these pictures during fall 2005.

b) We finalized the case study to test existing diatom models and metrics on sewage treatment plants and to develop site-specific criteria. We tested the diatom indices on a reach above, below and at the discharge of two selected sewage treatment plants that are part of New Jersey's current Phosphorus Evaluation studies. We evaluated model and metric performances on a site-by-site basis, and reported on how the results can be used to help develop site specific criteria. The study was presented by T. Belton at the TMDL Speciality Conference, arranged by the Water Environment Federation (WEF) and Pennsylvania Water Environment Association June 26-29, Philadelphia, Pennsylvania. All results are summarized in a manuscript published in the Proceedings of the TMDL Speciality Conference (Belton et al. 2005, see Appendix).

c) All algae data, water samples and field data were collected and databased. Data analysis and a final report in the form of a manuscript submitted to a peer-reviewed journal will be produced during the fall. The final paper will contain the following content: Development of diatom indicators and metrics in the Coastal Plain (Inner and Outer Coastal Plain, e.g. year 4 and 5). This paper will complement an existing manuscript that focuses on the project results for the Northern Part of the State (Ponader et al., in review). We are planning to submit it to the Journal "Freshwater Biology".

This new manuscript will focus on three different subjects: First, it will describe the models and metrics developed for the NJ Coastal Plain, and the problems and challenges posed by the different ecoregions, e.g. substrates. We will compare the results with those obtained for the northern part of the State. Second, we will analyze differences between diatometer and natural substrate samples for both biomass and diatom species composition. We will provide recommendations as to which sampling method provides better results for model development, and how these models should be used and interpreted by the State. Third, if it does not exceed the size of the paper, we will include a synthesis on the project as a whole, summarizing the results of all 5 project years. We will draft a detailed outline for approval by TJB after first results of the upcoming analyses are available. The draft manuscript will be sent out to Tom Belton on 31 September '05. All authors will review the draft carefully, and they will then discuss and agree on the final content of the paper.

Deliverables

Timing of submission of deliverables is expressed as months after effective contract date (April 1, 2004). Updated Year 5 deliverables (as agreed upon by Tom Belton per e-mail on 8/13/2004) are shown in the table below. Their submission extends into the sixth calendar year of the project. During a phone conversation with Karin Ponader on 14 July, Tom Belton agreed that the submission deadline for the final deliverable will be pushed back by one month (final deliverable now due on 31 October, 2005).

Due date	Deliverable	Status
5 months: 9-1-04	Revised QA Project Plan	delivered
6 months: 10-1-04	Year 5 - 1 st Quarterly report	delivered
9 months: 1-1-05	Year 5 - 2 nd Quarterly report	delivered
12 months: 4-1-05	Year 5 - 3 rd Quarterly report	delivered
15 month: 7-1-05	Draft Final Report	delivered
18 month: 10-31-05	Final Report	

Tasks left to do (year 5)

Tasks	Planned time frame		Person in charge	Status
	from	to		
1. Data login	1-Oct-04	28-Feb-05	Ponader/ Support staff	Done
2. Laboratory analysis/ preparations	1-Oct-04	31-Jan-05	Support staff/ PCER Geo-Chemistry section	Done
3. Diatom sample analysis	1-Dec-04	28-Feb-05	Ponader	Done
4. Preparing protocols	1-April-05	22-July-05	Ponader	Partly done
5. Developing year 5 model/ metrics, site specific criteria	1-Sept-05	31-Sept-05	Ponader	Partly done
6. Preparing manuscript (final report)	1- Oct-05	31-Oct-05	Ponader/ Charles/ Belton	to do
7. Taxonomic documentation (Flora)	1-July-05	31-Nov-05	Ponader/ Charles/ Belton	to do

References

- Barbour, M.T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish, Second Edition. EPA 841-B-99-002, Washington, D.C., U.S. Environmental Protection Agency, Office of Water.
- Belton, T.J., Ponader, K.C., and Charles D.F. 2005. Trophic Diatom Indices (TDI) and the development of site-specific criteria. Proceedings of the TMDL 2005 Speciality Conference, Water Environment Federation (WEF) and Pennsylvania Water Environment Association. June 26-29, Philadelphia, Pennsylvania. pp. 1042-1056.
- Charles, D., D. Winter, and M. Hoffman. 2000. Field Sampling procedures for the New Jersey Algae Indicators project. PCER Procedure P-13-64. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 18 Pages.
- Kiry, P., D. Velinsky and A.-M. Compton. 1999. Determination of dry weight and percent organic matter for sediments, tissues and benthic algae. PCER Procedure P-16-113. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 3 Pages.
- Moulton, S. R., J. G. Kennen, R. M. Goldstein, and J. A. Hambrook. 2002. Revised Protocols for Sampling Algal, Invertebrate and Fish Communities as Part of the National Water-Quality Assessment Program. Open-File Report 02-150. US Geological Survey, Reston, Virginia. 75 Pages.
- Patrick Center for Environmental Research. 2000. Quality Assurance Project Plan for the project "Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams", Academy of Natural Sciences, Philadelphia, PA. 21 Pages.
- Ponader, K., and D. Charles. 2001. Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams - Final Report Year 1. PCER Report 01-26. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 23 Pages.
- Ponader, K., and D. Charles. 2003. Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams - Final Report Year 2. Report No. 03-04. Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA. 74 Pages.
- Ponader, K., and D. Charles. 2004. Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams - Final

Report Year 4. Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA. 46 Pages.

Ponader, K., D. Winter, and D. Charles. 2003. Field Sampling procedures for the New Jersey Algae Indicators project: Use of Diatometer Artificial Substrates. PCER Procedure P-13-66. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 32 Pages.

Ponader, K. C., D. F. Charles and T. J. Belton. (in review). Diatom-based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. Ecological Indicators. Submitted May, 2005 (in review).

Ponader, K., D. Winter. 2004. Field Sampling procedures for the New Jersey Algae Indicators project: Sampling method for collection of qualitative diatom samples and algal biomass samples from epipsammic/epipellic habitat in the field. PCER Procedure P-13-67. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 7 Pages.

Velinsky, D. 2000. Syringe water sampling and filtration for the collection of filtered nutrient samples and unfiltered nutrient samples. PCER Procedure P-16-119. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 4 Pages.

Velinsky, D., and J. DeAlteris. 2000. Benthic algae and sediment chlorophyll *a* preparation and analysis. PCER Procedure P-16-117. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 4 Pages.

Appendix 1: List of pre-slected sites for field reconnaissance in Outer Coastal Plain

SS stations

AMNET #	Latitude	Longitude	Stream Name	County	Site sampled (s) /rejected (r)
AN 0575	39 39' 52"	74 45' 56"	Cedar Brook	Atlantic	s
AN 0593	39 34' 16"	74 39' 50"	Indian Cabin Creek	Atlantic	r
AN 0617	39 26' 23"	74 33' 59"	S Br Absecon Creek	Atlantic	r
AN 149A	39 58' 35"	74 34' 36"	Ong Run	Burlington	s
AN 0153	39 51' 34"	74 35' 53"	S. Br. Burrs Mill Brook	Burlington	r
AN 0156	39 55' 24"	74 43' 03"	S Br Rancocas Branch	Burlington	r
AN 0119A	40 01' 39"	74 33' 36"	South Run	Burlington	r
AN 0624	39 40' 04"	74 57' 38"	Squankum Branch	Gloucester	r
AN 0686	39 39' 44"	75 13' 51"	Oldmans Creek	Gloucester	s
AN 0503	40 08' 47"	74 11' 57"	Haystack Brook	Monmouth	s
AN 0532	40 00' 44"	74 18' 09"	Manapauqua Brook	Ocean	s
AN 0550	39 49' 02"	74 17' 34"	Long Branch	Ocean	r

EWQ stations

AMNET #	Latitude	Longitude	Stream Name	County	Site sampled (s) /rejected (r)
AN0617	39 ⁰ 26' 23"	74 ⁰ 33' 59"	S. Br. Absecon Creek	Atlantic	r
AN0621	39 ⁰ 44' 02"	74 ⁰ 57' 05"	Great Egg Harbor River	Camden	s AN0623 instead
AN0751	39 ⁰ 26' 53"	75 ⁰ 04' 20"	Maurice River	Cumberland	r
AN0740	39 ⁰ 29' 44"	75 ⁰ 04' 35"	Maurice River	Cumberland	s
AN0510	40 ⁰ 07' 36"	74 ⁰ 16' 40"	S Br Metedeconk River	Ocean	s
AN0744	39 ⁰ 33' 25"	75 ⁰ 10' 27"	Palatine Br	Salem	s
AN0699	39 ⁰ 35' 28"	75 ⁰ 17' 46"	Alloway Creek	Salem	r

Appendix 2: List of sites sampled in 2004

a) Outer Coastal Plain Sites

Date sampled	NJ Site ID	Waterbody	Location	AMNET Impairment1	AMNET Impairment2
9/14/2004	AN0149A	Ong Run	West Lakeshore Dr.	Non-impaired	Moderate
9/15/2004	AN0503	Haystack Brook	Southard Rd	Moderate	Moderate
9/15/2004	AN0510	S Br Metedeconk River	Bennetts Mill Rd (out. Lake Enno)	Severe	Moderate
9/15/2004	AN0532	Manapaqua Brook	Rt. 70	Severe	Severe
9/14/2004	AN0575	Cedar Brook	Myrtle Ave (Columbia Rd)	Moderate	Moderate
9/14/2004	AN0623	Great Egg Harbor River	Winslow Rd	Non-impaired	Moderate
9/13/2004	AN0686	Oldmans Ck	Swedesboro Rd	Moderate	-
9/13/2004	AN0740	Maurice River	Almond Ave. (USGS gauge)	Non-impaired	-
9/13/2004	AN0744	Palatine Br	Dubois Rd	Moderate	-

b) Sites re-sampled in 2004 (sites in Piedmont)

Date sampled	NJ Site ID	Waterbody	Location	Impairment1	Impairment2
8/25/2004	AN0115	Miry Run	Rt 533 (Quakerbridge Rd)	Moderate	Moderate
8/25/2004	AN0234	Whippany River	Ridgedale Ave W of Rt I-287	Severe	Non-impaired
8/25/2004	AN0374	N Br Raritan River	Rt. 202	Non-Impaired	Non-impaired

c) Sewage Treatment Plant sites

Date sampled	NJ Site ID	Waterbody	Location	AMNET Impairment1	AMNET Impairment2
9/16/2004	AN0039	Pequest River	Rt 615	Non-impaired	Non-impaired
9/16/2004	HydroQual 3	Pequest River	Freeborn Lane extension	-	-
9/16/2004	HydroQual 4	Pequest River	Alphano Rd	-	-
9/16/2004	AN0063	Musconetcong River	Waterloo Rd	Moderate	Non-impaired
9/16/2004	Najarian 4	Musconetcong River	Belton St.	-	-
9/16/2004	Najarian 5	Musconetcong River	Contitnental Drive	-	-

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

Thomas J. Belton (New Jersey Department of Environmental Protection)
Karin C. Ponader and Donald F. Charles (Patrick Center for Environmental Research,
The Academy of Natural Sciences, Philadelphia, PA)

Primary Contact: Thomas J. Belton, Research Scientist
New Jersey Department of Environmental Protection (NJDEP)
Division of Science Research and Technology
401 East State Street
PO Box 409
Trenton NJ 08625

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 (NO₃-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.

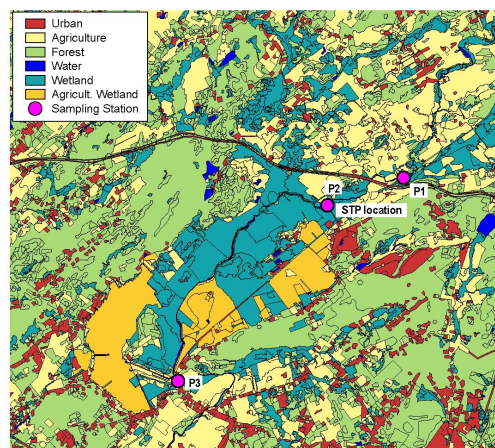
In this study, we tested diatom TP and TN models and indices developed for the Piedmont, Highlands and the Ridge and Valley physiographic provinces of New Jersey (Ponader and Charles, 2003, 2004) on reaches above, below and at the discharge of two selected STPs on the Pequest and Musconetcong Rivers.

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



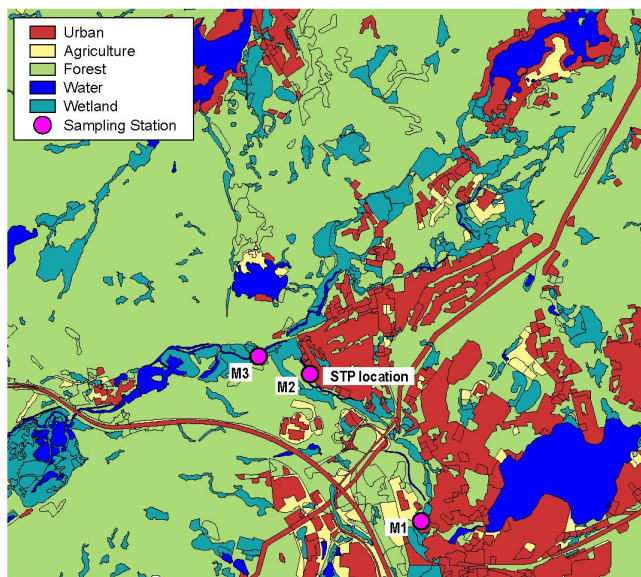
concentrated primarily in the river valleys along the riparian corridors. In addition to point sources, such as the STPs, nutrients can come from non-point sources such as agricultural fertilizer, storm water run off, and suburban lawn maintenance. In this present context, due to the large number of 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 (km²): watershed size.

Site ID	Avg width (m)	Open (%)	Flow	Bo (%)	Cob (%)	Gra (%)	San (%)	Si (%)	Urb (%)	%For (%)	Ag (%)	Wet (%)	Area (km ²)
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, NH₃-N, NO₃-N, TKN and TN), alkalinity, hardness and chloride following Patrick Center water chemistry sampling procedures (Velinsky, 2000). Samples for analysis of alkalinity, Cl⁻, 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 C², (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 ¹(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 ²(Van Dam, et al. 1994): 1) oligosaprobous, 2) β -mesosaprobous, 3) α -mesosaprobous, 4) α -meso/ polysaprobous, 5) polysaprobous. Trophic State index ²(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 ³(Potapova and Charles, 2003).

Taxon Code	Diatom Taxon Name	% Relative Abundance of Taxon at Site						TP opt. ¹ (μ g/L)	TP cat. ¹	Saprobity ²	Trophic State ²	Cond. opt. ³ (μ S/cm)	Cl ⁻ opt. ³ (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
NIIdissip	<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
REsinuta	<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
NACryten	<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
NAgregar	<i>Navicula gregaria</i> Donk.	4.0	7.6	2.0	0.8	0.0	1.7	55	3	3	5	392	20.2
CCplalin	<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
MEvarian	<i>Melosira varians</i> Agardh	4.6	2.3	0.5	0.8	0.0	0.7	60	4	3	5	309	12.8
NIamphib	<i>Nitzschia amphibia</i> Grunow	0.3	3.2	0.3	4.1	1.3	2.3	63	3	3	5	400	22.3
RSabbre	<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
NAMinima	<i>Navicula minima</i> Grunow	0.3	4.0	4.3	0.3	0.7	4.0	65	3	4	5	319	12.4
NAlancel	<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
AMPedcls	<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
SFseminu	<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
GOkobaya	<i>Gomphonema kobayashii</i> Kociolek & Kingston	4.1	2.0	2.7	7.8	1.7	3.5	1152	5	NA	NA	NA	NA
AMovalis	<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.

Figure 3a

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, NO₂+3 and NH₃-N. All other variables were entered as passive variables. Of the six variables, TKN had the highest influence with a λ_1/λ_2 ratio of 1.01, capturing 27% of the variation in the species data. SRP had a λ_1/λ_2 ratio of 0.84, capturing 24% of the variation in species data. All other variables had lower λ_1/λ_2 ratios: NH₃-N (0.78) TP (0.65), TN (0.41), NO₃-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.

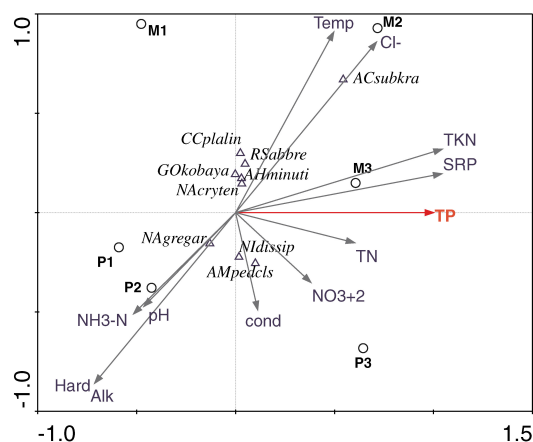
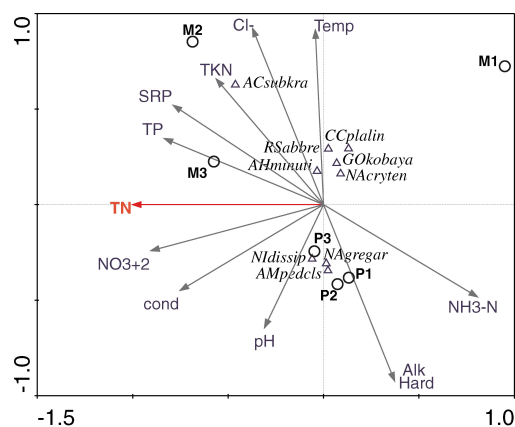


Figure 3b

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 C² (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
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.

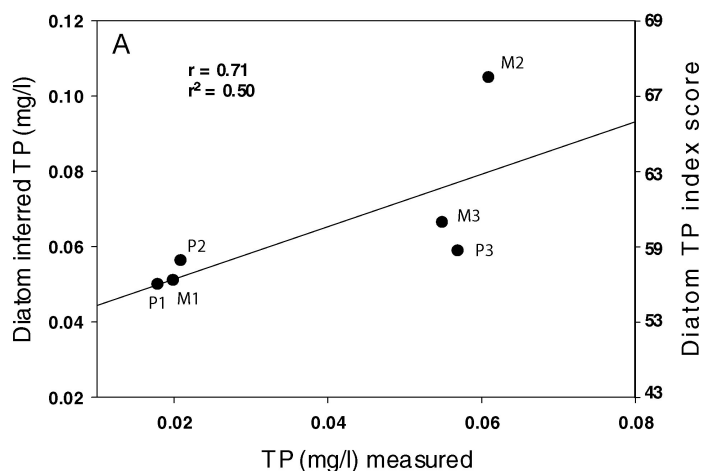
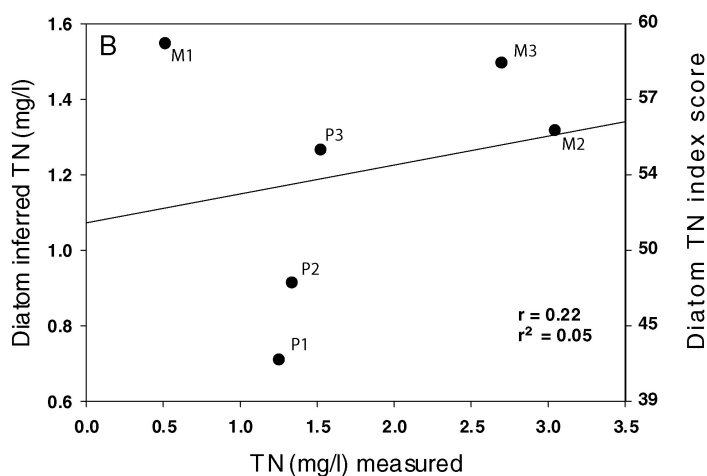


Figure 4b.

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.

ACKNOWLEDGEMENTS: Funding for this project came from the New Jersey Department of Environmental Protection, Watershed Research Fund (CBT).

REFERENCES

- Birks, H. J. B. (1995) Quantitative Paleoenvironmental Reconstructions; Pages 161-254 in D. Maddy; Brew, J. S. (editors); *Statistical Modelling of Quaternary Science Data. Technical Guide 5*; Quaternary Research Association: Cambridge, UK.
- Birks, H. J. B.; Line, J. M.; Juggins, S.; Stevenson, A. C.; ter Braak, C. J. F. (1990) Diatoms and pH Reconstructions. *Phil. Trans. Royal Soc. London, series B*, **327**:263-278.

Charles, D. F.; Winter, D.; Hoffman, M. (2000) *Field Sampling Procedures for the New Jersey Algae Indicators Project*; PCER Procedure P-13-64; Patrick Center for Environmental Research, Academy of Natural Sciences: Philadelphia, Pennsylvania.

Juggins, S. (2003) *C² User guide. Software for Ecological and Paleoecological Data Analysis and Visualization*; University of Newcastle: Newcastle upon Tyne, UK.

Moulton, S. R.; Kennen, J. G.; Goldstein, R. M.; Hambrook J. A. (2002) *Revised Protocols for Sampling Algal, Invertebrate and Fish Communities as Part of the National Water Quality Assessment Program*; Open-File Report 02-150; U.S. Geological Survey; Reston, Virginia.

Pan, Y.; Lowe, R. L. (1994) Independent and interactive effects of nutrients on benthic algae community structure. *Hydrobiologia*, **291**:201-209.

Pan, Y.; Stevenson, R. J.; Hill, B. H.; Herlihy, A. T.; Collins, G. B. (1996) Using Diatoms as Indicators of Ecological Conditions in Lotic Systems: A Regional Assessment. *J. North Amer. Benth. Soc.*, **15**: 481-495.

Patrick Center for Environmental Research (2000) *Quality Assurance Project Plan for the Project "Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams;"* Phycology Section, Academy of Natural Sciences; Philadelphia, PA.

Ponader, K.; Charles, D. F. (2003) *Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams. Final Report Year 2*; Report No. 03-04; Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, Pennsylvania.
<http://www.state.nj.us/dep/dsr/wq/wq.htm>

Ponader, K. C.; Charles, D. F. (2004) *Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams; Final Report Year 3*; Patrick Center for Environmental Research, Academy of Natural Sciences; Philadelphia, Pennsylvania.

Potapova, M.; Charles D. F. (2003) Distribution of Benthic Diatoms in U.S. Rivers in Relation to Conductivity and Ionic Composition. *Freshwater Biol.*, **48**: 1311-1328.

Potapova, M.; Charles, D. F.; Ponader K. C.; Winter D. M. (2004) Quantifying Species Indicator Values for Trophic Diatom Indices: A Comparison of Approaches. *Hydrobiologia*, **517**: 25-41.

Sprouffske, K.; Charles, D. F.; Potapova, M. (2004) *Phycological Indicators and Data Exploration Program (Phyco-AIDE) Version 1*. Phycology Section. Patrick Center for Environmental Research, Academy of Natural Sciences; Philadelphia, Pennsylvania.

Stevenson, R. J.; Pan, Y. (1999) Assessing Environmental Conditions in Rivers and Streams with Diatoms; Pages 11-40 in Stoermer, S.; Smol, J. P. (editors). *The Diatoms: Applications for the Environmental and Earth Sciences*; Cambridge University Press, Cambridge, UK.

Stevenson, R. J.; Bothwell, M. L.; Lowe, R. L. (editors) (1996) *Algal Ecology: Freshwater Benthic Ecosystems*; Academic Press: San Diego.

ter Braak, C. J. F.; Juggins, S. (1993) Weighted Averaging Partial Least Squares Regression (WA-PLS): An Improved Method For Reconstructing Environmental Variables From Species Assemblages. *Hydrobiologia*, **269/270**:485-502.

ter Braak, C. J. F.; Šmilauer, P. (2002) *CANOCO Reference Manual and CanoDraw for Windows. User's Guide: Software for Canonical Community Ordination*. Version 4.5; Microcomputer Power: Ithaca, New York.

U.S. Environmental Protection Agency (2000) *Nutrient Criteria Technical Guidance Manual – Rivers and Streams*; EPA-822-B-00-002; Washington, D.C.

Van Dam, H.; Mertens, A.; Sinkeldam, J. (1994) A Coded Checklist and Ecological Indicator Values of Freshwater Diatoms from The Netherlands. *Neth. J. Aquat. Ecol.*, **28**:117-133.

Velinsky, D. (2000) *Syringe Water Sampling and Filtration for the Collection of Filtered Nutrient Samples and Unfiltered Nutrient Samples*; PCER Procedure P-16-119; Patrick Center for Environmental Research, Academy of Natural Sciences; Philadelphia, Pennsylvania.