Development of Nutrient Endpoints for the Northern Piedmont Ecoregion of Pennsylvania: TMDL Application

Prepared for

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Region 3

Philadelphia, PA

By

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Introduction

The United States Environmental Protection Agency (USEPA) in Region 3 is overseeing the development of nutrient Total Maximum Daily Loads (TMDLs) to protect aquatic life uses for several streams in the Northern Piedmont ecoregion of southeastern Pennsylvania. Specifically TMDLs are being developed for the following watersheds: Chester, Indian, Neshaminy, Skippack, Southampton, and Wissahickon Creeks. Tetra Tech, Inc (Tt) was approached to assist USEPA in establishing appropriate TMDL endpoints for nutrients that are both protective of aquatic life uses in this region and defensible. This document describes the process that was applied, the results of those analyses, and recommended nutrient endpoints for the TMDLs in question.

Nutrients affect aquatic systems in diverse ways, and the effects on most non-primary producer aquatic life uses are indirect (Figure 1).

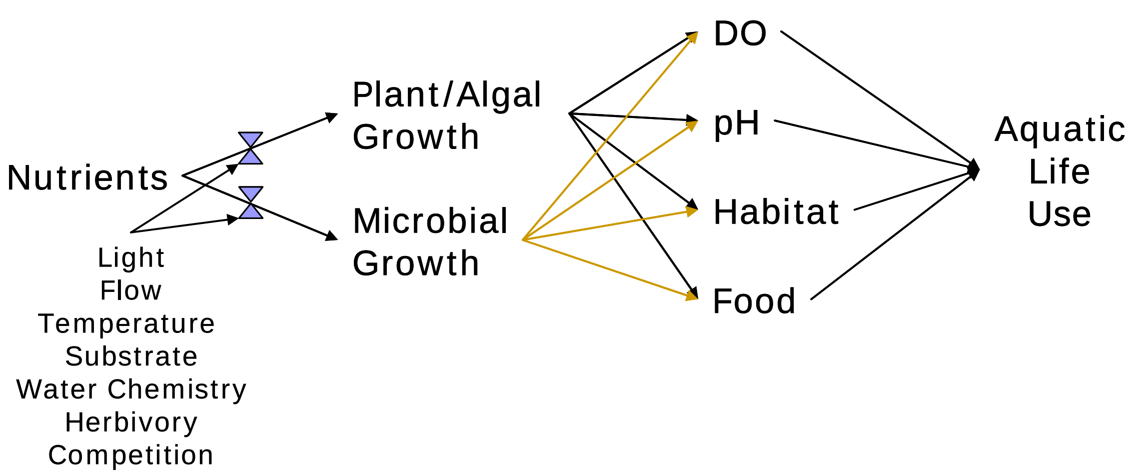

Figure 1 – Simplified diagram illustrating the causal pathway between nutrients and aquatic life use impacts. Nutrients enrich both plant/algal as well as microbial assemblages, which lead to changes in the physical/chemical habitat and food quality of streams. These effects directly impact the insect and fish assemblages. The effects of nutrients are influenced by a number of other factors as well, such as light, flow, and temperature.

Nutrients cause enrichment of primary producer and decomposer biomass and productivity, the increase of which leads to changes in the physical and chemical stream environment (e.g., reduced oxygen, loss of reproductive habitat, alteration on the availability of palatable algal taxa, etc.). It is these effects which directly result in changes to the biological stream community (e.g., loss of disturbance sensitive taxa), and ultimately impair the use of a stream for aquatic life.

Traditionally, water quality endpoints to protect aquatic life use were developed using toxicological approaches. Such approaches have been applied for a range of pollutants to develop water quality endpoints, for example. However, as explained above, nutrient enrichment does not have a direct toxicological effect on non-primary producer aquatic life. It is worth mentioning that nutrients do, however, affect algal and plant aquatic life directly, altering the diversity and composition of those assemblages radically. For insects, fish and other aquatic life, however, the mode of action of nutrients is indirect and through a causal pathway that involves alteration of physical, chemical, and biological attributes of their habitat. As a result, traditional toxicological approaches are not appropriate.

The USEPA has published guidance on nutrient endpoint development for the protection of designated uses for a range of waterbody types including rivers and streams (USEPA 2000a), but also for lakes and reservoirs (USEPA 2000b), estuaries (USEPA 2001), and wetlands (USEPA 2007). The principal method described in those documents is the use of a frequency distribution-based approach (often called the reference approach), where a percentile of a distribution of values is used to identify a nutrient endpoint. The sample distributions were typically either from least disturbed reference sites (sensu Stoddard et al. 2006) or the entire population of sample sites. These documents, however, clearly encourages the use of alternative scientifically defensible approaches and, especially, the application of several approaches in a multiple-lines-of-evidence framework, to establish defensible and protective endpoints. The document states that, "a weight of evidence approach that combines (multiple) approaches…will produce endpoints of greater scientific validity." The approaches recommended include the frequency distribution approach, stressor-response analyses, and literature based values.

In determining nutrient endpoints for developing TMDLs to protect aquatic life uses of northern piedmont streams in southeastern Pennsylvania, we relied on a multiple lines of evidence approach using all of the following approaches: frequency distribution based analysis, stressor-responses analyses, and literature based values. The following sections describe these approaches in detail including the methods used for each and the results. The resulting candidate values were then considered and a weight-of-evidence selection process applied to develop final endpoint recommendations.

Due to the limitation of watershed sizes and the difficulty in obtaining stressor response gradients (especially for reference sites) in the six target watersheds, we proposed using an ecoregional nutrient endpoint development approach similar to that applied for nutrient criteria development to identify nutrient targets that would protect aquatic life uses in these watersheds. The USEPA, in their recommendations for nutrient endpoint development, specified that "Ecoregional nutrient criteria will be developed to account for the natural variation existing within various parts of the country." (USEPA 2000a)

They go on to explain the importance of ecoregions:

"Ecoregions serve as a framework for evaluating and managing natural resources. The ecoregional classification system developed by Omernik (1987) is based on multiple geographic characteristics (e.g., soils, climate, vegetation, geology, land use) that are believed to cause or reflect the differences in the mosaic of ecosystems."

The six targeted watersheds are located within the Northern Piedmont ecoregion. We collected data from across the same ecoregion but used data from only selected sites within Pennsylvania, Maryland, and New Jersey—three states that have similar geology to the six watersheds. We also selected these sites because they have similar climatic conditions. We made the assumption that nutrient dynamics in the six watersheds should be similar to nutrient dynamics in this portion of the Northern Piedmont ecoregion.

Frequency Distribution Based Approach

For frequency distribution based approach, we identified water quality samples collected by a variety of agencies from streams in the northern piedmont ecoregion stored in a variety of databases including the USEPA STORET and EMAP databases, United State Geological Survey (USGS) National Water Inventory System (NWIS) and National Water Quality Assessment (NAWQA) program, and the Maryland Biological Stream Survey (MBSS) database (Figure 2). Two populations of sites were developed. The first was all sites for which nutrient samples were available (All Sites). The second was all sites for which watershed land cover was available and for which reference criteria could be applied (Reference Sites).

The All Sites population included samples from all of the agencies in Table 1. For sites with multiple samples, samples were averaged to estimate an average site nutrient concentration. This reduced the influence of any one site on the percentiles. After all the sites were prepared, we calculated the 25th percentile nutrient concentration of total phosphorus (TP) and total nitrogen (TN).

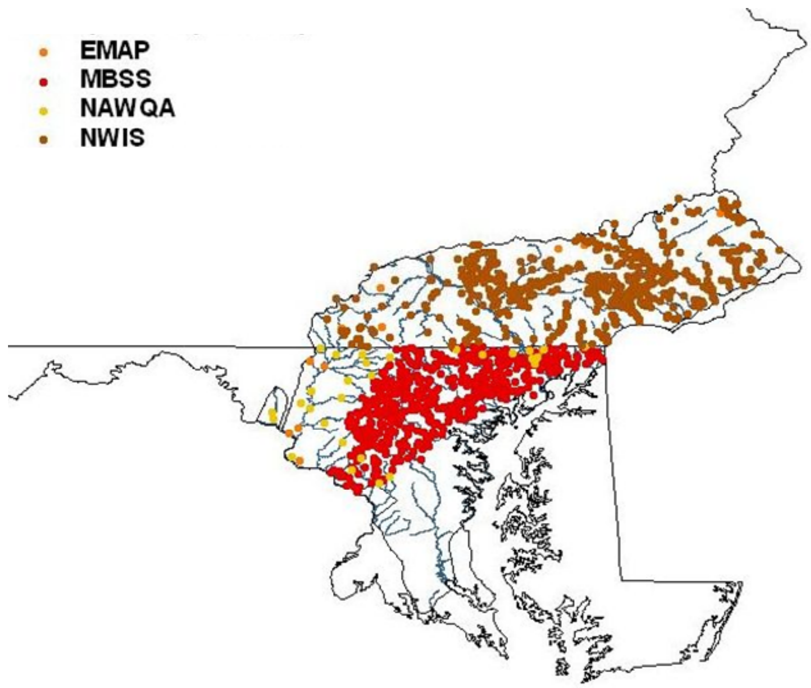

Figure 2 – Map of the sample sites used in the development of nutrient endpoints using the distribution based approach in this study, labeled by agency affiliation.

For sites where land cover information was available (USEPA EMAP, USGS NAWQA, and MBSS), we developed land cover screening criteria to identify least disturbed watersheds (sensu Stoddard et al. 2006). Least disturbed sites represent those watersheds with minimal human disturbance and, therefore, provide the best empirical estimate of chemical integrity. We developed two different reference criteria: >80% Forest, <5% urban (N=7) and >70% Forest, <5% urban (N=24). We then calculated the 75th percentile of total phosphorus and total nitrogen concentrations associated with these populations.

The results of the distribution based analyses gave comparable results whether the All Sites or either Reference Site population was used (Figure 3, Table 1). Total phosphorus concentrations were between 16 and 17 μg/L and total nitrogen concentrations between 1.3 and 1.5 mg/L (Table 1).

Table 1 – Values of TN and TP candidate endpoints derived using the distribution based approach.

Parameter	Reference Sites >80% Forest <5% Urban	Reference Sites >70% Forest <5% Urban	All Sites
	75th Percentile	75th Percentile	25th Percentile
TN (mg/L)	1.5	1.3	1.5
TP (μg/L)	17	16	17
N	7	24	782 (TN) 836 (TP)

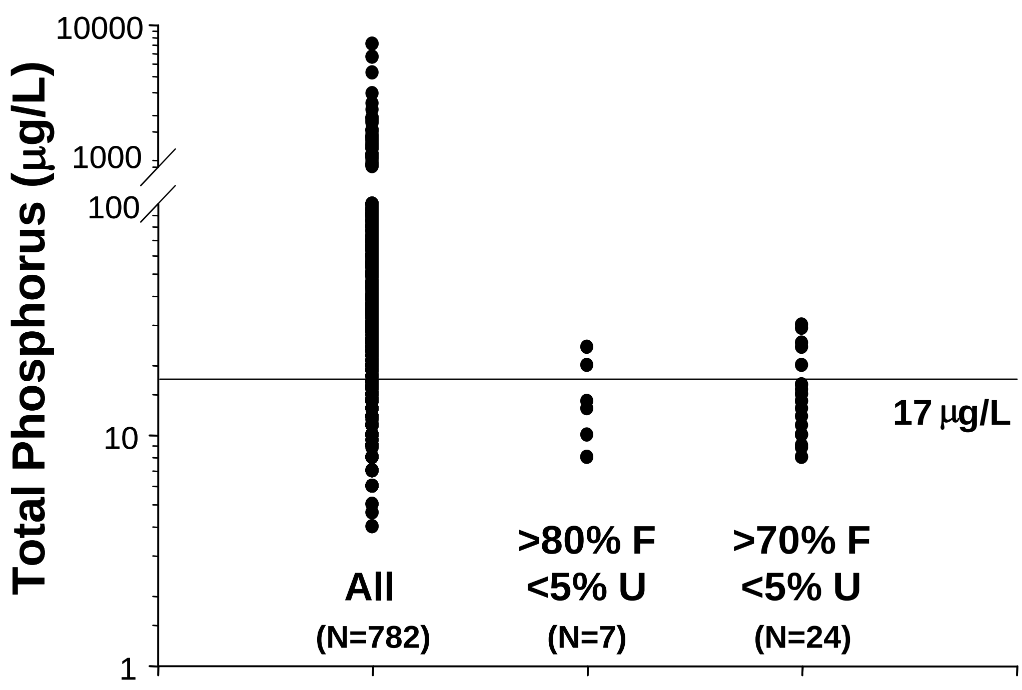

Figure 3 – Plot of total phosphorus samples in the All Sites and two Reference Site populations used to estimate candidate endpoints with the distribution based approach. Sample sizes are shown and the 25th percentile of All Sites and 75th percentile of Reference Sites was equal (17 μg/L).

Modeled Reference Expectation Approach

Another approach that falls under the rubric of "reference approaches" is the modeled reference expectation approach (Dodds and Oakes 2004). In this approach, multiple regression models of total nutrients versus human land cover (agriculture and urbanization) are built and then solved for the condition of no human land cover (i.e., the intercept). This approach has been used to estimate nutrient concentrations in the absence of human disturbance in the Midwest (Dodds and Oakes 2004).

We developed modeled reference expectation models for the northern piedmont region using data from the MBSS, USGS NAWQA and USEPA EMAP programs. The final equation for total nitrogen was:

$$\mathrm{Log}_{10}(\mathrm{TN}+1) = 0.1 + 0.49(\mathrm{arcsine}\sqrt{\%\ \mathrm{Agriculture}}) + 0.14(\mathrm{arcsine}\sqrt{\%\ \mathrm{Urban}});$$

($R^2 = 0.43$, F=125, $p<0.001$).

Solving for the undisturbed condition leads to a modeled reference total nitrogen concentration of 260 μg/L.

No significant model for total phosphorus could be created with the land cover data, so we estimated the TP value for this approach based on N:P ratios.

N:P Ratios Suggest P Limitation Dominates the Northern Piedmont Ecoregion

We calculated N:P ratios for two populations of sites: All Sites in the northern piedmont dataset and Reference Sites in the northern piedmont dataset (Table 2). The average molar N:P ratio for All Sites was 259:1 and for Reference Sites was 184:1 or 208:1, depending on which reference criteria were used. We applied these ratios to the TN value estimated from the modeled reference expectation value for TN, which yielded TP values of 2, 3 and 3 μg/L TP, respectively. The molar ratio of N:P based on the recommended USEPA nutrient criteria for this ecoregion (TP=36 μg/L, TN=690 μg/L) is 43:1. Applying this value, as well as the Redfield molar N:P ratio (16:1), to the value of TN estimated using the modeled reference expectation approach above led to estimated TP values of 14 and 37 μg/L, respectively. We would defend the use of natural ratios rather than Redfield given uncertainties in the applicability of Redfield to freshwater systems combined with the fact that Northern Piedmont average N:P ratios are much higher than Redfield. The 5th percentile of N:P ratios across all sites was 17 – meaning 95% of the streams sampled in the region have values above Redfield.

Table 2 – Values for molar N:P ratios estimated from the All Sites and the two Reference Site populations developed for use in this study. Molar N:P ratios were calculated as the ratios of moles TN: moles TP for each site.

Parameter	Reference Sites >80% Forest <5% Urban	 >70% Forest <5% Urban	All Sites
	N:P	N:P	N:P
Average	184	208	259
Median	186	186	158
25th Percentile	181	159	57
10th Percentile	141	87	25
5th Percentile	111	81	17

Stressor-Response Approach

Stressor-response approaches refer to a suite of analytical techniques that derive candidate endpoints by exploring the relationships between response variables and nutrient concentrations. Typical response variables in the context of nutrient endpoint development include water chemical aquatic life use indicators (dissolved oxygen, pH, etc.), algal biomass and/or algal assemblage metrics (e.g., percent nutrient sensitive diatoms), and aquatic life use indicators or biocriteria indicators (e.g., algal multimetric indices or individual metrics scores, invertebrate multimetric indices or individual metrics, etc.). The value of these indicators is their direct linkage to aquatic life use designations. They, therefore, provide a way to connect nutrient concentrations directly to aquatic life use protection. We used a few different stressor-response analytical techniques to develop candidate nutrient endpoints using algal and invertebrate response indicators.

We selected two important nutrient variables to examine biological responses: total nitrogen (TN) and total phosphorus (TP). TN and TP are two of the four primary variables EPA recommended for nutrient endpoint development and are likely to limit aquatic primary producers. TP and TN may reflect stream trophic status better than inorganic P and N because nutrient depletion can be partially offset by increases in particulate fractions of TP and TN resulting from benthic algal drift and suspension in the water column (Dodds 2002). In addition, TN and TP are also measured more frequently in most of the national and state programs than other nutrient variables.

The primary response variable of interest for stream trophic state characterization is algal biomass, which is most commonly reported as mg m^{-2} Chl a. Chl a is a photosynthetic pigment and is a sensitive indicator of algal biomass. It is considered an important biological response variable for nutrient-related problems (USEPA 2000a). Periphyton is also often analyzed for dry mass (DM) and ash free dry mass (AFDM), which includes non-algal organisms. EPA also recommends a measure of turbidity as the response variable. However, turbidity is often associated with total suspended solids (TSS) and other environmental factors and is less commonly used as a direct response variable. In addition to these, algal species composition often responds dramatically to excess nutrients, including the proliferation of eutrophic and nuisance algal taxa. As a result, algal metrics are frequently used as direct indicators of nutrient enrichment (van Dam et al. 1994, Pan et al. 1996). The last response variable we considered was macroinvertebrate metrics from multimetric indices. Macroinvertebrate indices are the most reliable and frequently used bioindicators, and many macroinvertebrate metrics are sensitive to nutrient enrichment.

Data:

We collected data from seven different national and state programs, similar to those used in the distribution based analyses (Table 3):

- USEPA Environmental Monitoring and Assessment Program (EMAP)
- United States Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program
- USGS National Water Information System (NWIS)
- USEPA STORET database
- EPA national nutrient center (NNC) database (include Legacy STORET data)
- Maryland Biological Stream Survey (MBSS) program
- Pennsylvania Department of Environmental Protection (PADEP) periphyton biomass data

Two national projects, the USEPA EMAP and USGS NAWQA programs, simultaneously collected nutrients, periphyton, and macroinvertebrate composition data, which were valuable for exploring both algal and invertebrate assemblage responses to nutrients. The MBSS collected hundreds of macroinvertebrate samples from its statewide stream survey. This dataset was valuable for evaluating macroinvertebrate responses. Algal biomass data from NNC, PADEP, NWIS, and EMAP were used to evaluate nutrient algal biomass responses.

Table 3 - Biological data and their related chemical measurements in the stations in Northern Piedmont ecoregion. Numbers in parentheses are numbers of samples.

	EMAP	USGS NAWQA	USGS NWIS	STORET	MBSS	NNC	PADEP
TN	20	76	380		372	55	72
TP	20	76	347		372	54	72
Algal biomass	20		48			15	93
Algal species composition	20(47)	76(142)					
Macroinvertebrate composition	20(47)	77(106)		12(27)	658		

Data Analysis: Overview

Establishing definitive stressor-response relationships is a valuable line of evidence in the multiple lines of evidence approach. We first used Spearman correlation analysis to examine relationships between response and stressor variables. Correlation analyses identified significant relationships between biological response and nutrient variables. However, correlation may or may not indicate the real relationship. Numerous relationships were examined; only a subset of which was correlated. There were also results that were considered potentially important but showed weaker relationships (Appendix A).

We selected correlations of interest and performed visual scatter plots to further examine the relationships. We used either linear regression or a locally weighted average regression line to examine the trend of change along the environmental gradients. The locally weighted scatterplot smoothing (LOWESS) technique (Cleveland 1979) models nonlinear relationships where linear methods do not perform well. LOWESS fits simple models to localized subsets of the data to construct a function that describes, essentially, the central tendency of the data. LOWESS fits segments of the data to the model. Tension, which describes the portion of data being used to fit each local function, was set at 0.50 for LOWESS regression.

We also used conditional probability analysis (Paul and MacDonald, 2005) to examine changes in the biological community along stressor gradients. Conditional probability provides the likelihood (probability) of a predefined response when a specific value of a pollutant stressor (condition) is exceeded. Conditional probability is the likelihood of an event when it is known that some other event has occurred. To estimate conditional probability of an impairment, we first had to define impairment as a specific value for a response variable (e.g., EPT < 8 genera). We used preexisting biocriteria thresholds as our response thresholds (MDNR 2005). Conditional probability answers the question: for a given threshold of a stressor, what is the cumulative probability of impairment? For example, if the total phosphorus concentration is greater than 30 μg/L, what is the probability of biological impairment (defined as < 8 EPT Taxa) for each site under consideration? All observed stressor values (in this example, all observed values of total phosphorous) are used to develop a curve of conditional probability (Paul and MacDonald, 2005). Because of its ability to identify risks of impact associated with given nutrient concentrations, the approach is suited to identifying nutrient thresholds protective of aquatic biological condition.

We also used nonparametric deviance reduction (change point analysis) to identify thresholds in biological responses to nutrients (Qian et al. 2003). This technique is similar to regression tree models, which are used to generate predictive models of response variables for one or more predictors. The change-point, in our application, is the first split of a tree model with a single predictor variable (nutrient concentration). The loss function of regression trees can be evaluated by the proportion of reduction in error (PRE), which is analagous to the multiple R^2 of general linear models.

Data Analysis: Metric Calculation

Algal Metrics

A number of algal metrics were calculated to evaluate algal assemblage characteristics and their response to nutrient enrichment. Algal density, total algal species richness, diatom species richness, Shannon's Diversity index and evenness were calculated to measure abundance and diversity. The following diatom autoecological indices were calculated to characterize different specific algal assemblage responses:

Nitrogen uptake metabolism index**:** This index relates to the flow of nutrients, particularly nitrogen, within a waterbody. It is based upon the nitrogen cycling rate from autotrophs to heterotrophs to provide a measure of the nutrient input and processing occurring in a waterbody. The nitrogen uptake metabolism index (Van Dam et al. 1994) increases with elevated concentrations of organically bound nitrogen.

Saprobity index. This is a pollution tolerance index for algal species related to oxygen, organic matter, products of septic decay, and products of mineralization. The density of oligosaprobous diatoms and polysaprobous diatoms (Van Dam et al. 1994) is impacted by waters where oxygen is saturated or absent. The percentage of alpha-mesosaprobous to polysaprobous diatoms will increase as organic loads from human disturbance (e.g., from agriculture and wastewater discharges) increase.

Trophic state index (TSI). Eutraphentic diatoms (Van Dam et al. 1994) indicate elevated concentrations of nutrients that are important for diatom growth, including nitrogen, phosphorus, inorganic carbon, and silica. As concentrations of these nutrients increase due to human disturbance, the TSI will also increase.

The TSI is one of the most important nutrient enrichment indices. Van Dam's TSI was developed in Europe and has been adapted in many parts of the United States. In addition, Jan Stevenson of Michigan State University, developed a TSI for the Mid-Atlantic highland region. He compiled a new TSI based on van Dam's TSI and Mid-Atlantic Highland (MAH) TSI into a new TSI. The Academy of Natural Science also developed a diatom TP inference model for the Northern Piedmont ecoregion. We calculated all the available TSI values to examine the relationship between observed TP concentrations and diatom inferred trophic states based on their indicator values and relative abundance. The percent sensitive taxa (indicator value from 1 to 2) were calculated (van Dam et al. 1994).

<u>Oxygen requirement index.</u> Several diatom taxa prefer conditions of high oxygen availability and will decrease in response to oxygen deficiency (Van Dam et al. 1994). Lower oxygen requirement index values implicate nutrient influx and subsequent eutrophication.

<u>*Macroinvertebrate Metrics*</u>

Approximately 40 macroinvertebrate assemblage metrics, including total taxa, Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, Ephemeroptera taxa, percent Clingers, urban Intolerant %, percent Chironomidae, and Hilsenhoff's Biotic Index (HBI), were calculated using data from various programs. After initial screening, we selected a subset of benthic macroinvertebrate indicators, including the Maryland Benthic IBI score (B-IBI) and mayfly-stonefly-caddisfly (EPT) taxon richness. Other indicators were all responsive general indicators of stress but were not diagnostic of any particular stressor.

Results: Algal Biomass – Nutrient Relationships

Similar to the distribution based approach, the molar N:P ratio of sites in the study region used for this analysis ranged from 5 to 2298 and averaged 259 and the 5th percentile of N:P ratios was greater than 16. As noted earlier, deviations from the Redfield ratio (41:7:1 by weight or 106:16:1 molar) are frequently used to determine N and P limitation (Redfield 1958). High N:P ratios indicate P is limiting growth, and low N:P ratios suggest that N is limiting growth. In addition to the strong evidence of P limitation from nutrient ratios, our examination of all the metrics with TN and other nitrogen parameters did not find strong correlations with biological variables. As a result, we considered Northern Piedmont streams as principally P-limited systems and focus on relationships with TP concentrations.

Not surprisingly, a strong algal biomass–nutrient relationship was not present in our examination of the datasets (Figure 4). A reasonable wedge shaped relationship was found between algal biomass and TP in the USGS NWIS dataset (black stars). The wedge shaped relationship is often found in large scale investigations when multiple stressors/constraints are present. It is possible that at some of the high nutrient concentration sites there was a light and flow limited accumulation of algal biomass. The wedge shaped relationship also indicated that elevated levels of algal biomass can exist at relatively low nutrient concentrations (<100 μg/L).

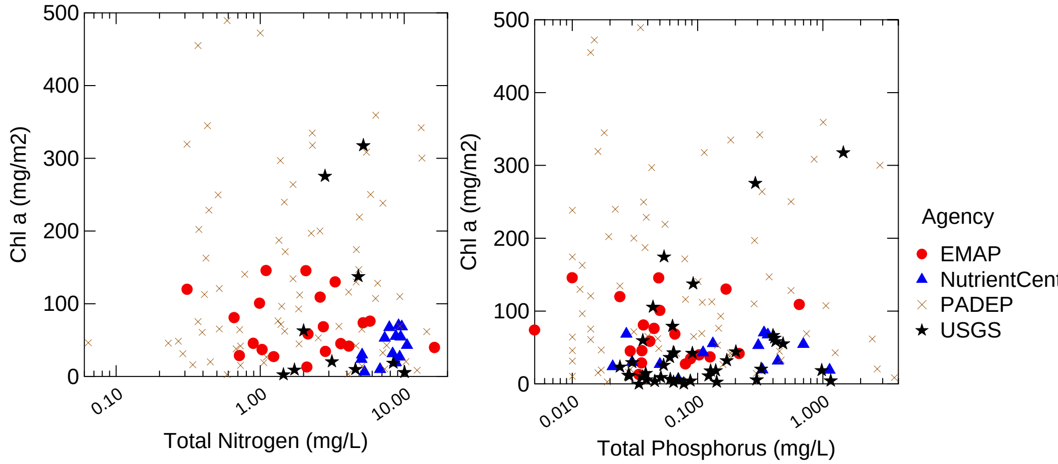

Figure 4 - Site average Chl a concentrations in relation to average TN and TP concentrations in the Northern Piedmont ecoregion. Data were collected by four different programs.

The samples with the highest algal biomass were collected by the PADEP - Pennsylvania State University periphyton study, which focused on the targeted watersheds. Surprisingly, the highest algal biomass occurred at sites where TP concentrations were relatively low (14–35 μg/L). It is possible that algal growth has been saturated even at this low level. The difference in magnitude of algal biomass among programs is probably due to different protocols being used by different programs.

Results: Algal Metrics – Nutrient Relationships

Algal compositional data from the EMAP and NAWQA programs were combined to obtain larger sample size. Two types of samples were collected and analyzed: depositional habitat and riffle habitat. Overall, four nutrient based metrics were significantly related to TP concentrations (Table 4).

Table 4 - Spearman Correlation matrices between environmental parameters and algal metrics. Significant Correlations are highlighted. (PANS = Philadelphia Academy of Natural Sciences, TSI = Trophic State Index, MSU = Michigan State University, MAIA = Mid-Atlantic Integrated Assessment)

	TN	*TP*	*NH4*	*NO3*	*DO*	*PH*	*COND*
Algal density	0.076	0.166	-0.023	0.064	-0.083	-0.083	0.147
Total taxa	0.004	0.236	0.279	-0.04	0.063	-0.131	0.133
Algal biovolume	-0.16	0.031	0.042	-0.131	0.035	-0.137	0.15
Diatom taxa	-0.03	0.243	0.283	-0.065	0.124	-0.127	0.12
N uptake index	-0.099	0.299	0.18	-0.13	0.267	-0.119	0.142
Oxygen index	-0.169	0.235	0.251	-0.184	0.255	-0.131	-0.006
Saprobity index	-0.142	0.208	0.229	-0.177	0.184	-0.127	-0.003
TSI index	-0.093	**0.402**	0.318	-0.107	0.243	-0.195	0.158
PANS TP model	-0.037	**0.515**	0.23	-0.037	0.072	0.163	**0.518**
MSU TSI index	-0.029	**0.505**	0.341	-0.039	0.188	-0.117	0.274
MSU-MAIA TSI index	-0.009	**0.454**	0.312	-0.063	0.17	-0.228	0.256

The Michigan State University (MSU) TSI index had the strongest correlation with TP concentrations among the three TSI indices. The diatom TP inference index from the Philadelphia Academy of Natural Science was the best predictor of TP concentrations in the Northern Piedmont ecoregion. However, since the model was developed from the same dataset, we did not use it for further analysis.

MSU TSI was plotted against TP concentrations in both riffle and depositional samples (Figure 5a and 5c). The significant relationship between TSI and TP concentrations in both sample types supports the prediction that TP is associated with increased trophic state in these streams. The relatively small variance explained by the regression models (R^2=0.22 and 0.35 respectively) is likely due to other stressors coexisting in the streams that are affecting diatom species compositions. A conditional probability analysis was performed for each of these sample types (Figures 5b and 5d). This analysis identifies TP thresholds associated with an increase in the probability of adverse ecological conditions, in this case, a shift in the TSI from meso- to eutraphentic conditions. According to van Dam et al (1994), TSI=4 indicates a meso-eutraphentic condition and 5 indicates eutraphentic condition. We defined the adverse condition as TSI=4.5, which is the transition from meso-eutraphentic to eutraphentic. The conditional probability analyses indicates that the probability of impairment (TSI>4,5) increases with elevated TP concentrations, i.e., diatom species composition shifts from meso-eutraphentic taxa to eutraphentic taxa. The threshold associated with the change point of this relationship was 36 μg/L for both riffle and depositional samples.

Results: Benthic Macroinvertebrate Metrics – Nutrient Relationships

The largest dataset available for analyzing macroinvertebrate responses to nutrient concentrations was the Maryland Biological Stream Survey (MBSS) dataset. The MBSS sampled more than 500 macroinvertebrate samples with 372 corresponding nutrient samples in the Northern Piedmont ecoregion. The MBSS also developed a benthic macroinvertebrate IBI for this ecoregion based on the scores of six metrics: total taxa, Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, Ephemeroptera taxa, % Clingers, % Intolerant Urban, % Chironomidae. For each metric, scoring criteria were developed based on the distribution of values from least disturbed reference sites (score of 5). We selected the middle point of the distribution as the impairment threshold for each metric (Table 5).

Of the six metrics above, only EPT taxa (Pearson r= -0.293, p<0.001), %intolerant urban taxa (r=-193, p=0.005), and % clingers (r=0.191, p=0.006) were negatively correlated with log TP concentrations. The other three metrics were either not sensitive to nutrient enrichment or more sensitive to other stressors.

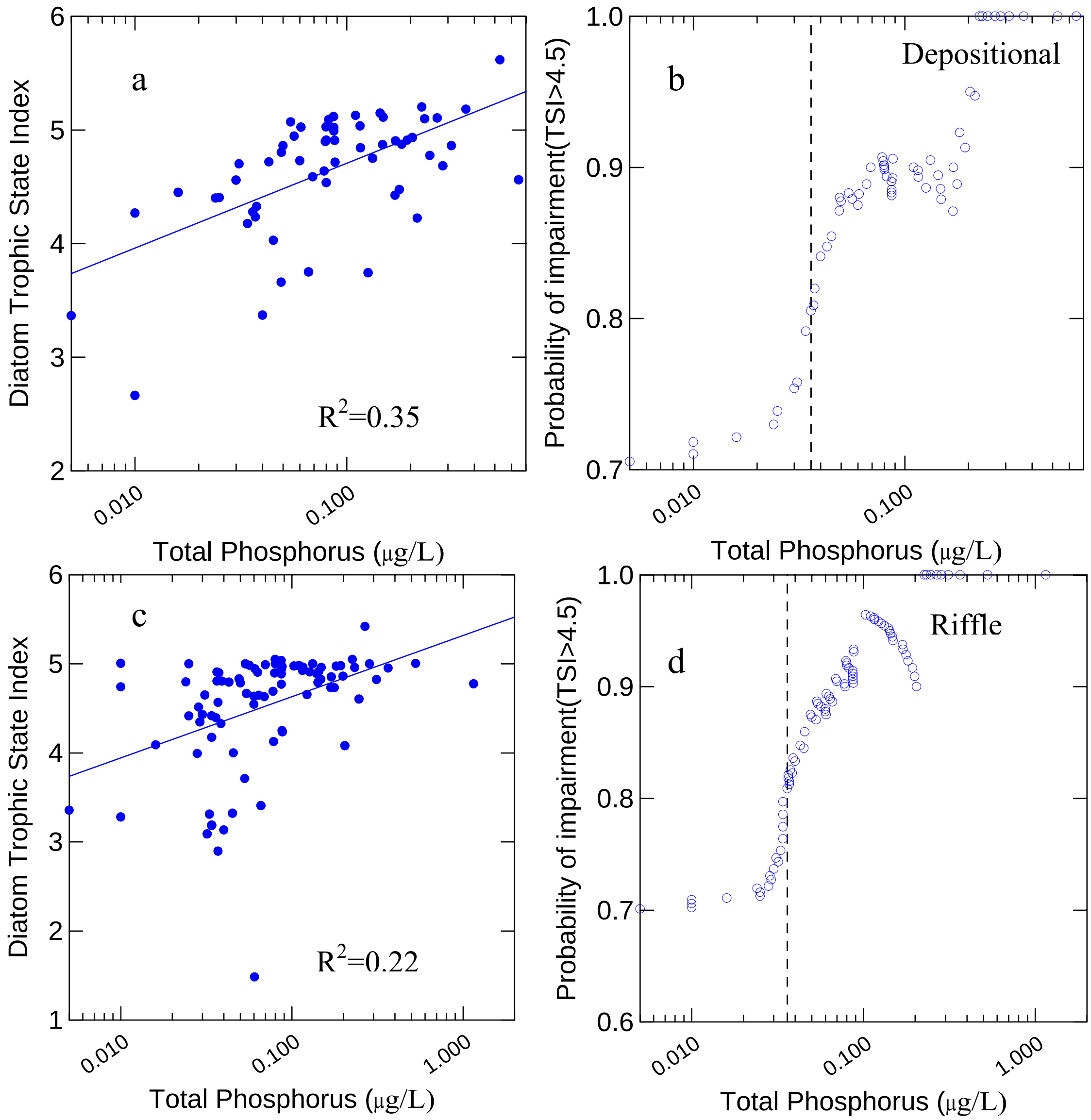

Figure 5 - Response of diatom trophic state index (MSU_TSI) to total phosphorus concentrations in depositional habitats and riffles. The figures on the right show the conditional probability of exceeding a TSI value of 4.5 with increased TP concentration.

Table 5 – Threshold values for the MBSS benthic macroinvertebrate IBI metrics in the Northern Piedmont ecoregion.

Scoring criteria	5	3	1	Mid Point
Number of Taxa	≥ 25	15 – 24	<15	20
Number of EPT	≥ 11	5 – 10	< 5	8
Number of *Ephemeroptera*	≥ 4	4– 3	< 2	3
% Chironomidae	≤ 4.6	4.7 – 63	> 63	38.6%
% Intolerant Urban	≥ 51	12 – 50	< 12	31.5%
% Clingers	≥ 74	31 – 73	< 31	52.5%

For example, EPT taxa declined with increased TP concentrations (Figure 6a). The scatterplot relationship exhibited a traditional wedge shape decline, while the conditional probability graph (Figure 6b) clearly indicated that the probability of impairment (EPT taxa<8) increased from 45 to 88% when TP concentrations increased from 30 to 80 µg/L TP. Change point analysis indicated a threshold at 38 µg/L.

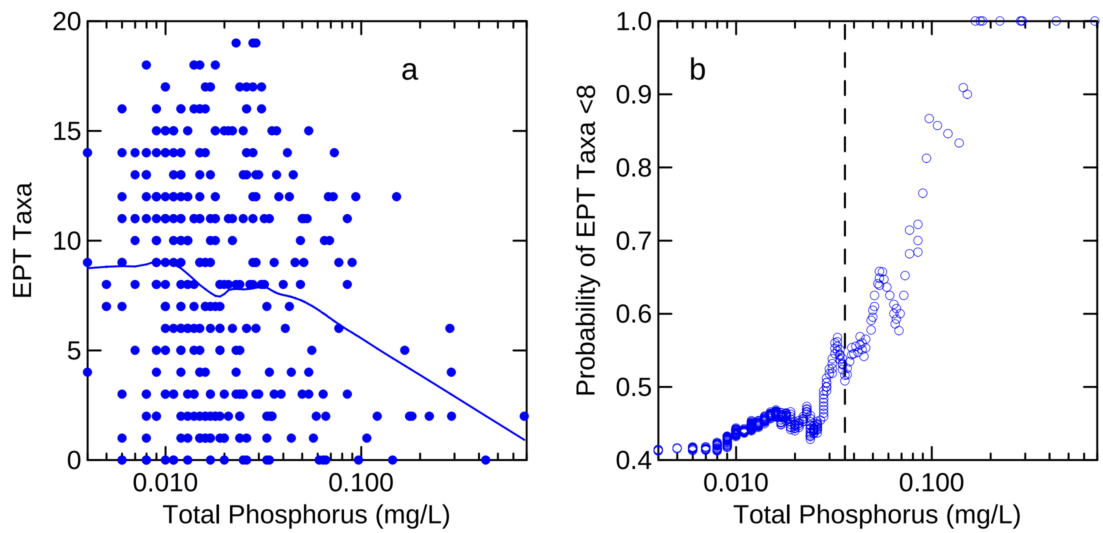

Figure 6 - Response of EPT taxa metric to the phosphorus gradient in the Northern Piedmont Ecoregion. Figure on the right shows the conditional probability of having fewer than 8 EPT taxa as TP concentrations increase.

The percent of clinger taxa in the streams responded similarly to increased TP concentrations (Figure 7). The conditional probability graph (Figure 7b) showed that the probability of impairment (% Clinger taxa <52.5) would likely increase from 45 to 70% when TP increased from 30 to 80 µg/L. Change point analysis identified a threshold at 39 µg/L.

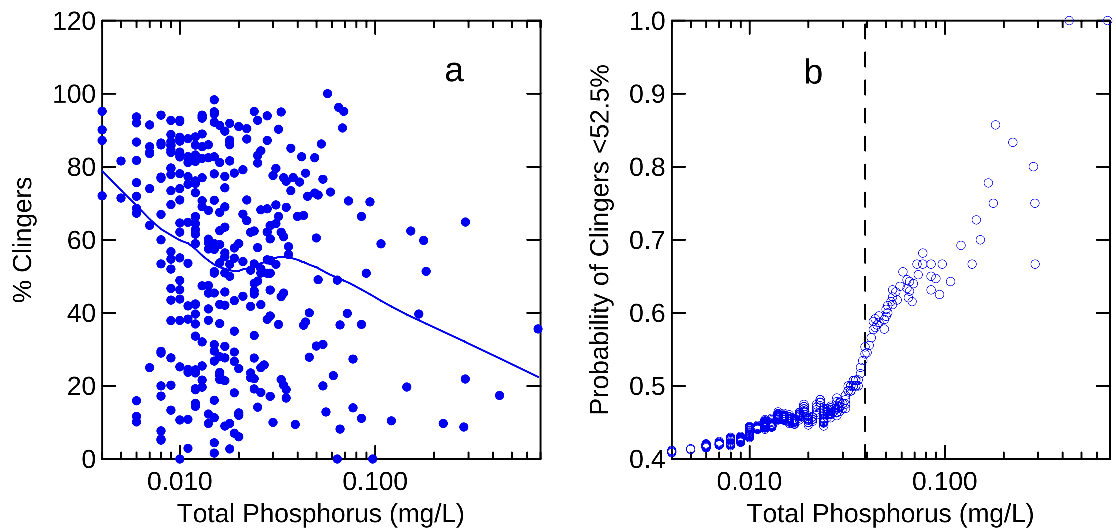

Figure 7 - Response of % clingers in benthic samples along phosphorus gradient in the Northern Piedmont Ecoregion. The figure on the right shows the conditional probability of having fewer than 52.5% clinger taxa in a stream as TP concentration increases.

The % urban intolerant taxa metric was based on the response of taxa intolerant of urbanization (Figure 8). Although TP concentration likely increases along the urban gradient, this metric is less sensitive to nutrient concentrations since it includes some taxa that are insensitive to nutrients but sensitive to other stressors. The conditional probability graph (Figure 8b) and change-point analysis indicated that probability of impairment would likely increase from 50% to much higher after TP concentration exceeded 64 µg/L TP.

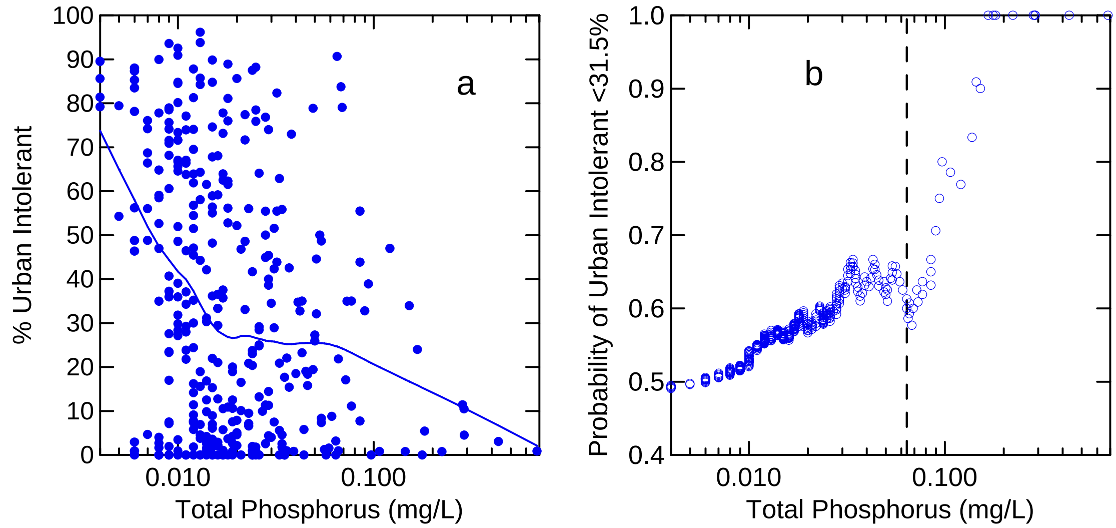

Figure 8 - Response of % intolerant urban taxa in benthic samples along phosphorus gradient in the Northern Piedmont Ecoregion. The figure on the right shows the conditional probability of having fewer than 31.5% urban intolerant taxa in a stream as TP concentration increases.

Responses of macroinvertebrate metrics to nutrient enrichments have been examined for other databases. The Spearman correlation matrices are included in Appendix 1 and 2. Due to relatively small sample size and a lack of relationships of interest, we did not examine these further.

<u>Literature Based Analysis: Current Existing Endpoints or Threshold Values</u>

In this last analytical section, we present several studies relevant to the development of nutrient endpoints in the northern Piedmont region of Pennsylvania. These are taken principally from the peer-reviewed literature and reflect increasing experimental and theoretical interest in the impact of nutrients on natural stream systems. We attempted to extract information from these studies that could recommend specific endpoints.

In natural, shaded streams [such as those evaluated in the Dodds et al. (2002) model], it is difficult to assess the full growth potential of algae. Algal growth potential has been evaluated using artificial stream channels that are fully exposed to nutrient and light gradients. Previous studies (Horner et al. 1983, Bothwell 1989) demonstrated that in artificial streams, algal growth could be saturated (i.e., achieved maximum growth rate) at 25–50 µg/l phosphorus. Rier and Stevenson (2006) found that at 16 µg/L soluble reactive phosphorus (SRP) or 86 µg/L dissolved inorganic nitrogen (DIN), algal growth was at 90% of its maximum rate. They also found that saturation concentrations were 3–5 times lower than concentrations needed to produce maximum algal biomass (i.e., 430 µg/L DIN and 80 µg/L SRP for growth saturation). However, these values were derived mostly on the basis of diatom and bluegreen algae growth. We expect that green algae (i.e., *Cladophora*) would have higher nutrient saturation concentrations for peak growth (Borchardt 1996).

Studies in adjacent regions have shown consistently low values for TP required for the control of benthic chlorophyll. An ongoing study on the Jackson River in Virginia (Louis Berger Group, pers. comm) is proposing an ortho-phosphorus endpoint of 38 µg/L. This is based on a regression equation developed using local data. In New Jersey, a trophic diatom index (TDI) was developed that identified a TP below 25 µg/L as a low, protective TDI value and a range from 75 µg/L to 100 µg/L for a high TDI value (Ponader et al. 2005). These authors also found that within this region, concentrations above 50 µg/L TP were sufficient to produce nuisance algal growth.

EPA's nutrient threshold recommendation for the Northern Piedmont was 690 µg/L for TN and 37 µg/L for TP. Ponader and Charles (2003) applied EPA's reference approach to the Northern Piedmont in New Jersey and found fairly good agreement with the EPA recommended numbers. The Ponader and Charles (2003) estimates using the distribution approach were 1.3 μg/L for TN and 40-51 μg/L for TP.

Dodds and Welch (2000) conducted a meta-study including values from a range of areas nationwide. These were combined into regression equations to predict chlorophyll. They found that if a mean of 50 mg m^{-2} of chlorophyll is the target (thus insuring chlorophyll is less than 100 mg m^{-2} most of the time), TN should be 470 μg/L and TP should be 60 μg/L. Even lower numbers should be considered for more pristine waters. These estimates were more general in scope. These authors further noted that lower TN and TP values associated with these chlorophyll concentrations were obtained when using a detailed, smaller data set than those from a larger data set (55 μg/L TP from a large dataset versus 21 μg/L for a more specific, local data set).

USGS conducted a study in 2001 for a broad area of the US, including the New River and Big Sandy River in Virginia (Robertson et al. 2001). They looked at 234 sites using the reference approach and found that a TP of 20 μg/L was appropriate for what they define as Environmental Nutrient Zone 2. Similarly, Ponader et al. (2005) in a study of over 35 streams in Virginia observed changes in the diatom assemblages and suggested threshold limits of 500 μg/L for TN and 50 μg/L for TP to protect against conditions that they termed as "nutrient impaired", based on a variety of factors.

ENSR (2003) developed endpoints for rivers in New England by combining the distribution based approach (using a database of 569 stream and river nutrient and trophic parameter data) and effects-based approach (based on a weight-of-evidence review of literature, models and TMDL studies)(Table 6). Using the EPA approach for calculating ambient water quality recommendations, the 25th percentile of all rivers and streams and the 75th percentile of the reference waters provided relatively similar values. ENSR (2003) suggested, based on the weight-of-evidence, that 40 μg/L TP and 800 μg/L TN would be an upper bound for nutrient endpoints (i.e., approaching impaired aquatic community status).

Table 6 - Comparison of New England Water Quality Recommendations, after ENSR (2003) with EPA recommended regional criteria. All values in μg/L.

		New England Ecoregion All Season		EPA Recommended Criteria
Sub-Ecoregions		25th Percentile	75th Percentile	
83	Chl a	1.6	1.5	1.6
	TN	470	538	480
	TP	31	44	24.1
82	Chl a	1.7	2.5	
	TN	33	325	390
	TP	14	12	12
59	Chl a	4.9		
	TN	560	458	570
	TP	20	22	23.5
58	Chl a	2.2		3.4
	TN	360	121	420
	TP	10	12	5
Composite	Chl a	1.9	1.8	
	TN	460	520	
	TP	20	23	

Rohm et al. (2002) conducted a national study to demonstrate how regional reference conditions and draft nutrient endpoints could be developed. They divided the country into 14 regions and analyzed available nutrient data as a case study, using EMAP data from Central and Eastern Forested Uplands, an area that includes much of central Pennsylvania. This case study suggested a criterion of 375 μg/L for TN and 13 μg/L for TP. Rough estimates from the data presented for their Region IX that includes Eastern Pennsylvania gives estimates of 500 μg/L TN and 20 μg/L TP.

Several states have developed nutrient standards or guidelines (see companion report). These values range from a maximum TP of 100 µg/L to a summer average TP of 25 µg/L. Delaware uses TP in assessing their waters for reporting under Section 305(b) of the Clean Water Act. They list segments as impaired when one or more stations whose lower confidence limit is at or above the moderate value of 1.0 to 3.0 mg/L TN and 50 to 100 µg/l TP. Hill and Devlin (2003), in a preliminary study in Virginia, suggest a TN threshold for benthic impairment somewhere between 350 and 900 µg/L TN. The Dodds et al. (2006) TN breakpoint fits within this range for TN.

<u>**Recommended Endpoints**</u>

Total Phosphorus (TP)

<u>*Endpoint (magnitude)*</u>

Our analyses relied on a weight-of-evidence analysis drawing on many different analytical approaches. Each of the different approaches produced slightly different endpoints and these are summarized in Table 7.

In a weight-of-evidence approach, the different analyses are weighted based, essentially, on their applicability and the strength of the analysis. The Reference Approach analyses we weight less for a few reasons. The Reference Site 75th percentile estimate was based on few sites and the All Sites 25th percentile analysis included a variety of data spanning many different periods. The modeled reference expectation approach could not produce a significant model for TP and the TP endpoint for this method was derived, instead, from the TN model using an estimate based on a variety of TN:TP molar ratios. Lastly, the reference approach is less easy to link directly to use protection, given that it is based on percentiles of a frequency distribution. Reference sites arguably reflect the "indigenous" or "natural" condition, which is the goal of aquatic life use standards in Pennsylvania, but this is an indirect measure.

Table 7 – Summary of candidate endpoints for each of the analytical approaches discussed.

	Approach	TP Endpoint (μg/L)
Reference Approach		**2-37**
	Reference Site 75th Percentile	16-17
	All Sites 25th Percentile	17
	Modeled Reference Expectation	2-37
Stressor-Response		**36-64**
	Conditional Probability – EPT taxa	38
	Conditional Probability - % Clingers	39
	Conditional Probability - % Urban Intolerant	64
	Conditional Probability - Diatoms TSI	36
Other Literature		**13-100**
	USEPA Recommended Regional Criteria	37
	USEPA Regional Criteria Approach – Local Data	40-51
	Algal Growth Saturation	25-50
	Nationwide Meta-Study TP-Chlorophyll	21-60
	USGS Regional Reference Study	20
	USGS National Nutrient Criteria Study	13-20
	New England Nutrient Criteria Study	40
	Virginia Nutrient Criteria Study	50
	New Jersey TDI	25-50
	Delaware Criteria	50-100

The stressor-response analyses carry more weight because we could link nutrient concentrations to specific aquatic life endpoints – both invertebrate and algal. Using invertebrate taxa metrics, conditional probability analyses evaluated those TP concentrations which increased the risk of exceeding degradation thresholds developed for these macroinvertebrate metrics in comparable piedmont streams in Maryland. For the diatom Trophic State Index (TSI), the same analysis was used to identify the TP concentration associated with a shift from meso- to eutrophic conditions.

The scientific literature was variably weighted, since it included data from regions proximate to Pennsylvania, as well as data less applicable to Pennsylvania.

Based on greater weight to the stressor-response models, we recommend TP endpoints for northern piedmont streams in southeastern Pennsylvania of 40 μg/L. This value is comparable to the majority of the stressor-response analyses, on the high end of the reference approaches, and intermediate to the scientific literature values, but comparable to regionally relevant literature values.

- **Recommended endpoint: 40 μg/L TP**

Sample period

We recommend applying the endpoint over the algal growing season (April to October), which in streams is typically the time during which the greatest risk of deleterious algal growth exists.

- **Endpoint applies from April to October**

Sample duration

Unlike toxics, there is less literature to recommend appropriate sample duration and frequencies for nutrients. Toxics, with chronic and acute criteria, have a longer history of implementation. Their mode of action is also very different than nutrients. As a result, it was more difficult to recommend an appropriate sample period than to derive the endpoints themselves.

Humans tend to sample nutrients at temporal scales that are different than those to which stream organisms respond. Streams respond both to pulsed as well as chronic nutrient concentrations. For example, algae possess mechanisms to store nutrients and use these stored nutrients for growth over time – so they can respond to episodic inputs.

Moreover, the responses to episodic inputs include both assemblage responses (for example, development the nuisance algal taxa) as well as population and individual responses (biomass).

The nutrient data we analyzed for the invertebrate and plant responses were based on single grab samples associated with biological sampling. These analyses, therefore, represent a space for time substitution of sorts, estimating what would occur in a piedmont stream as nutrient concentrations increase.

• Endpoint is assessed as the average TP concentration during the growing period over one year.

These factors would recommend a not-to-exceed criterion. However, water velocity affects nutrient delivery in streams and elevated nutrients associated with high flows may not be as accessible to benthic algae. We also recognize that there is resistance to not-to-exceed standards and concern about the risk of capturing false positives, even though the risk of false negatives is similarly great. These concerns would recommend averaging multiple samples over some time period. Algal and microbial responses to nutrients can occur rapidly, but these can be offset by floods that scour the bottom and remove algae. At this time, there is limited information and we have had insufficient time to investigate appropriate averaging periods, especially those that result in conditions detrimental to uses.

As a result, for the purposes of these TMDLs, we recommend that the TP endpoint be applied as an average of water samples taken over the growing season. Realize, again, that there is less information to guide this recommendation, which is based principally on our professional judgment and in an attempt to be consistent with other typical duration procedures. A more conservative alternative would be to use the recommended endpoint as a not-to-exceed value, but again, we have had insufficient time to evaluate this.

We feel that this approach will be protective, but we strongly encourage the state and EPA to investigate this issue more fully for the purposes of regional criteria development. For the TMDLs, this approach is sufficient, but it deserves more attention and resources before being applied to regional criteria.

Total Nitrogen (TN)

Endpoint

The focus of our work was the development of total phosphorus endpoints. This was principally due to the fact that TP was assessed as the cause of impairment. Our analyses support the conclusion that these streams are P limited, based on instream N:P molar ratios evaluated against Redfield. The distributional statistics of N:P ratios taken from more than 552 stream sites across the northern piedmont region in Pennsylvania and Maryland are shown in Table 2.

The traditional, critical N:P Redfield molar ratio is 16:1, values below indicating N limitation and those above, P limitation. Ratios have to be considered in relation to supply and become less meaningful as nutrient supplies exceed uptake capacity of streams. Even so, clearly more than 95 percent of the streams sampled in the northern piedmont region were P limited, relative to Redfield.

Because these systems are not N limited, relationships between TN and response measures are less well etablished. The fact that N is not limiting also means that TN likely contributes less to use impairment from eutrophication in this region. Endpoints are best derived when clear connections to use impairment can be made. It is most likely that N contributes to use issues in the tidal and estuarine waterbodies downstream of rivers and streams in this region (e.g., Delaware Bay). Those systems are where data and analyses will be able to suggest an appropriate N target for upstream systems. That being the case, there is some risk in setting stream TN endpoints in this region that may ultimately be inconsistent with those needed to protect uses from TN enrichment in the Bay. While we cannot recommend an N target, we can recommend a few TN endpoints using different approaches:

Ratio-based

This approach assumes it is protective to reduce N in proportion to P based on ambient molar nutrient ratios. This, again, may not sufficiently reduce N to protect downstream uses, but it would keep the ratios consistent.

Based on the distributional statistics on stream molar N:P ratios (Table 2), one could decide that keeping the ratio consistent with average Piedmont streams would be appropriate. The average piedmont TN:TP molar ratio for reference streams (minimal human disturbance using 70% forest cover and 5% urban cover) was 208:1. Based on a 40 ug/L TP target, that would recommend a TN value of 3.8 mg/L TN. Note that this value would be consistent with other Piedmont streams, which is on the higher end for TN, especially for export to an estuary. One could also use the ratio based on EPA recommended criteria (43:1). Based on that ratio, the TN concentration would be 780 μg/L.

Modeled Reference Expectation

We ran an analysis of human disturbed land cover against TN and found a significant linear regression model (see above), where TN increased with non-forested land cover. The equation was:

$$\mathrm{Log}_{10}(\mathrm{TN}+1) = 0.1 + 0.49(\mathrm{asin}\sqrt{\%\ \mathrm{Agriculture}}) + 0.14(\mathrm{asin}\sqrt{\%\ \mathrm{Urban}})$$

Solving for the y-intercept (all forested land) leads to a value of LogTN = 0.1 or 260 μg/L. This would be the expected average TN concentration without human disturbance in the watershed, based on this modeling approach. Note that this value is much less than the ratio-based number, but is likely more along the lines of what might have existed in the absence of land-cover disturbance.

EPA Regional Recommendation

The EPA recommended criteria based on their reference population derived approach was 690 μg/L. That is another value that could be used.

Distribution Based

This approach looked at the distribution of TN values in reference sites and across all sites we gathered for this analysis, comparable to those used for the TP endpoint. The 75$^{\mathrm{Th}}$ percentile of the reference population values ranged from 1.3 to 1.5 mg/L TN based on what land cover criteria were used (Table 1). The value based on the 25$^{\mathrm{th}}$ percentile of all sites would be 1.5 mg/L.

Overall

The endpoints ranged from 260 μg/L to 3.7 mg/L. Again, the less established linkage to use protection as seen for the TP endpoint stressor-response analysis, makes the selection difficult. A value approximate to 1.5 mg/L would seem to be sensibly consistent with reference conditions (perhaps the strongest analysis in this context). This would be applied over the same time period and sample duration as TP.

Again, we cannot be as definitive as we were with TP, because there appears to be little reason to think that TN is limiting uses in these northern piedmont freshwater stream systems – but rather the effects of N are likely manifested downstream. It is those systems that should be driving the choice of protective TN targets upstream. Given that, the strategy would be to err on the low side, and this would argue for something more along the 1-1.5 mg/L range.

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Appendix A. Spearman Correlation matrices among enrichment related environmental variables and a few macroinvertebrate metrics in Northern Piedmont Ecoregion in EMAP database. Significant correlations are highlighted.

	Conductivity	DOC	NH4	TN	TP
Total Richness	-0.22	-0.387	-0.486	-0.014	-0.134
Chironomid richness	-0.233	-0.411	-0.343	-0.043	-0.229
Ephemeroptera richness	-0.004	-0.157	-0.431	0.107	0.138
EPT richness	-0.177	-0.321	-0.438	0.005	-0.044
Non-insect richness	0.107	0.081	-0.078	0.181	0.054
Megaloptera richness	0.066	0.034	-0.112	-0.177	-0.261
Oligochaete/leech richness	-0.417	-0.153	-0.265	-0.32	-0.095
Plecoptera richness	-0.405	**-0.616**	-0.379	-0.114	-0.373
Trichoptera richness	-0.158	-0.269	-0.334	-0.005	-0.038
Collector-filterer richness	0.021	-0.256	-0.259	0.172	-0.04
Collector-gatherer richness	-0.325	-0.43	-0.478	-0.027	-0.187
Mixed functional richness	-0.241	**-0.537**	-0.499	0.091	-0.067
Omnivore richness	0.317	0.124	0.166	0.235	0.058
Predator richness	-0.154	-0.244	-0.177	-0.301	-0.3
Scavenger richness	-0.333	-0.091	-0.213	-0.217	-0.05
Shredder richness	-0.083	-0.306	-0.489	0.136	0.007
Scraper richness	0.056	-0.042	-0.289	-0.055	-0.147
Intolerant taxa richness	-0.381	**-0.508**	**-0.543**	-0.065	-0.201
Facilitator richness	-0.163	-0.372	-0.429	0.011	-0.19
Tolerant richness	0.083	0.138	-0.047	-0.05	0.084
Tolerant %	0.283	0.449	0.268	-0.017	0.23
Dominant Taxon %	0.204	0.246	0.182	-0.037	0.183
Shannon's diversity	-0.194	-0.387	-0.333	0.036	-0.22
Simpson's diversity	0.194	0.302	0.211	-0.058	0.203
Dominant 3 Taxa %	0.205	0.293	0.202	-0.088	0.201
Hilsenhoff BI	0.245	0.289	0.312	0.059	0.194
Sample Size	47	45	45	47	47

Appendix B. Spearman Correlation coefficients among enrichment related environmental variables and a sub-sample of macroinvertebrate metrics from Northern Piedmont Ecoregion stream samples in the USGS NAWQA database. Significant correlations are highlighted.

	Conductivity	NH4	TKN	NOX	TN	DP	TP
Beck's BI	**-0.586**	-0.147	-0.29	0.039	0.012	-0.238	-0.151
Bivalves %	0.274	0.21	**0.668**	-0.317	-0.254	**0.518**	0.205
Chironomid %	0.217	0.28	0.064	0.111	0.133	-0.033	-0.05
Chironomid richness	0.028	0.264	0.163	-0.009	0.036	-0.022	0.039
Collector %	0.143	-0.125	-0.164	0.289	0.388	-0.233	-0.24
Collector richness	-0.273	-0.038	-0.318	0.086	0.089	-0.3	-0.195
Clinger richness	**-0.551**	-0.04	-0.264	-0.08	-0.076	-0.15	-0.138
Coleoptera %	0.042	-0.07	0.346	0.137	0.115	0.415	**0.511**
Coleoptera richness	-0.417	0.148	-0.016	-0.126	-0.201	0.127	0.26
Percent Corbicula	0.471	0.434	0.401	-0.064	0.015	0.386	0.37
Crustacea+Mollusca %	0.157	0.409	0.459	-0.46	-0.429	0.424	0.286
Margalef Diversity	-0.467	-0.073	-0.294	-0.067	-0.045	-0.239	-0.14
Diptera richness	-0.065	0.247	0.135	0.07	0.098	-0.032	0.033
Dominant Taxon %	0.271	0.148	0.293	-0.054	-0.099	0.33	0.331
Ephemeroptera richness	-0.424	-0.274	-0.113	0.062	0.056	-0.036	-0.165
EPT richness	-0.47	-0.198	-0.289	0.036	0.006	-0.163	-0.139
Evenness	-0.278	-0.254	-0.387	0.174	0.217	-0.369	-0.214
Filterer %	-0.002	0.434	0.163	-0.168	-0.153	0.113	0.175
Filterer richness	-0.231	0.154	0.012	-0.252	-0.289	0.014	0.133
Hilsenhoff BI	0.225	0.032	-0.004	-0.066	-0.057	-0.203	0.055
Intolerant richness	**-0.577**	-0.156	-0.304	-0.006	-0.024	-0.272	-0.155
Noninsect %	0.171	-0.269	-0.042	-0.004	0.039	-0.112	0.03
Odonate %	-0.173	-0.104	-0.049	0.023	0.019	-0.032	-0.057
Oligochaete %	-0.009	-0.12	-0.159	-0.058	-0.062	-0.24	-0.208
Oligochaete richness	-0.115	-0.02	-0.284	0.012	0.041	-0.283	-0.225
Plecoptera %	**-0.525**	-0.229	-0.346	0.066	0.078	-0.417	-0.315
Plecoptera richness	**-0.533**	-0.173	-0.331	0.048	0.06	-0.399	-0.318
Predator %	-0.236	-0.336	-0.423	0.183	0.21	-0.493	-0.256
Predator richness	-0.424	-0.051	-0.065	-0.255	-0.252	-0.051	-0.159
Scraper Richness	-0.184	0.222	0.101	-0.417	-0.444	0.262	0.175
Shannon diversity	-0.397	-0.222	-0.423	-0.061	-0.011	-0.421	-0.324
Swimmer %	**-0.578**	-0.337	**-0.508**	-0.001	-0.023	-0.398	-0.392
Swimmer richness	**-0.564**	-0.362	-0.477	0.041	-0.004	-0.376	-0.297
Tolerant %	0.099	-0.098	-0.07	0.092	0.098	-0.158	0.114
Tolerant richness	0.258	0.281	0.344	-0.313	-0.367	0.322	0.251
Total richness	-0.441	0.074	-0.174	-0.194	-0.184	-0.106	-0.037
Trichoptera richness	-0.319	0.006	-0.264	-0.216	-0.243	-0.15	-0.047
Sample size	77	52	44	52	44	44	38