

Development of Nutrient Endpoints for the Northern Piedmont Ecoregion of Pennsylvania: TMDL Application Follow-up Analysis

**Prepared for
United States Environmental Protection
Agency
Region 3
Philadelphia, PA**

**Prepared by
Michael J. Paul, James Robbiani, Lei
Zheng, Teresa Rafi, Sen Bai, and Peter
Von Loewe
Tetra Tech, Inc.
400 Red Brook Boulevard, Suite 200
Owings Mills, MD 21117**



18 July 2012

TABLE OF CONTENTS

Table of Contents	ii
List of Figures	iii
List of Tables	v
1. Introduction	1
2. Revised Guidance	1
3. Conceptual Model	2
4. Data Analysis – Correlations	5
5. Data Analysis – Multiple Regression	6
6. Data Analysis – regression within bins	9
7. Model validation	19
8. Mechanistic Modeling	20
9. Scientific Literature	23
10. Updated Endpoint Summary	24
11. Literature Cited	26

LIST OF FIGURES

Figure 1- Steps in stressor-response analysis (USEPA 2010).....	1
Figure 2 – Simplified diagram illustrating the causal pathway between nutrients and aquatic life use impacts (Paul and Zheng 2007).....	3
Figure 3 - More detailed conceptual model of the causal relationship between nutrients and responses in streams (after USEPA 2010).....	4
Figure 4 – Plot of first two principal components generated indicating axis 1 was associated with the principal urban factors related to water chemistry (conductivity), flow alteration, and overall urbanization (LDI scores and Imperviousness). Habitat condition was predominantly orthogonal to the principal urban stressors. The percent value indicates how much of the variance in the data is explained by each axis.....	10
Figure 5 – Plot of first two principal components generated from second PCA indicating axis 1 was again associated with the principal urban factors related to water chemistry (conductivity), flow alteration, and overall urbanization (LDI scores and Imperviousness).....	11
Figure 6 – Box and whisker plot of TP concentrations (mg/L) across the three principal component groups.....	12
Figure 7 – Scatterplot of standardized TP concentration versus EPT taxa richness for MBSS piedmont sites. red triangles are group 3 (most urban), blue circles group 2, and black crosses group 1 (least urban). Black line is a loess smoothed fit through all the data.....	13
Figure 8 – Scatterplot of standardized TP concentration versus EPT taxa for Group 1, Group 2, and Groups 1 and 2 combined. Lines indicate TP lower quartile and average TP concentrations associated with the EPT richness endpoint (8). Hatched lines are the 50% prediction interval.....	14
Figure 9 - Scatterplot of standardized TP concentration versus the percent intolerant urban metric for MBSS piedmont sites. Red triangles are group 3 (most urban), blue circles group 2, and black crosses group 1 (least urban).....	15
Figure 10 - Scatterplot of standardized TP concentration versus the percent clinger individuals metric for MBSS piedmont sites. Red triangles are group 3 (most urban), blue circles group 2, and black crosses group 1 (least urban).....	16
Figure 11 – Scatterplot of standardized TP concentration versus percent intolerant urban metric for Groups 1. Hatched lines are the 50% prediction interval.	17
Figure 12– Scatterplot of standardized TP concentration versus percent clinger metric for Group 2. Hatched lines are the 50% prediction interval.	18

Figure 13 – Plot of TP versus EPT taxa richness for the MBSS (black triangle) and USGS Chester County datasets. The black lines indicate the locations of the approximate wedge shaped relationship between invertebrate response and TP concentration. The outer decline in EPT richness begins at a concentration of approximately 30-40 ug/L TP.....20

Figure 14 - Longitudinal profile of modeled and observed orthophosphate concentration (mg/L) in Indian Creek. The distance is meters from the mouth of Indian Creek. Red dots are data and blue line is model results.....21

Figure 15 - Longitudinal profile of modeled and observed ammonia concentration (mg/L) in Indian Creek. The distance is meters from the mouth of Indian Creek. Red dots are data and blue line is model results.....21

Figure 16 - Longitudinal profile of modeled and observed nitrate concentration (mg/L) in Indian Creek. The distance is meters from the mouth of Indian Creek. Red dots are data and blue line is model results.....22

Figure 17 - Comparison of modeled and observed DO (mg/L) at Bergey Rd. Red dots are data and blue line is model results.....22

Figure 18 - Predicted periphyton (chlorophyll *a*) before and after simulated phosphorus reductions (Indian Creek, Bergey Rd sampling location).23

LIST OF TABLES

Table 1 - Correlation matrix among physical and chemical variables in the MBSS dataset used. Values highlighted in blue were significantly correlated and > 0.5.....	6
Table 2 - Correlation matrix between physical and chemical variables and the biological metrics that compose the MBSS Piedmont multimetric index. Values highlighted in red were significantly correlated.....	6
Table 3 - MLR model summary of stepwise regression for the intolerant percent urban invertebrate metric.	8
Table 4 - MLR model summary of stepwise addition for the intolerant percent urban invertebrate metric.	8
Table 5 - MLR model summary of stepwise regression for the Ephemeroptera taxa invertebrate metric.	8
Table 6 - MLR model summary of stepwise addition for the Ephemeroptera taxa invertebrate metric.	9
Table 7 – Summary of interpolated TP concentrations ($\mu\text{g}/\text{L}$) associated with target response metric thresholds for different groups (bins) of sites based on urban intensity.....	19
Table 8 - Summary of candidate endpoints for each of the analytical approaches discussed.....	24

1. INTRODUCTION

The United States Environmental Protection Agency (USEPA) in Region 3 continues to oversee the development of nutrient TMDLs to protect aquatic life use for several streams in the Northern Piedmont ecoregion of southeastern Pennsylvania. Tetra Tech, Inc (Tt) was contracted to establish appropriate and defensible TMDL endpoints for nutrients that protect aquatic life uses in this ecoregion. For that original work Tt developed TMDL endpoints using a multiple lines of evidence approach consistent with USEPA guidance (USEPA 2000a, 2000b) that included reference distribution based, stressor-response based, and scientific literature based evidentiary lines and which were reported to USEPA (Paul and Zheng 2007).

In 2010, USEPA published revised guidance for conducting stressor-response analyses in support of nutrient criteria derivation (USEPA 2010). In response to that revision, Tt was asked to conduct additional analyses in support of the original report and to recommend values associated with the additional analyses that may be considered with the original lines of evidence in revising the TP endpoints. It is important to note that this report does not replace the original analysis, but rather adds to it. In particular, no additional reference distribution based endpoints are being derived, and only one additional piece of scientific literature is being added that was published subsequent to the original report and may have relevance for this region. Lastly, results from a mechanistic model of Indian Creek targeting specific algal endpoints are being included in this report as an additional line of evidence.

2. REVISED GUIDANCE

The revised guidance lays out a 4 step process (Figure 1) which was essentially followed in the original analysis: developing a conceptual model, assembling and exploring the data, analyzing the data to derive candidate criteria, and reviewing and documenting the analysis. This document begins with a discussion of the conceptual model in more detail following the 2010 guidance. It then skips the second step since no additional data were added and the data

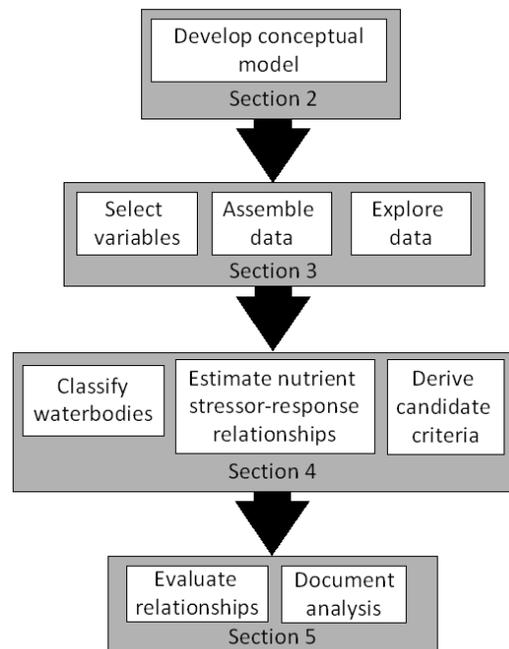


Figure 1- Steps in stressor-response analysis (USEPA 2010).

selection, assembly, and exploration, covered in the original document, remain unchanged. Step 3 is the principal focus of the document, explaining and reporting on the additional analyses conducted. This report satisfies step 4's element related to documenting the analysis.

Ultimately, the stressor-response modeling efforts are conducted to identify a nutrient threshold to protect Piedmont stream macroinvertebrates from nutrient impacts. It is preferable that this be done in a way that is not confounded by other stressors, which could result in errors in estimating a protective nutrient concentration, and the USEPA (2010) provided guidance to better achieve this goal. It is well known that certain sources, for example urbanization, produce a range of stressors, including, but not limited to, nutrients that can affect aquatic life in streams. The revised conceptual model effort in this report builds off the original conceptual model and attempts to identify other stressor pathways by which the response variable could be impacted by the dominant stressors sources in the watershed and ecoregion. This is done, in part, to help guide the consideration of other stressors for modeling so the unique effect of nutrients on responses relative to the other stressors could be better estimated. After identifying potential additional stressors with the conceptual model, their relationship to both nutrients and responses was estimated with correlation analysis. Multiple regressions were then conducted to compare the predictive strength of these different stressors and evaluate how significant nutrients remained. Finally, sites were classified into dominant stressor source classes to better isolate the unique effect of nutrients on the response and estimate a more appropriate and protective nutrient concentration for protecting aquatic life in Piedmont streams.

3. CONCEPTUAL MODEL

Nutrients affect aquatic systems in diverse ways, and the effects on most non-primary producer aquatic life uses are indirect. The original ecoregional TMDL analysis was based on a simplified conceptual model that was, nonetheless, used to “depict accepted scientific knowledge regarding the effects of nitrogen/phosphorus pollution in surface waters” (USEPA 2010) and thereby reinforce the presumptive causal relationship and guide the analysis (Figure 2).

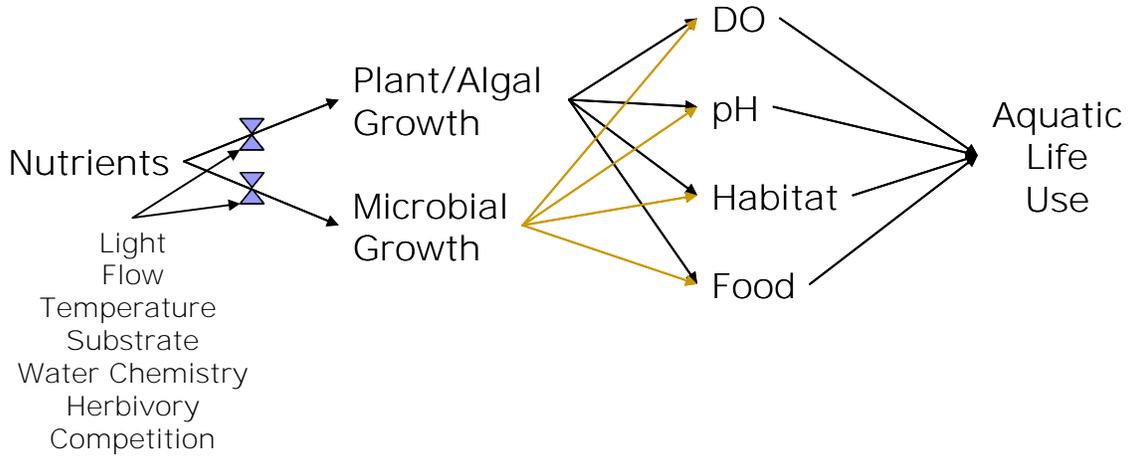


Figure 2 – Simplified diagram illustrating the causal pathway between nutrients and aquatic life use impacts (Paul and Zheng 2007).

The revised guidance provides a more detailed conceptual model that can be adapted for similar application (Figure 3). Blue boxes indicate primary elements relevant to the current analysis. Specifically detailed are the dominant urban point and non-point pollutant sources generating nutrient stressors (orange box), as well as stressors that co-occur with nutrients such as sediment, flow, and toxics arising from similar sources that may confound the stressor-response analyses. The model is consistent with the presumptive causal model presented earlier and the guidance reviews the substantial literature in support of the causal linkages (USEPA 2010). An essential insight from the causal model in Figure 3 is the identification of alternate potential stressors that co-vary with nutrients such as flow, sediment, and toxics data. If available, these should be evaluated for their potential to confound results. As explained above, these other variables have negative effects on macroinvertebrates, their co-occurrence with nutrient stressors could interfere with the nutrient response and this needs to be evaluated to the extent possible. Toxics data were not available within the ecoregional dataset, so conductivity was used as a surrogate for other dissolved pollutants. Also, habitat data were considered to control for the confounding effect of sediment and scouring on habitat mediated impacts on macroinvertebrates. The goal of subsequent analyses, therefore, was to consider and account for some of these covariate effects.

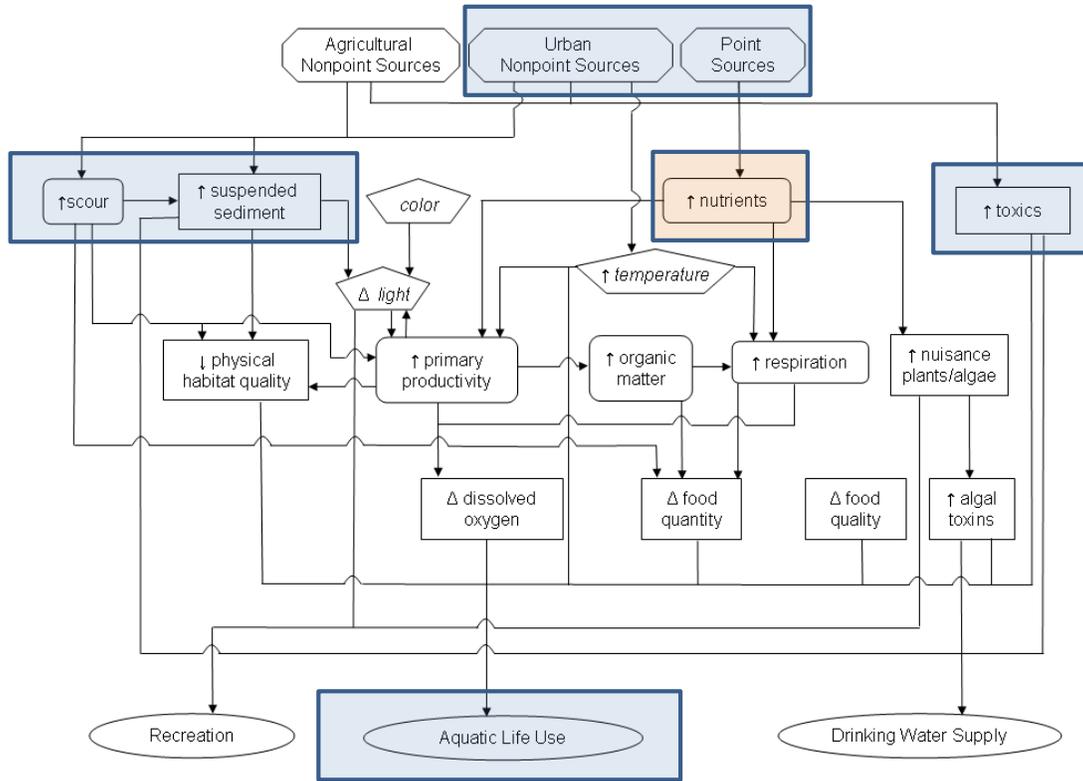


Figure 3 - More detailed conceptual model of the causal relationship between nutrients and responses in streams (after USEPA 2010)

In the original empirical models, biological metrics from the Maryland Biological Stream Survey (MBSS) piedmont index were used since this was the most substantial dataset available for the piedmont ecoregion (MDNR 2005). Since the goal of the analysis was to identify thresholds inimical to aquatic life, these data were appropriate. Also, absent specific numeric aquatic life use endpoints for these metrics in Pennsylvania, the middle of the MBSS index and component metric scoring ranges were used as response goals in the regression models, where such endpoints were needed. For example, the median of the Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa richness metric scoring range was 8 taxa (MDNR 2005), this is the midpoint between poor and good scores for this metric in the MBSS index and was used as the response target in the regression models below. Higher, more stringent targets could have been developed to assure greater likelihood of protection, but this value was defensible as a mid-range threshold. These middle values were also between the 10th and 50th percentile of MBSS piedmont reference site biological metric values, which is consistent with thresholds commonly used for defining biological targets and within the range ultimately proposed by Pennsylvania Department of Environmental Protection (PADEP) for their own evolving macroinvertebrate index (Barbour et al. 1999, PADEP 2009).

The metrics selected by MBSS for their piedmont ecoregion index include the number of taxa, number of EPT, the number of Ephemeroptera taxa, the percent of individuals of intolerant urban taxa, the percent of individuals that are chironomids, and the percent of

individuals classified as clingers. The PADEP (2009) used similar metrics: number of taxa, number of EPT taxa, Beck's index, Shannon diversity, Hilsenhoff's biotic index, and the percent individuals from intolerant taxa.

4. DATA ANALYSIS – CORRELATIONS

The goal of this subsequent analysis was to evaluate the effects of confounding or covarying stressors on nutrients, to attempt to refine the analysis to account for those effects, and to document the results. One approach recommended in the revised USEPA guidance was to attempt to classify the data into bins or classes of similar covariate distributions to control for the effects of these covariates and isolate, as much as possible, the independent effect of nutrients. This was attempted in two ways: propensity scores and manual binning.

Propensity score analysis is explained in the guidance (USEPA 2010) and is an analytical approach that controls for the effects of covariates by using them to generate predicted nutrient concentrations, called propensity scores, whose predicted value is a function of the covariation. Propensity scores are then split into several bins, within which the approximate distribution of covariates is similar and the effects of nutrients can be independently evaluated. The first step in the analysis is identifying nutrient covariates. A correlation analysis between nutrients and other likely stressors was conducted. Data were standardized to the mean and standard deviation of all values and log-transformed as necessary beforehand to meet assumptions of constant variance for the correlation, multiple regression, and principal components analysis. Those analyses were performed using Statistica software. This standardization allowed for an equal comparison of variable effects independent of differences in magnitude and range, which helped these analyses. For the simple linear regression model to estimate TP endpoints, however, log₁₀-transformed TP concentrations were used as in the original report.

Little or no correlation between nutrients and other stressors, both chemical and physical, was observed. Highest correlations with TN were with DO (positive), sulfate (negative), and flow (positive). With TP, the highest correlations were with turbidity (positive), sulfate (positive), embeddedness (positive), and epibenthic substrate habitat (negative). These results are consistent with a covarying effect of nutrients and sediment, but were insufficiently strong to recommend propensity score analysis.

Table 1 - Correlation matrix among physical and chemical variables in the MBSS dataset used. Values highlighted in blue were significantly correlated ($p < 0.05$ and $r > |0.5|$)

Spearman Rank Order Correlations												
Variable	Nitrate	Total Nitrogen	Total Phosphorus	Dissolved Oxygen	pH	Conductivity	Sulfate	Turbidity	Instream	EPI Substrate	Embedded	Flow
Nitrate		0.988	0.061	0.248	-0.099	-0.081	-0.236	-0.027	0.015	0.061	-0.080	0.130
Total Nitrogen	0.988		0.099	0.239	-0.109	-0.070	-0.220	-0.011	0.002	0.043	-0.077	0.142
Total Phosphorus	0.061	0.099		-0.059	0.051	0.120	0.269	0.283	-0.145	-0.194	0.231	0.128
Dissolved Oxygen	0.248	0.239	-0.059		-0.029	-0.130	-0.181	-0.234	0.209	0.293	-0.193	0.232
pH	-0.099	-0.109	0.051	-0.029		0.548	0.518	-0.141	0.162	0.049	0.115	0.274
Conductivity	-0.081	-0.070	0.120	-0.130	0.548		0.730	-0.065	-0.205	-0.255	0.263	-0.112
Sulfate	-0.236	-0.220	0.269	-0.181	0.518	0.730		0.001	-0.182	-0.236	0.213	-0.034
Turbidity	-0.027	-0.011	0.283	-0.234	-0.141	-0.065	0.001		-0.101	-0.156	0.163	0.138
Instream	0.015	0.002	-0.145	0.209	0.162	-0.205	-0.182	-0.101		0.808	-0.423	0.512
EPI Substrate	0.061	0.043	-0.194	0.293	0.049	-0.255	-0.236	-0.156	0.808		-0.571	0.339
Embedded	-0.080	-0.077	0.231	-0.193	0.115	0.263	0.213	0.163	-0.423	-0.571		-0.109
Flow	0.130	0.142	0.128	0.232	0.274	-0.112	-0.034	0.138	0.512	0.339	-0.109	

Correlations in blue are more than or less than 0.5
Missing data is deleted pairwise

Given the weak correlation between nutrients and other stressors, and essentially only weak covariation, it was decided that propensity score analysis was not necessary. However, correlations between biological responses and stressors other than nutrients were evident (Table 2). As a result, it was felt that it was still necessary to attempt to tease apart the effect of nutrients after controlling for other stressors. This was done in two different ways.

Table 2 - Correlation matrix between physical and chemical variables and the biological metrics that compose the MBSS Piedmont multimetric index. Values highlighted in red were significantly correlated.

Spearman Rank Order Correlations												
Variable	Nitrate	Total Nitrogen	Total Phosphorus	Dissolved Oxygen	pH	Conductivity	Sulfate	Turbidity	Instream	EPI Substrate	Embedded	Flow
Intolerant Urban %	0.137	0.104	-0.268	0.235	-0.393	-0.612	-0.522	-0.164	0.247	0.371	-0.345	-0.086
Chironomid %	-0.082	-0.058	0.147	-0.232	0.284	0.483	0.373	0.200	-0.231	-0.332	0.300	0.007
Clinger %	0.093	0.057	-0.183	0.242	-0.212	-0.448	-0.351	-0.218	0.327	0.412	-0.353	0.075
Total Taxa	0.242	0.225	0.022	0.058	-0.280	-0.389	-0.411	0.025	0.111	0.138	-0.077	0.051
EPT Taxa	0.270	0.239	-0.159	0.263	-0.286	-0.553	-0.492	-0.171	0.289	0.383	-0.328	0.089
Ephemeroptera Taxa	0.286	0.263	-0.107	0.257	-0.231	-0.569	-0.434	-0.061	0.240	0.302	-0.238	0.112

Correlations in red are significant at $p < .05$
Missing data is deleted pairwise

The first was through the use of multiple regression models to compare and explore the contributory effects of different stressors simultaneously and the second was through binning sites by urban intensity, which will be discussed in sections 5 and 6.

5. DATA ANALYSIS – MULTIPLE REGRESSION

Multiple linear regression (MLR) models were generated to predict invertebrate metric scores across MBSS piedmont sites using forward stepwise selection (F to enter = 4). This analysis was intended to compare stressor predictors and identify and support the basis for the independent effects of nutrients in this multi-stressor environment.

Simple linear regression is a statistical method that generates a predictive empirical model that estimates the effect of a single predictor (e.g., nutrients) on a response variable (e.g., a benthic metric). Alternatively, one may believe that multiple predictors (e.g., sediment and nutrients) influence the response variable and one can construct a model that predicts the effect of many variables on the response simultaneously. In these multiple regression models, then, the resultant model identifies more than one predictor to estimate a response condition. Various statistical methods exist that guide selection of the order of predictors in a multiple regression model. For this expression, a forward stepwise procedure was used, which adds predictors to a model based on the significance of their effect on the response. The most significant effect is added first, then the model compares the significance of the remaining predictors and adds the one providing the second most significant prediction, and so on until no additional predictors that meet the significance requirement for entry in the model are identified. The result is a multiple predictor model that predicts the responses. Multiple regression modeling allows one to compare the significance of different predictors, but it was also used here to verify the continued significance of nutrient predictors after accounting for other stressors on macroinvertebrates, which was important in continuing to argue for the importance of nutrients to invertebrate responses. For the intolerant percent urban individual taxa metric, MLR models still included TP as a significant negative predictor in the model (Tables 3 and 4); in fact, TP was the second most predictive variable after conductivity. Conductivity is a frequent stressor associated with urbanization and was highly correlated with pH. Other predictors included turbidity, flow, and habitat conditions, but the latter all explained less additional variance than TP.

The other metric for which TP entered an MLR as a significant predictor was for Ephemeroptera taxa (Tables 5 and 6). In this case, TP explained less variance than for intolerant percent urban taxa, but TP had a significant negative effect on the model prediction.

These results strengthen the argument for an independent effect of TP on macroinvertebrate taxa in Piedmont streams that is consistent with the causal conceptual model presented in the original report and the updated model presented above. There are several variables that contribute to predicting invertebrate declines in the Piedmont, but TP is defensibly one of them. The next analysis attempts to develop TP thresholds while controlling for these other stressors and focused specifically on urban effects and binning sites by urban intensity.

Table 3 - MLR model summary of stepwise regression for the intolerant percent urban invertebrate metric.

Regression Summary for the Dependent Variable of Intolerant Urban %						
	b*	Standard Error of b*	b	Standard Error of b	t(330)	p-value
Intercept			27.139	1.912	14.195	0.00000
Conductivity	-0.499	0.049	-14.854	1.459	-10.182	0.00000
Total Phosphorus	-0.139	0.043	-4.251	1.308	-3.249	0.00128
Turbidity	-0.157	0.042	-4.637	1.255	-3.694	0.00026
Flow	-0.198	0.047	-10.666	2.500	-4.266	0.00003
EPI Substrate	0.221	0.047	6.604	1.404	4.702	0.00000
pH	-0.101	0.049	-3.026	1.477	-2.049	0.04123
R= .69996489 R ² = .48995084 Adjusted R ² = .48067722						
F(6,330)=52.833 p<0.0000 Standard Error of estimate: 21.445						
N=337						

Table 4 - MLR model summary of stepwise addition for the intolerant percent urban invertebrate metric.

Summary of Stepwise Regression of Intolerant Urban %							
Variable	Step	Multiple R	Multiple R-square	R-square change	F - to remove	p-value	Variables
Conductivity	1	0.576	0.332	0.332	166.171	0.00000	1
Total Phosphorus	2	0.631	0.398	0.066	36.785	0.00000	2
Turbidity	3	0.656	0.430	0.032	18.633	0.00002	3
Flow	4	0.672	0.451	0.021	12.914	0.00038	4
EPI Substrate	5	0.695	0.483	0.032	20.713	0.00001	5
pH	6	0.700	0.490	0.006	4.199	0.04123	6

Table 5 - MLR model summary of stepwise regression for the Ephemeroptera taxa invertebrate metric.

Regression Summary for the Dependent Variable Ephemeroptera Taxa						
	b*	Standard Error of b*	b	Standard Error of b	t(332)	p-value
Intercept			2.779	0.102	27.201	0.00000
Conductivity	-0.531	0.043	-1.261	0.103	-12.232	0.00000
Total Nitrogen	0.206	0.044	0.508	0.109	4.650	0.00000
Dissolved Oxygen	0.112	0.044	0.279	0.110	2.536	0.01166
Total Phosphorus	-0.090	0.044	-0.220	0.107	-2.063	0.03985
R= .62131600 R ² = .38603357 Adjusted R ² = .37863639						
F(4,332)=52.187 p<0.0000 Std. Error of estimate: 1.8712						
N=337						

Table 6 - MLR model summary of stepwise addition for the Ephemeroptera taxa invertebrate metric.

Summary of Stepwise Regression of Ephemeroptera Taxa							
Variable	Step	Multiple R	Multiple R-square	R-square change	F - to remove	p-value	Variables
Conductivity	1.000	0.565	0.319	0.319	156.770	0.00000	1.000
Total Nitrogen	2.000	0.604	0.365	0.046	24.038	0.00000	2.000
Dissolved Oxygen	3.000	0.615	0.378	0.014	7.302	0.00724	3.000
Total Phosphorus	4.000	0.621	0.386	0.008	4.258	0.03985	4.000

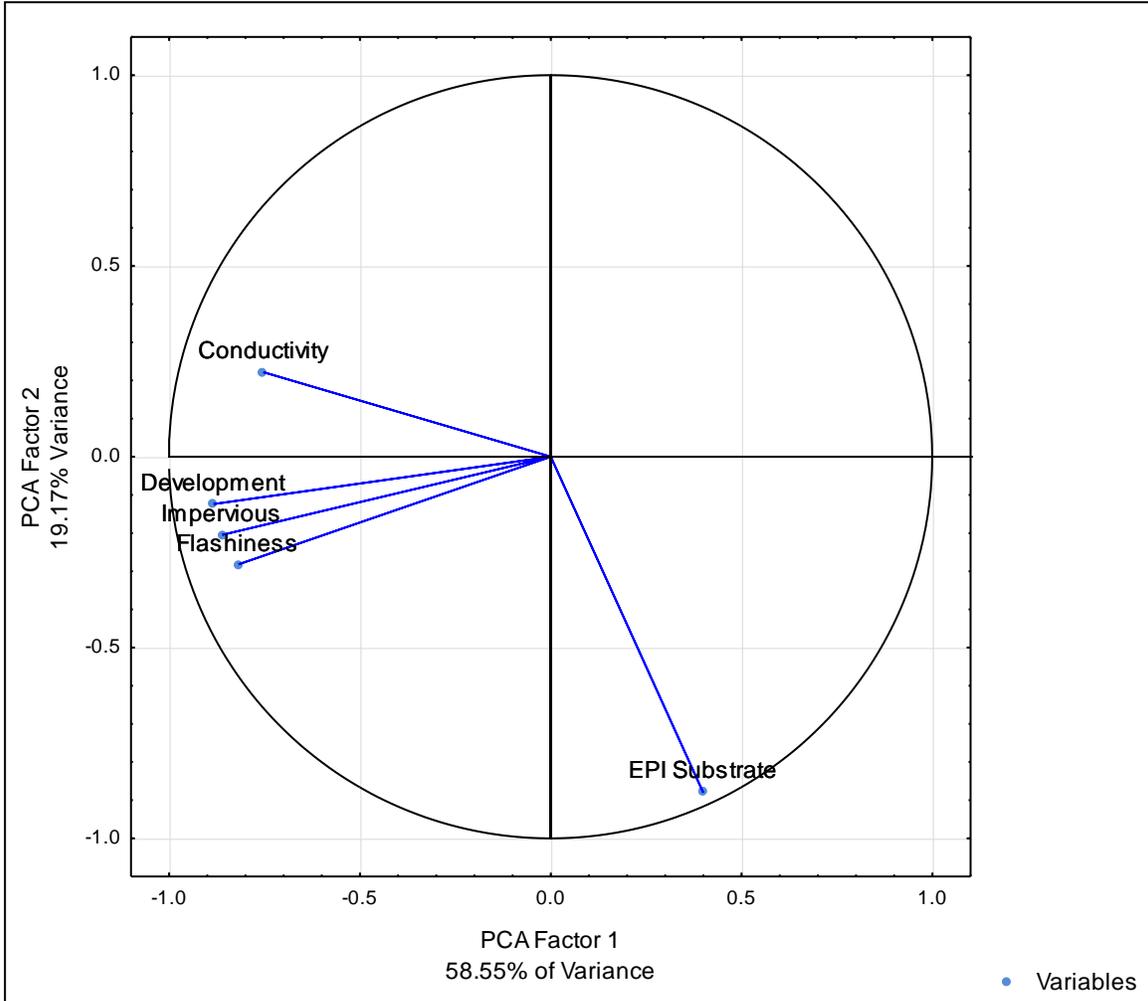
6. DATA ANALYSIS – REGRESSION WITHIN BINS

There was substantial evidence that, in this ecoregion, urbanization was associated with several stressors including nutrients and TP, consistent with the conceptual model. This is likely due to both point and non-point TP sources that have been demonstrated to deliver this particular pollutant. The conceptual model identifies some of these – namely flow alteration, sediment, and other toxics. Fortunately, variables related to these stressors were available and many were used in the MLR demonstration above to verify the independent significant effect of nutrients in the presence of these multiple stressors.

The ultimate goal of this analysis was to strengthen the defensibility of TP threshold concentrations developed to protect aquatic life in Piedmont streams for the purposes of TMDL modeling. A concern, indicated above, was that other stressors may be confounding the ability to identify the most defensible endpoints. One element that became clear during the analysis was that urbanization may actually be responsible for several stressors that co-occur with nutrients, likely impact invertebrates as well as nutrients, but may be confounding the ability to create the clearest model of nutrient response for the purposes of developing a TP target to protect aquatic life. Conceptually, if the impact of these urban stressors could be isolated and/or reduced, then a clearer model of nutrient response could be developed and TP thresholds identified for the target aquatic life use endpoints when these confounding effects were minimized. Such approaches are recommended in the new stressor-response guidance (USEPA 2010). Therefore, an attempt was made to identify the urban effect and focus on identifying a gradient minimally impacted by these co-occurring urban stressors. The first step was to identify the urban gradient and this was addressed with principal component analysis.

A principal components analysis (PCA) was used to construct a model that created a predominantly urban gradient (Figure 4). PCA is a multivariate analysis that reduces the variance among multiple factors into a few dimensions associated with the dominant gradients. Only the first principal component was used since it represented the majority of the variance (59%) and was associated with urbanization (e.g., imperviousness, conductivity, development, and flashiness). Given the orthogonal effect of the habitat metric to urbanization, it was removed, and a second PCA was conducted to construct the

final urban gradient, the first axis of this second PCA explained 70% of the variability in the



data (Figure 5).

Figure 4 - Plot of first two principal components generated indicating axis 1 was associated with the principal urban factors related to water chemistry (conductivity), flow alteration, and overall urbanization (LDI scores and Imperviousness). Habitat condition was predominantly orthogonal to the principal urban stressors. The percent value indicates how much of the variance in the data is explained by each axis.

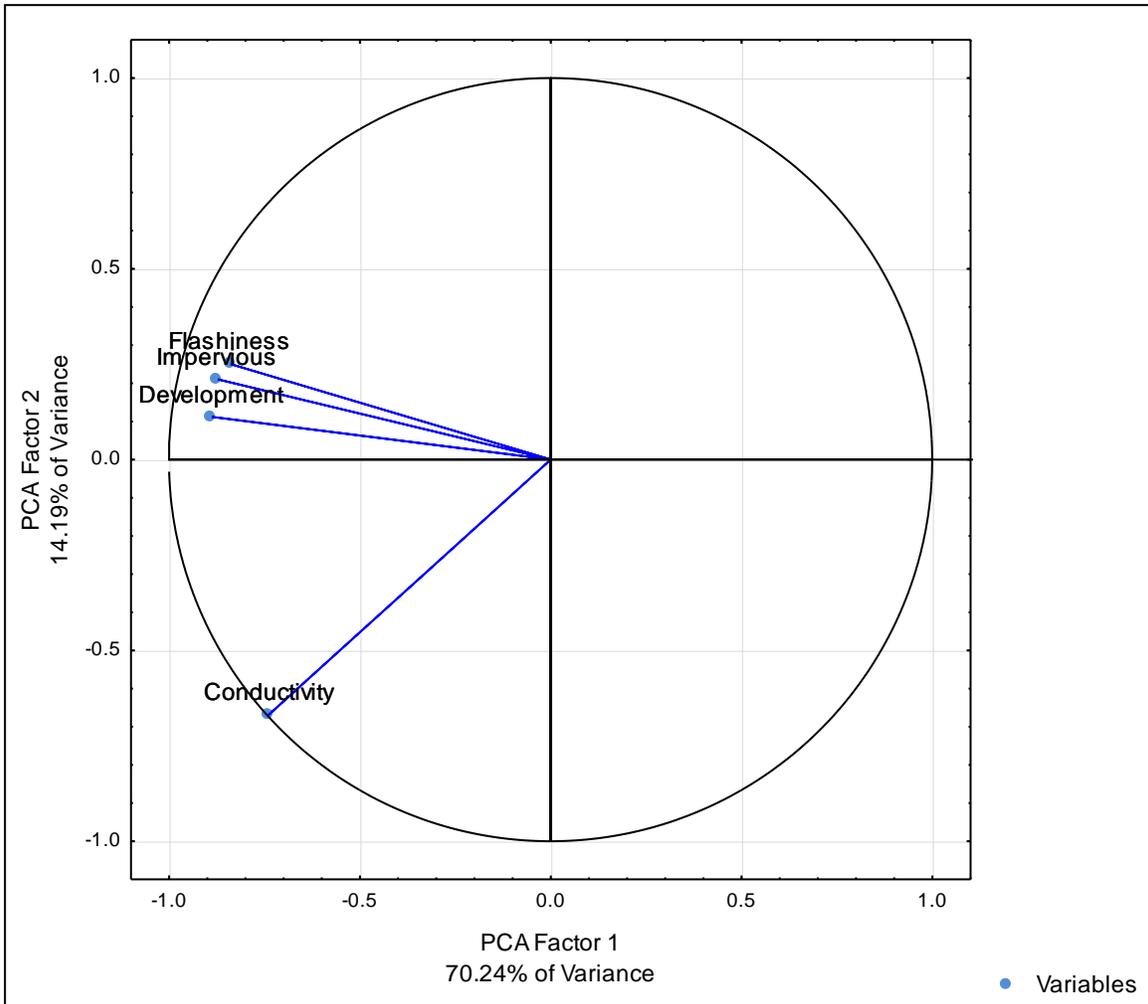


Figure 5 - Plot of first two principal components generated from second PCA indicating axis 1 was again associated with the principal urban factors related to water chemistry (conductivity), flow alteration, and overall urbanization (LDI scores and Imperviousness).

The first axis of the second PCA was split into 3 equal sized bins from group 1 (highest third of principal component axis 1 values, least urban) to group 3 (lowest third of principal component axis 1 values, most urban). Total phosphorus concentrations overlapped across the three groups, but central tendencies were highest for groups 2 and 3 (Figure 6). The bin groups were used to color-code the sites in plots of TP versus EPT taxa richness and percent intolerant urban taxa, two of the metrics more strongly related to TP.

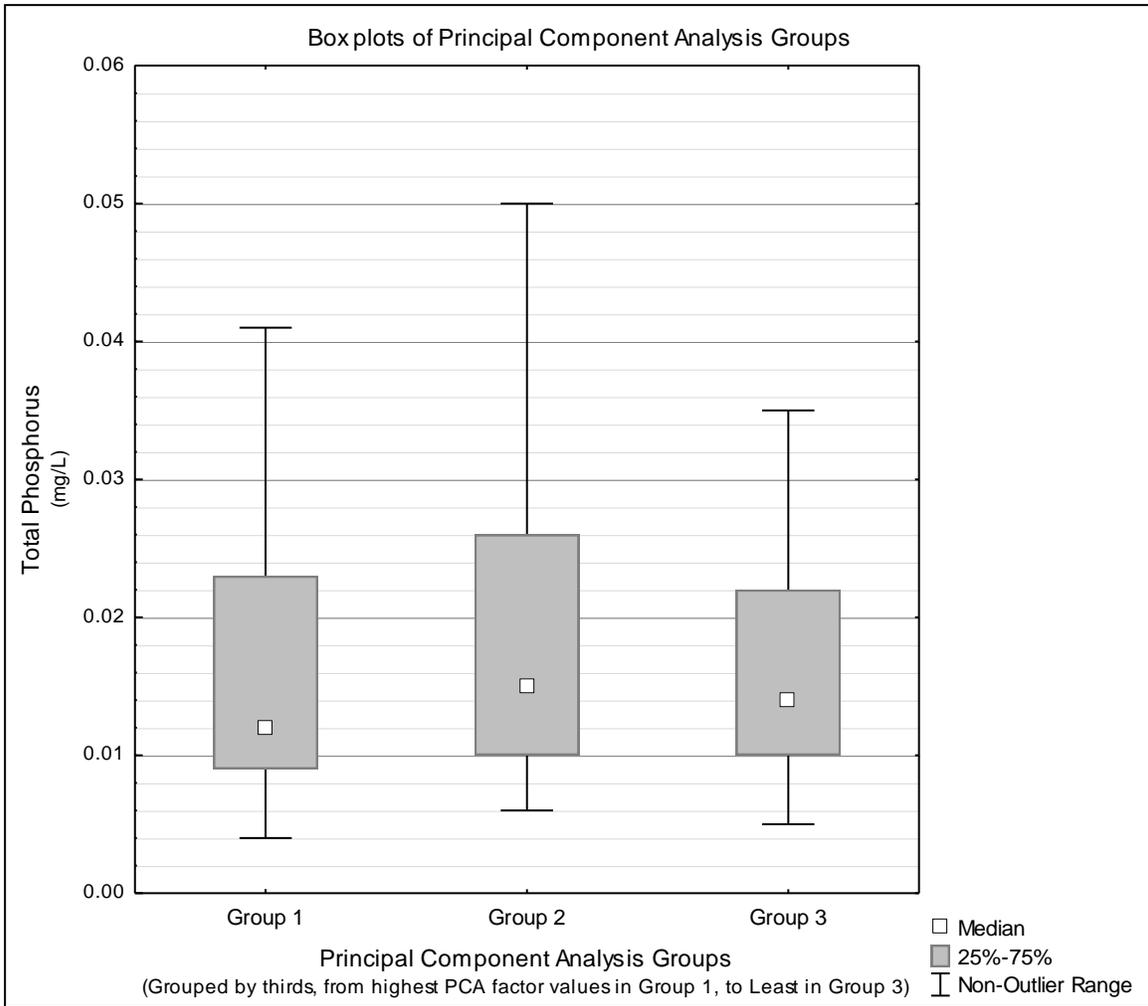


Figure 6 - Box and whisker plot of TP concentrations (mg/L) across the three principal component groups.

The resultant figure (Figure 7) indicates that group 3 sites (most urban) were frequently associated with sites scoring low for EPT taxa richness when TP concentrations were low. This group represents a large confounding effect on the EPT vs. nutrient relationship for Piedmont streams because these sites have generally low TP and low EPT richness, so are presumably primarily impacted by other stressors than nutrients. This analysis allowed us to isolate the conflicting effects of the urban stressors and focus more specifically where TP effects were strongest in order to better identify a protective TP threshold estimate for this region. Simple linear regression interpolative models (Figure 8), as recommended in the revised guidance (USEPA 2010), were then used to infer protective concentrations of TP associated with the adverse response condition for this metric (8 EPT taxa) with the confounding group 1 sites removed, and it can be seen that the nutrient response was even stronger (steeper slope and increased regression coefficient than the original models). The range in TP concentrations associated with the interpolation of EPT taxa richness of 8 taxa

with the lower 50th percentile prediction interval, a conservative prediction interval estimate, and the average predicted value was 10 to 85 µg/L. Groups 1 and 2 also produced independent linear models that were significant. The group 2 model was the most precise ($r^2 = 0.16$) and the predicted TP values associated with the EPT taxa richness endpoints for the lower quartile and average predictions ranged from 10 to 60 µg/L.

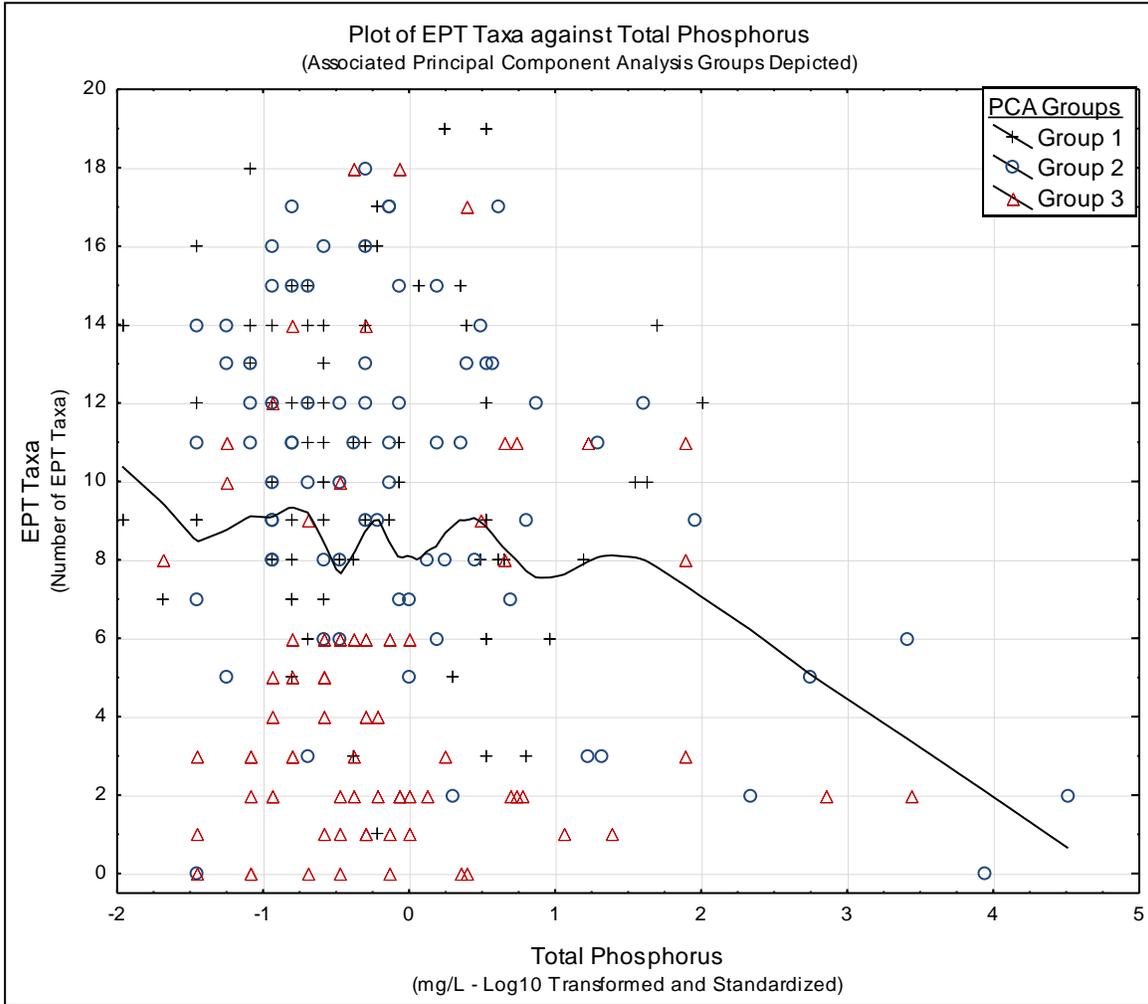


Figure 7 – Scatterplot of standardized TP concentration versus EPT taxa richness for MBSS piedmont sites. red triangles are group 3 (most urban), blue circles group 2, and black crosses group 1 (least urban). Black line is a loess smoothed fit through all the data.

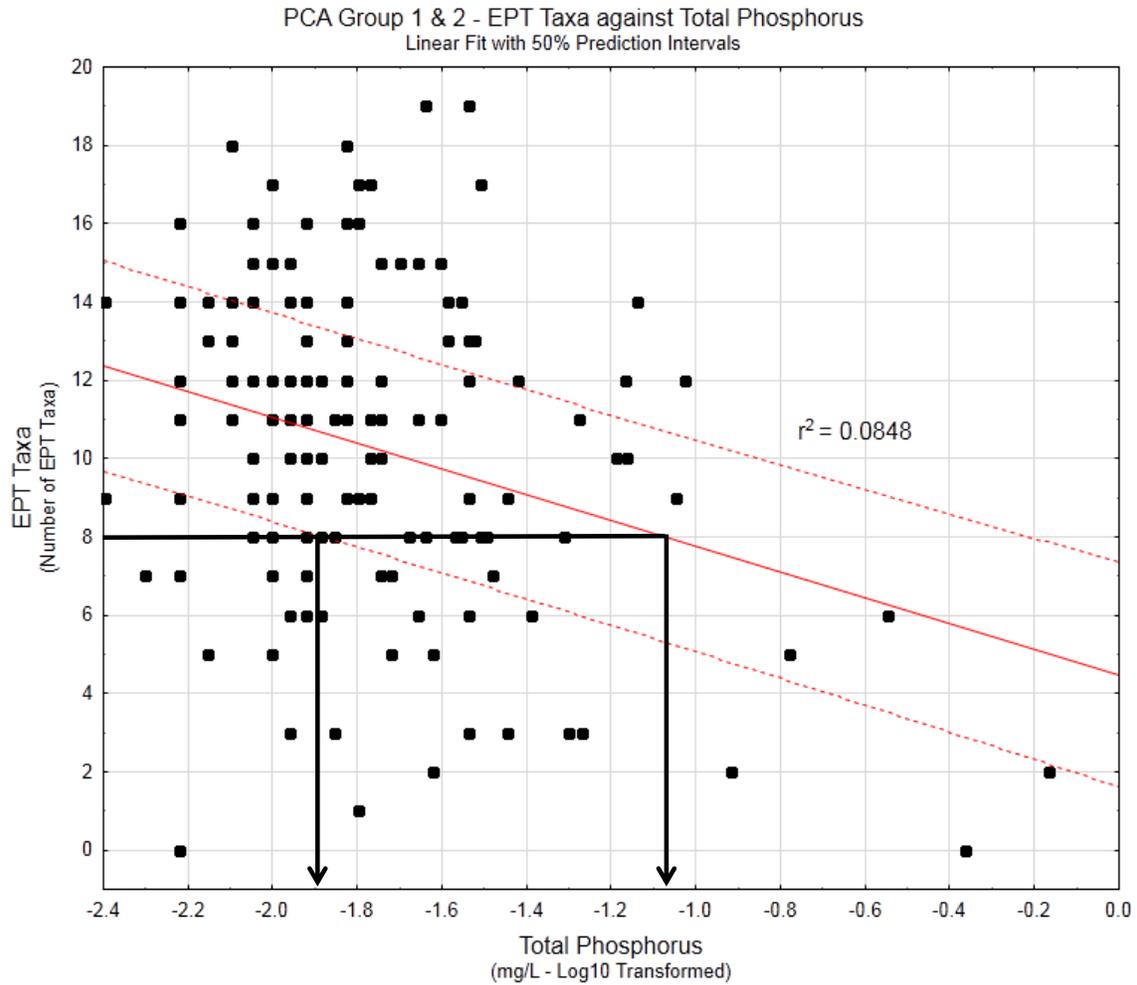


Figure 8 – Scatterplot of standardized TP concentration versus EPT taxa for Group 1, Group 2, and Groups 1 and 2 combined. Lines indicate TP lower quartile and average TP concentrations associated with the EPT richness endpoint (8). Hatched lines are the 50% prediction interval.

Similar analyses were conducted for percent intolerant urban and percent clinger metrics (Figures 9-12). These same metrics once again indicate a strong confounding effect of the most urban group (group 3), affecting the sites with low metric values and low TP values (Figures 9 and 10). Once again, by looking at the groups independently and groups 1 and 2 combined, the confounding effects of these urban covariates could be reduced allowing a clearer focus on the threshold TP concentration associated with adverse metric conditions in piedmont streams (Figures 11 and 12).

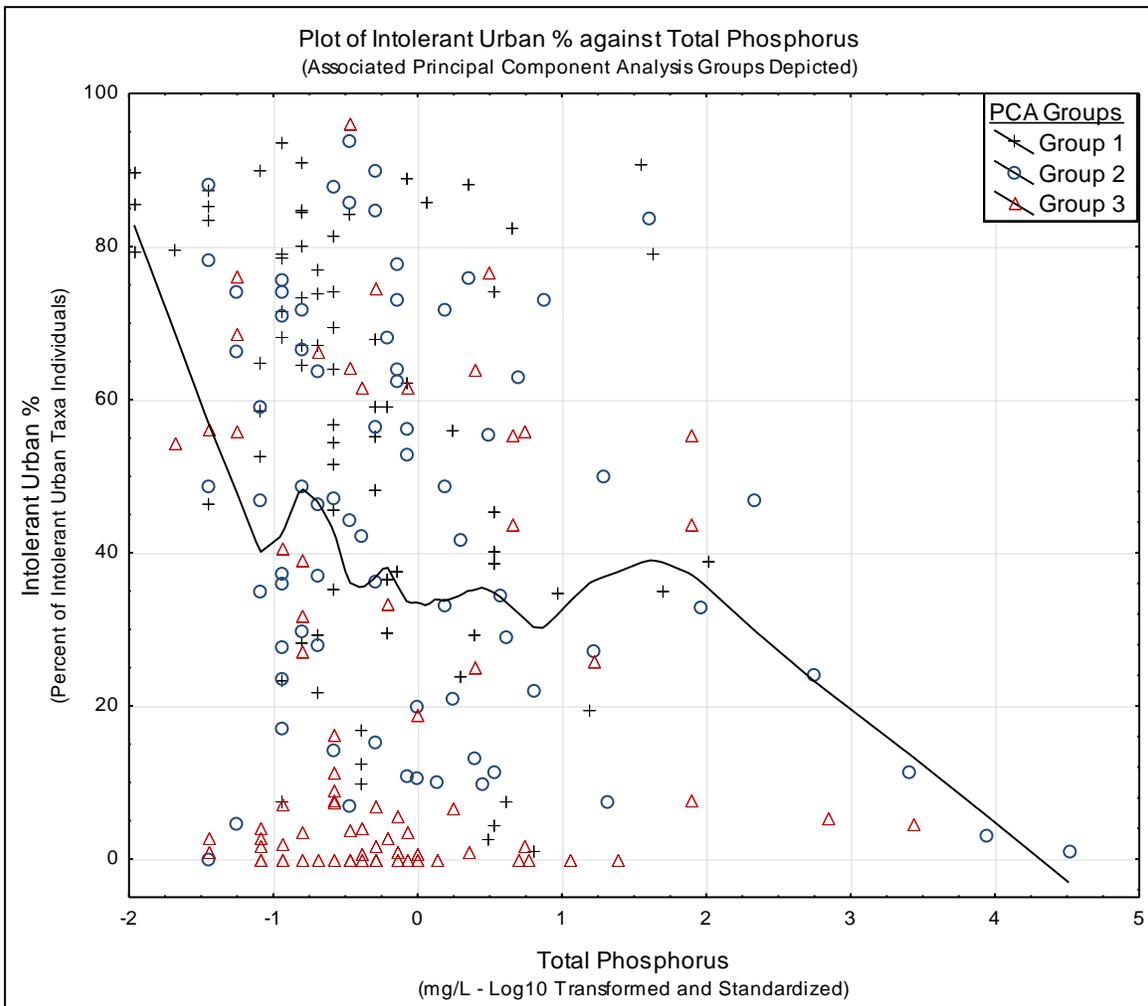


Figure 9 - Scatterplot of standardized TP concentration versus the percent intolerant urban metric for MBSS piedmont sites. Red triangles are group 3 (most urban), blue circles group 2, and black crosses group 1 (least urban).

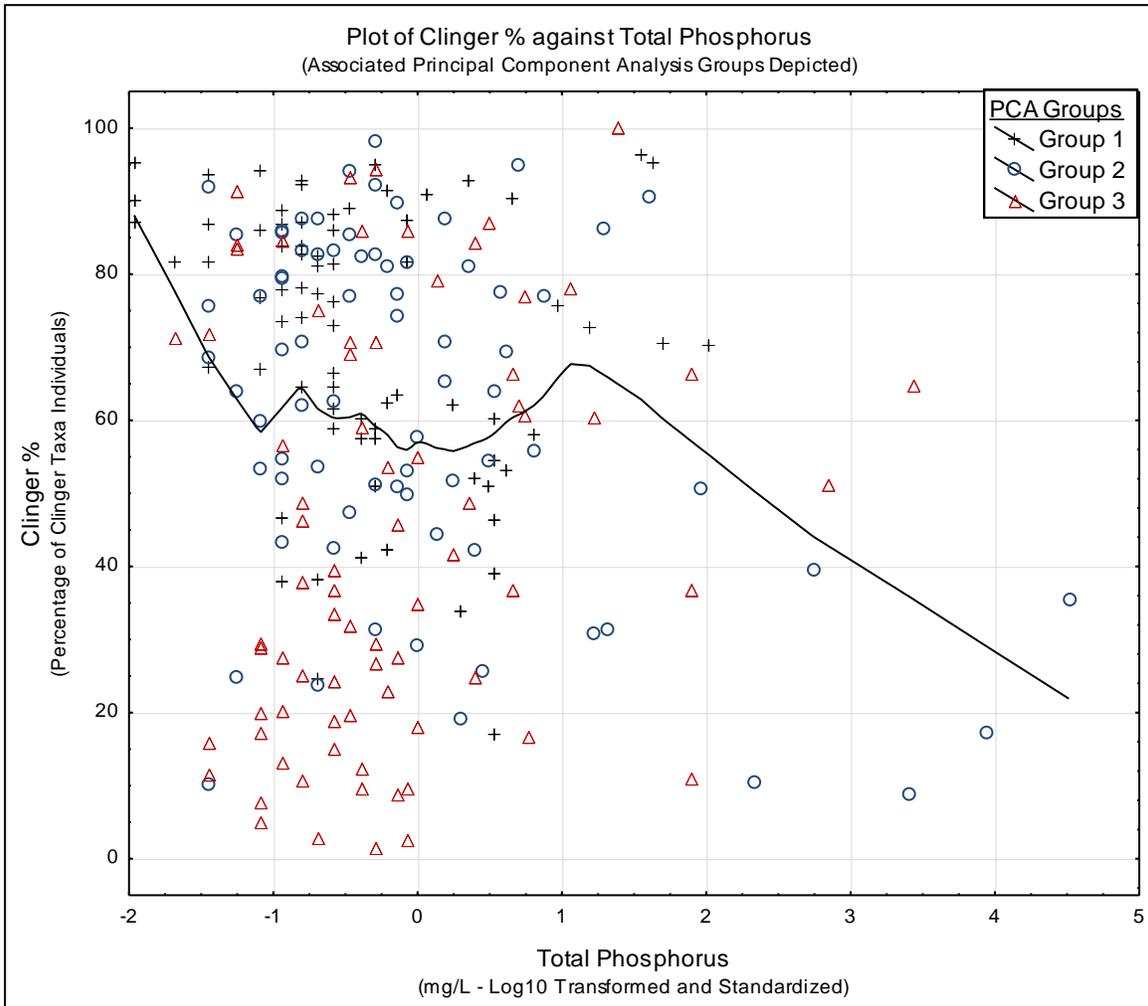


Figure 10 - Scatterplot of standardized TP concentration versus the percent clinger individuals metric for MBSS piedmont sites. Red triangles are group 3 (most urban), blue circles group 2, and black crosses group 1 (least urban).

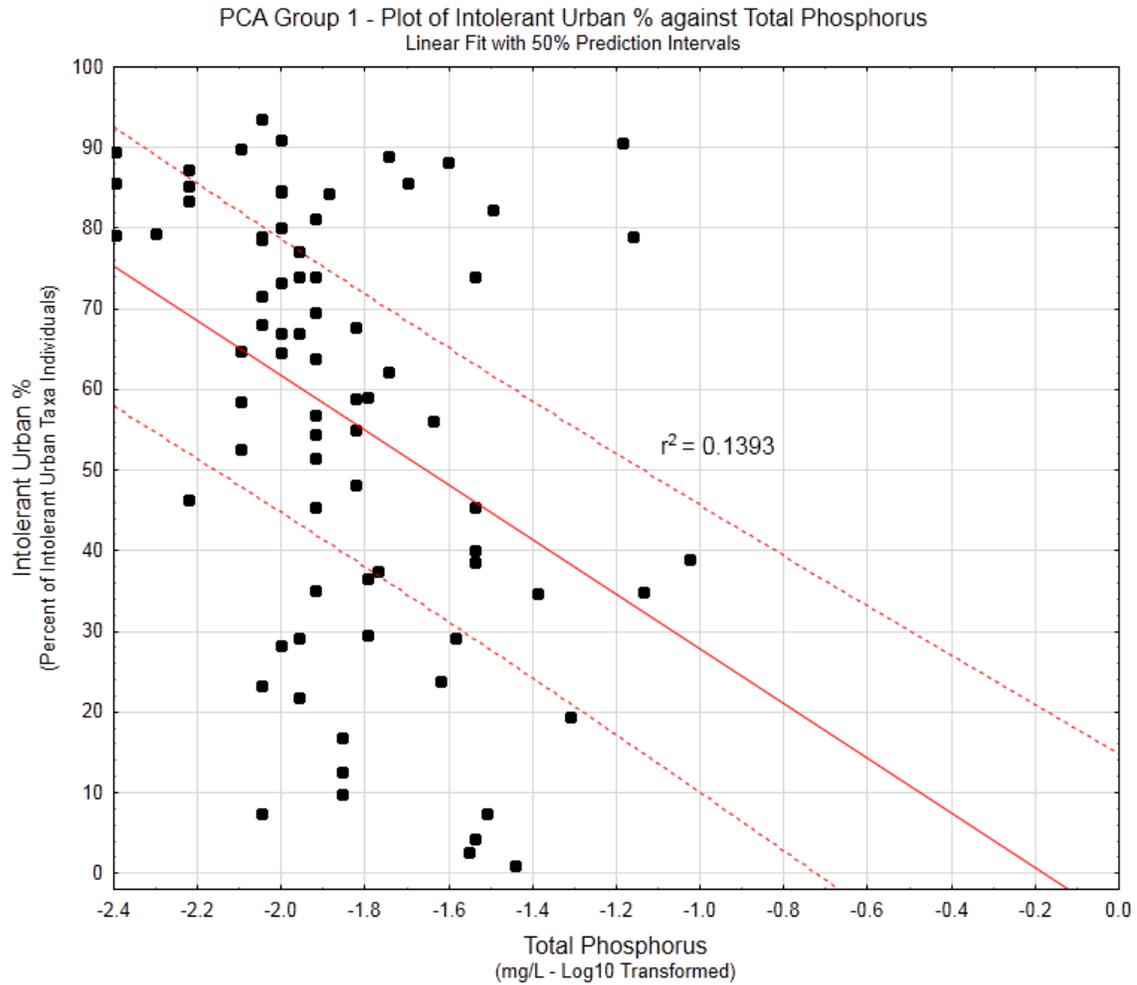


Figure 11 – Scatterplot of standardized TP concentration versus percent intolerant urban metric for Groups 1. Hatched lines are the 50% prediction interval.

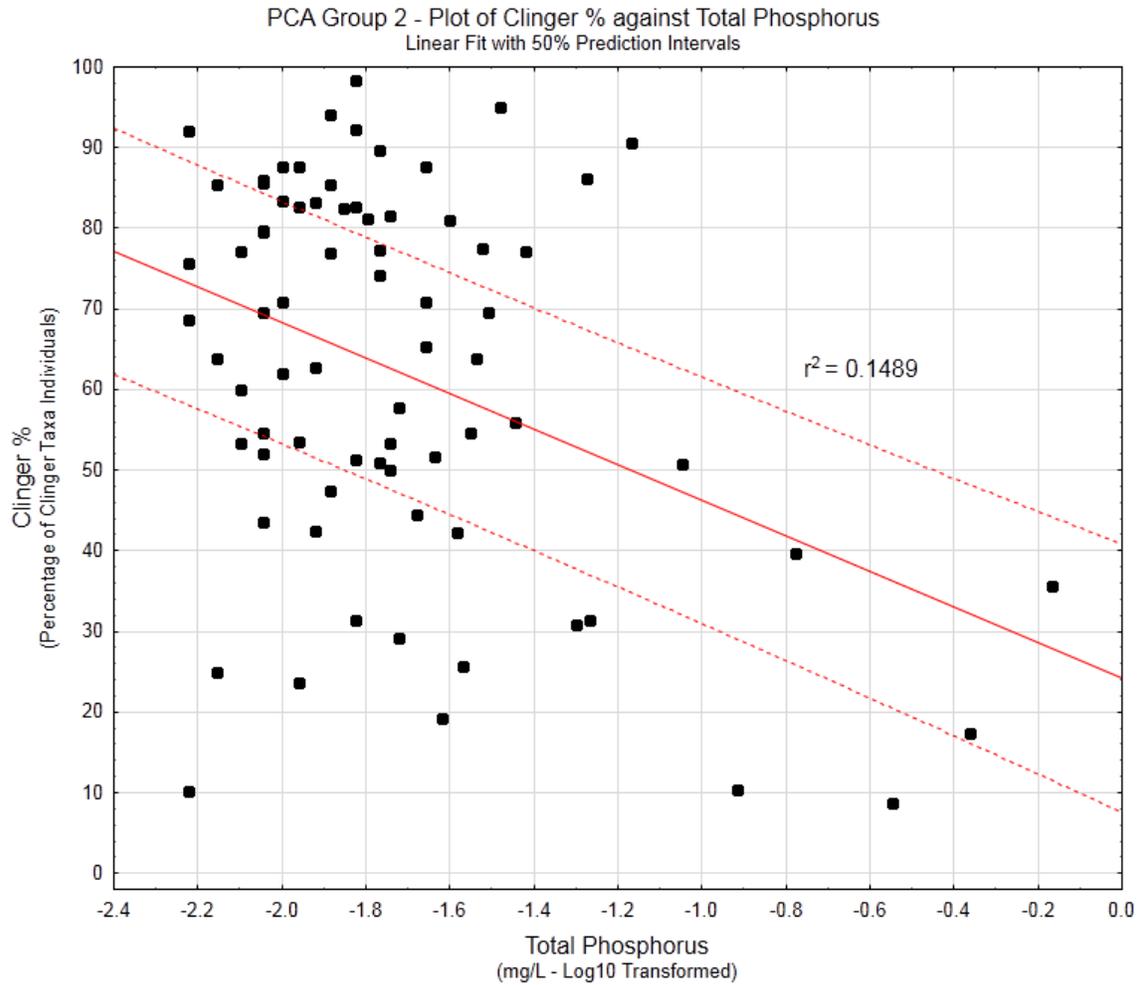


Figure 12- Scatterplot of standardized TP concentration versus percent clinger metric for Group 2. Hatched lines are the 50% prediction interval.

The range in TP concentrations interpolated from the intersection of the metric threshold values for percent intolerant urban (31.5%) for group 1 sites ($r^2=0.139$) was 16 to 78 $\mu\text{g/L}$ and for group 2 sites ($r^2=0.105$) was 8 to 82 $\mu\text{g/L}$. Similar interpolated values for the percent clingers metrics (metric threshold = 52.5%) was 8 to 52 $\mu\text{g/L}$ for the lower quartile and average prediction, respectively, for group 2 sites ($r^2=0.137$). Using group 1 and 2 combined for percent intolerant urban and group 1 and group 1 and 2 combined sites for the percent clinger models did not produce significant predictions.

Table 7 summarizes the prediction ranges from the different simple linear regression model predictions of TP for the three metrics explored.

Table 7 – Summary of interpolated TP concentrations (µg/L) associated with target response metric thresholds for different groups (bins) of sites based on urban intensity.

Metric	Groups	Interpolated TP (ug/L)	
		lower quartile	average
EPT Taxa	Group 2	10	60
	Groups 1 and 2	10	85
Percent Intolerant Urban	Group 1	16	78
	Group 2	8	82
Percent Clingers	Group 2	8	52

7. MODEL VALIDATION

There was a desire to provide some validation of the model linking invertebrate response to nutrients with independent data, consistent with revised USEPA guidance (USEPA 2010) to strengthen the basis for the inference. Data from USGS on stream nutrient concentrations and invertebrate metric response was made known to EPA (Rief 1999, 2000, 2002a, 2002b). These data were collected with similar but distinct sampling methods and to different fixed counts than the MBSS sample data. When the data were corrected with simple rarefaction to estimate taxa richness using a comparable number of individuals as recommended by basic ecological theory, what is evident from the rarefaction exercise is even given the difference in sampling habitat and sampling design, rarefied samples fit within the wedge shaped plot identified in the original relationship (Figure 13), supporting the original observation that invertebrate richness decreases with increasing nutrient concentrations and that this general decline begins at approximately 30-40 µg/L. Additional corrections for differences in the habitat and sampling design would likely improve the fit. These data support the trends observed in the MBSS data and provide independent reinforcement for the causal model.

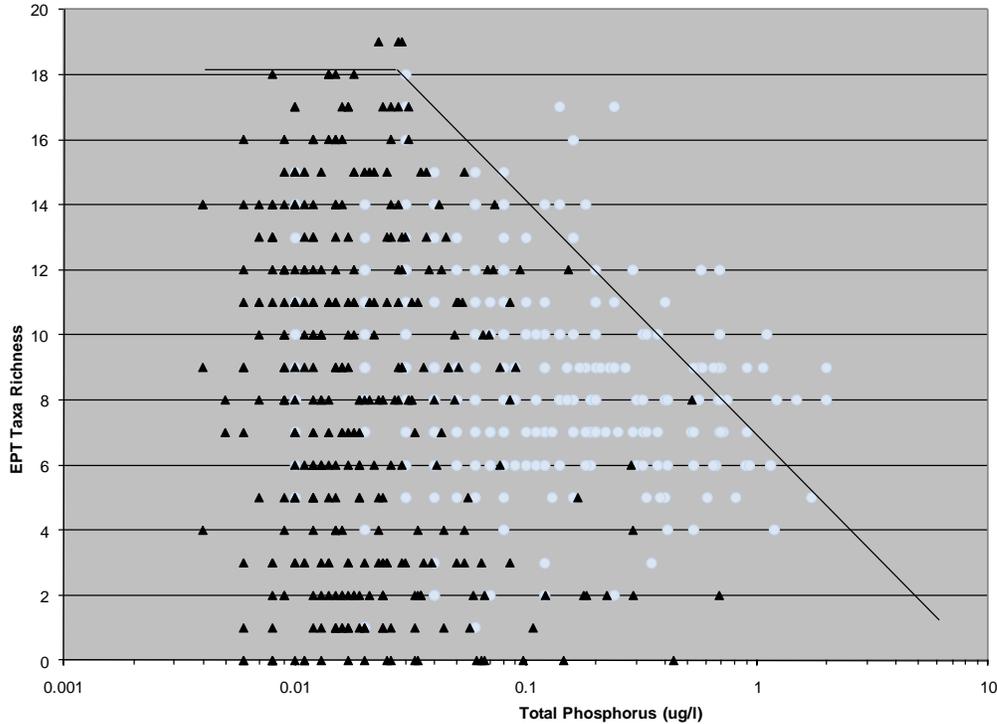


Figure 13 – Plot of TP versus EPT taxa richness for the MBSS (black triangle) and USGS Chester County datasets. The black lines indicate the locations of the approximate wedge shaped relationship between invertebrate response and TP concentration. The outer decline in EPT richness begins at a concentration of approximately 30-40 ug/L TP.

8. MECHANISTIC MODELING

Another line of evidence considered in the analysis of data for developing thresholds that was not included in the original report, was the use of a mechanistic model to estimate TP concentrations associated with adverse benthic algal concentrations in a Piedmont stream in Pennsylvania. A dynamic linked process model of Indian Creek using the Generalized Watershed Loading Functions (GWLf) and EPA’s Environmental Fluid Dynamics Code (EFDC) was developed and used to evaluate average TP concentrations associated with exceeding a target benthic chlorophyll *a* density of 100 mg/m², a density on the conservative end of that frequently cited as a nuisance concentration (e.g., Dodds and Welch 2000, Suplee et al. 2008).

Watershed loads were simulated using GWLf and the in-stream water quality results were predicted using EFDC. The linked modeling system considers the loadings of nitrogen and phosphorus from all sources including point sources and non-point sources. The point source discharges of flow and nutrients were obtained from USEPA’s DMR database. The non-point sources of runoff and nutrients are simulated using the GWLf model.

The GWLf model was first calibrated using observed data to ensure correct representation of the runoff and loading yield processes. Runoff and nutrient load predictions from GWLf

were then input to the EFDC model. The EFDC model simulates the transport of nutrients and other dissolved or particulate materials from upstream to downstream in the creek. It also simulates water temperature dynamically using weather data. The simulated water temperature is passed to EFDC's eutrophication module to model dynamics of benthic algae under the influence of water temperature, available solar radiation, and available nitrogen and phosphorus. Continuously observed DO data and grab samples of nutrients were used to support model calibration, which involved determining key benthic algae parameters including growth rate, metabolism rate, and excretion rate.

Figures 14 through 17 are examples of the Indian Creek EFDC model calibration results. No benthic chlorophyll *a* data were available for calibration; DO was used as an indicator of benthic algae based on the understanding that the DO fluctuation is mainly caused by the benthic algae in Indian Creek. A detailed discussion of model configuration, calibration, and application can be found in the EPA's Indian Creek TMDL report (EPA 2008).

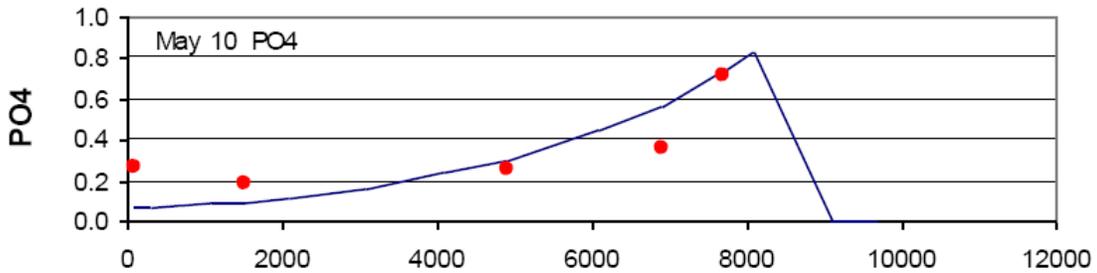


Figure 14 - Longitudinal profile of modeled and observed orthophosphate concentration (mg/L) in Indian Creek. The distance is meters from the mouth of Indian Creek. Red dots are data and blue line is model results.

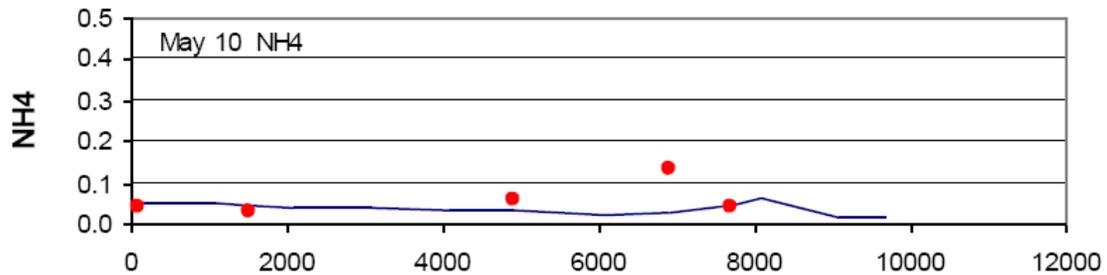


Figure 15 - Longitudinal profile of modeled and observed ammonia concentration (mg/L) in Indian Creek. The distance is meters from the mouth of Indian Creek. Red dots are data and blue line is model results.

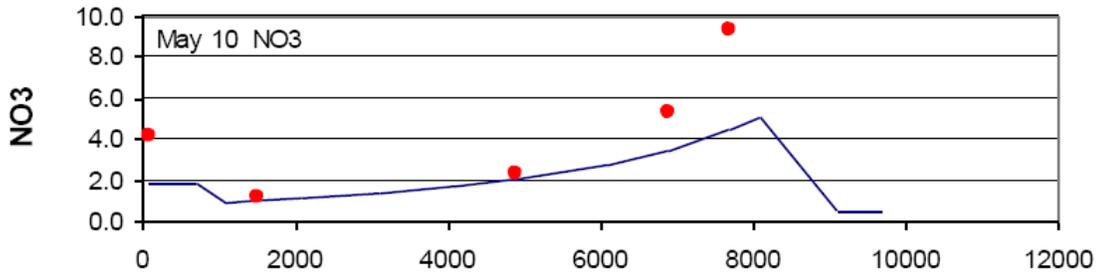


Figure 16 - Longitudinal profile of modeled and observed nitrate concentration (mg/L) in Indian Creek. The distance is meters from the mouth of Indian Creek. Red dots are data and blue line is model results.

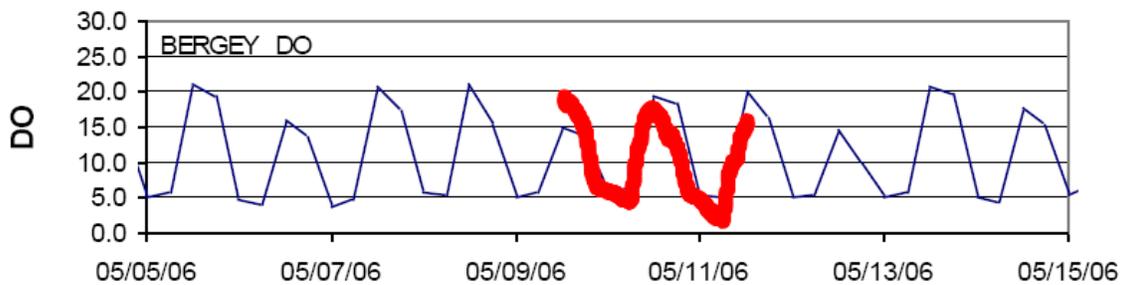


Figure 17 - Comparison of modeled and observed DO concentration (mg/L) at Bergey Rd. Red dots are data and blue line is model results.

After the model calibration parameters were determined, the model was applied to examine the level of nutrients required to achieve the desired average benthic algal density of 100 mg / m², of chlorophyll *a*. The process was iterative with some initial estimates regarding reduction of nutrients. No other factors, including solar radiation and water temperature were changed.

As nutrient loads were reduced, the resulting chlorophyll *a* levels were compared to the target benthic density (100 mg/ m²). Results indicate that when average TP concentrations are between 20-33 µg/L in Indian Creek, average benthic chlorophyll *a* levels are predicted to remain near the 100 mg/ m² desired threshold. These levels are slightly lower than/consistent with the average TP concentration targets derived by the multiple lines of evidence approach. Figure 18 shows predicted chlorophyll *a* levels for existing conditions and after reduction conditions at the Bergy Road location. These are directly resulting from reduced TP inputs.

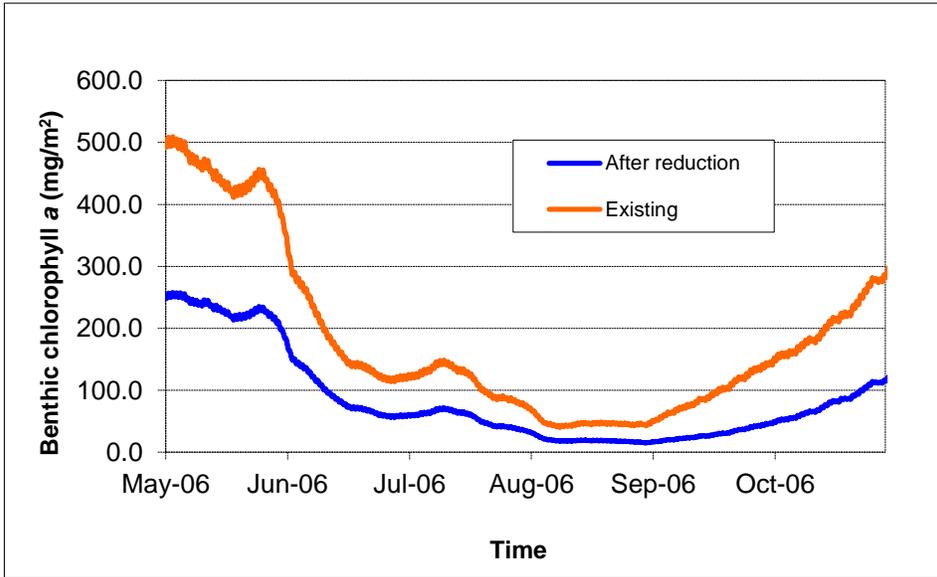


Figure 18 - Predicted periphyton (chlorophyll *a*) before and after simulated phosphorus reductions (Indian Creek, Bergey Rd sampling location).

9. SCIENTIFIC LITERATURE

The scientific literature was another line of evidence used in the original analysis. One study (Herlihy and Sifneos 2008), in particular, seemed relevant since the original PA TMDL TP target report was written; namely an analysis of national nutrient data collected as part of the USEPA Wadeable Streams Assessment (WSA, USEPA 2006). The WSA is the first comprehensive national probabilistic survey of streams in the US and included data collected from identified least disturbed reference streams (*sensu* Stoddard et al. 2006). The data collected in that study were used to estimate reference stream nutrient concentration upper quartiles, consistent with USEPA's original recommended regional criteria methodology and recommended in USEPA guidance (USEPA 2000). The 75th percentile TP concentrations in reference streams from the comparable nutrient ecoregion to the PA Piedmont were 60 µg/L.

10. UPDATED ENDPOINT SUMMARY

The following table updates the original report endpoint summary table with the additional analysis and information provided in this report.

Table 8 - Summary of candidate endpoints for each of the analytical approaches discussed.

	Approach	TP Endpoint (µg/L)
Reference Approach		2-37
	Reference Site 75 th Percentile	16-17
	All Sites 25 th Percentile	17
	Modeled Reference Expectation	2-37
Stressor-Response		8-85
	Conditional Probability – EPT taxa	38
	Conditional Probability - % Clingers	39
	Conditional Probability - % Urban Intolerant	64
	Conditional Probability - Diatoms TSI	36
	Simple linear regression interpolation – EPT taxa	10-85
	Simple linear regression interpolation – Percent intolerant urban individuals	8-82
	Simple linear regression interpolation – Percent Clinger individuals	8-52
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
	National Reference Criteria Study	60
Mechanistic Model		20-33
	Indian Creek	20-33

Given the resultant concentrations from the new stressor-response analyses and the fact that the range of endpoints derived with that method included the recommended endpoint (i.e., between the lower quartile and average estimate ranges), that distribution based values remain unaltered, that one additional scientific study estimating regional reference concentration recommends a value similar to the original value and in the range of previous literature, and that a process model of chlorophyll in streams used to derive a TP endpoint to meet acceptable benthic chlorophyll concentrations reached a comparable value, the recommended TP endpoint in the original report (40 µg/L) remains unaltered in the opinion of the authors.

11. LITERATURE CITED

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. United States Environmental Protection Agency, Office of Water, Washington, DC. EPA 841/B/99-002.

Dodds, W.K. and E. B. Welch. 2000. Establishing nutrient criteria in streams. *J. North Am. Benthol. Soc.* 19:186-196.

Herlihy, A.R. and J.C. Sifneos. 2008. Developing nutrient criteria and classification schemes for wadeable streams in conterminous United States. *Journal of the North American Benthological Society* 27(4):932-948.

Maryland Department of Natural Resources (MDNR). 2005. New Biological Indicators to Better Assess the Condition of Maryland Streams. Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment, Annapolis, MD. CBWP-MANTA-EQ-05-13.

Paul, M.J. and L. Zheng. 2007. Development of Nutrient Endpoints for the Northern Piedmont Ecoregion of Pennsylvania: TMDL Application. Prepared for US Environmental Protection Agency, Region 3, Philadelphia, PA.

Pennsylvania Department of Environmental Protection (PADEP). 2009. A Benthic Index of Biotic Integrity for Wadeable Freestone Riffle-Run Streams in Pennsylvania. Pennsylvania Department of Environmental Protection, Division of Water Quality Assessment and Standards, Harrisburg, PA.

Rief, A. 1999. Physical, Chemical, and Biological Data for Selected Streams in Chester County, Pennsylvania, 1981-94. USGS Open-File Report 99-216, USGS, Denver, CO.

Rief, A. 2000. Physical, Chemical, and Biological Data for Selected Streams in Chester County, Pennsylvania, 1995-97. USGS Open-File Report 00-238, USGS, Denver, CO.

Rief, A. 2002a. Assessment of Stream Conditions and Trends in Biological and Water-Chemistry Data from Selected Streams in Chester County, Pennsylvania, 1981-97. USGS Water Resources Investigations Report 02-4242, USGS, Denver, Colorado

Rief, A. 2002b. Assessment of Stream Quality Using Biological Indices at Selected Sites in the Delaware River Basin, Chester County, Pennsylvania, 1981-97. USGS Fact Sheet FS-116-02, USGS, Denver, C

Suplee, M., V. Watson, M. Teply, and H. McKee. 2008. How Green is too Green? Public Opinion of What Constitutes Undesirable Algae Levels in Streams. *J. American Water Resources Association*. 44(6):1-18.

United States Environmental Protection Agency (USEPA). 2000a. Nutrient Criteria Technical Guidance Manual. Rivers and Streams. United States Environmental Protection Agency, Office of Water, Washington, DC. EPA-822-B-00-002

USEPA. 2000b. Nutrient Criteria Technical Guidance Manual. Lakes and Reservoirs. United States Environmental Protection Agency, Office of Water, Washington, DC. EPA-822-B-00-001

USEPA. 2006. The Wadeable Streams Assessment: A Collaborative Survey of the Nation's Streams. United States Environmental Protection Agency, Office of Water, Office of Research and Development, Washington, DC. EPA-841-B-06-002

USEPA. 2010. Using Stressor-response Relationships to Derive Numeric Nutrient Criteria. United States Environmental Protection Agency, Office of Water, Washington, DC. EPA-820-S-10-001.