

# **A Screening-Value Approach to Nutrient Criteria Development for Freshwater Wadeable Streams in the Mountain and Piedmont Regions of Virginia: July 2008 – June 2009 Activities**

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## **I. Introduction**

Under the Clean Water Act, criteria are components of water quality standards. The U.S. Code of Federal Regulations (CFR) defines criteria as “elements of State water quality standards, expressed as constituent concentrations, levels, or narrative statements, representing a quality of water that supports a particular use. When criteria are met, water quality will generally protect the designated use” [40 CFR 131.3(b)]. The U.S. Environmental Protection Agency (EPA) requires that all states develop criteria to protect waters from impairment by nutrient enrichment using scientifically defensible approaches that consider the effects of nutrients on designated use within the stream segment being assessed (localized effects) and on receiving water bodies located further downstream (downstream-loading effects) (U.S. EPA 2000).

When present in surface water bodies at elevated concentrations, nutrients (nitrogen and phosphorus) are water pollutants. Excess nutrients cause negative effects in surface water bodies nationwide. Recent EPA reports to Congress have listed nutrients as prominent pollutants impairing freshwater rivers and streams nationwide (Table 1).

**Table 1.** Prevalence of nutrient impairments in assessed rivers and streams as documented by U.S. Environmental Protection Agency National Water Quality Inventory (U.S. EPA 2009).

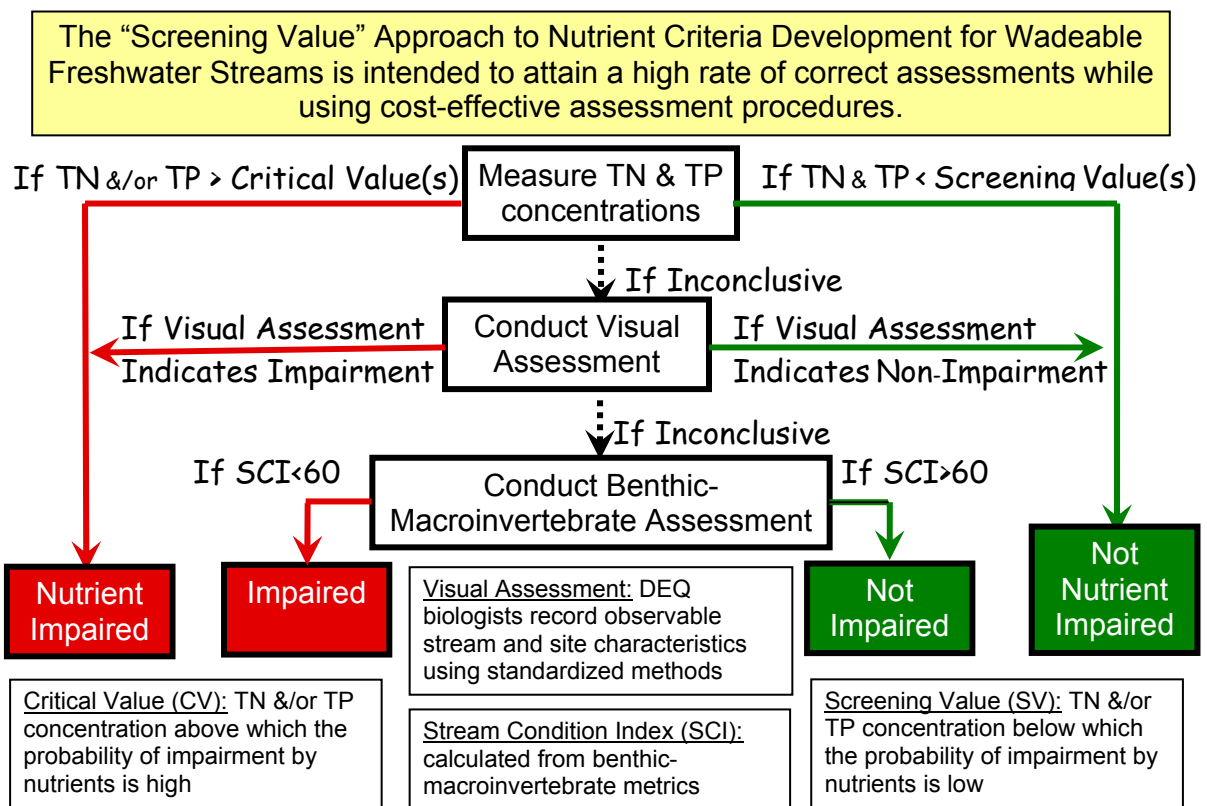
<b>Year</b>	<b>Stream Miles Assessed</b>	<b>Stream Miles Affected by Nutrient Impairment</b>	<b>Nutrient Impaired Streams (% of assessed)</b>
1998	842,246	84,071	10.0%
2000	699,946	52,870	7.6%
2002	695,540	52,228	7.5%
2004	563,955	38,632	6.9%

This report documents activities being conducted by the Water Quality Academic Advisory Committee (AAC) to Virginia Department of Environmental Quality (DEQ) in collaboration with Virginia DEQ for the purpose of developing nutrient criteria for wadeable, freshwater rivers and streams in the Mountain and Piedmont regions of Virginia. The Mountain region of Virginia is within the following Level III Ecoregions: Central Appalachians, Ridge and Valley, and Blue Ridge. The Piedmont region of Virginia is within the following Level III Ecoregions: Northern Piedmont and Piedmont.

## Background: Virginia’s Nutrient Criteria Development Process

In Virginia, all state waters are designated to support aquatic life. Virginia water quality standards define the aquatic-life designated use as “the propagation and growth of a balanced, indigenous population of aquatic life” (Virginia DEQ 2007). In accord with EPA guidance, Virginia has developed a biological-monitoring procedure to assess the suitability of freshwater rivers and streams for the aquatic-life use. Like many other state agencies, Virginia DEQ employs benthic macroinvertebrates in determining the support of the aquatic-life use (Tetra Tech, Inc. 2003; Virginia DEQ 2006).

The Virginia DEQ has requested advice from the AAC to aid in the development of nutrient criteria for freshwater rivers and streams. The AAC is recommending that nutrient criteria for freshwater wadeable streams be defined using a unique approach, termed as the “screening value approach” (AAC 2006). This approach employs a series of monitoring procedures to determine whether the amount of nutrients in a water body allows it to support the aquatic-life use (Figure 1).



**Figure 1.** A proposed screening-value approach for developing nutrient criteria in Virginia’s freshwater-wadeable streams. TN = total nitrogen; TP = total phosphorus

The first stage of the screening-value approach to water-quality assessment for nutrient effects, as recommended by the AAC (2006), would employ two sets of thresholds for nitrogen (N) and phosphorus (P):

- Screening Value(s): Streams with nutrient concentrations below the screening value(s) are assessed as “not impaired by nutrients.”
- Critical Value(s): Streams with nutrient concentrations above the critical value(s) are assessed as “impaired.”

Streams that cannot be assessed using the screening or critical values would be visually assessed.

- Visual Assessment: Nutrient impairments occur due to the effects of algal and plant growth stimulated by the nutrients. A visual procedure to assess the stream for impairment by nutrients would rely on the presence or absence of visible macrophytes and algae. As proposed by the AAC, the visual assessment can have three possible outcomes: impaired by nutrients, not impaired by nutrients, or inconclusive.

If a stream’s nutrient concentrations do not allow assessment using the screening or critical values, and if the visual assessment is inconclusive, a benthic-macroinvertebrate assessment would be employed to assess the stream.

- Benthic-Macroinvertebrate Assessments: Virginia DEQ uses the assessment of the benthic-macroinvertebrate community to determine that the stream meets the aquatic-life use.

A screening-value approach is recommended as an alternative to traditional fixed-threshold criteria because nutrient effects on aquatic systems differ from the effects of traditional stressors. Whereas traditional stressors tend to exert toxic influences at the organism level, nutrient overenrichment effects are systemic (i.e., nutrients, themselves, are not generally toxic, but overenrichment of nutrients affects the stream system, such as by depleting oxygen levels, and thus causes detrimental impacts on organisms). Furthermore, unlike traditional toxic stressors, nutrients are required in surface waters to support aquatic life. Nutrients are considered a stressor in surface waters only when present in excessive amounts. Thus, variations among physical characteristics of river-and-stream systems affect those systems’ responses to nutrient enrichment. As a result, biotic responses to nutrient enrichment at specific concentration levels are highly variable among river and stream systems.

The screening-value approach is applied with the intention of limiting assessment errors despite the inherent variability of aquatic systems’ responses to nutrients. The screening-value approach has a secondary goal of achieving efficiency in the DEQ resource expenditures necessary to meet the goals of the Clean Water Act.

The AAC has been consistent in recommending that DEQ develop nutrient criteria to limit assessment errors in recognition of the costs that result from incorrect assessments (Figure 2). When streams are assessed as impaired, a TMDL study is required. Thus, when non-impaired streams are incorrectly assessed as impaired (false-positive assessment, Type I error), the resulting costs of the TMDL study utilizes resources for enforcing the Clean Water Act that could otherwise be applied elsewhere for water-quality protection. False-positive assessments can also affect investment decisions by regulated point sources discharging into that stream

segment. When impaired streams are not assessed as impaired (Type II error, false negative), costs are borne by the public in the form of lost environmental services that result from failure of that water body to support its designated uses.

		<u>Actual Condition</u>	
		Impaired	Not Impaired
<b>Assessment Outcome:</b>	Impaired	Correct Assessment (true positive)	Incorrect Assessment (false positive, type I error)
	Not Impaired	Incorrect Assessment, (false negative, type II error)	Correct Assessment (true negative)

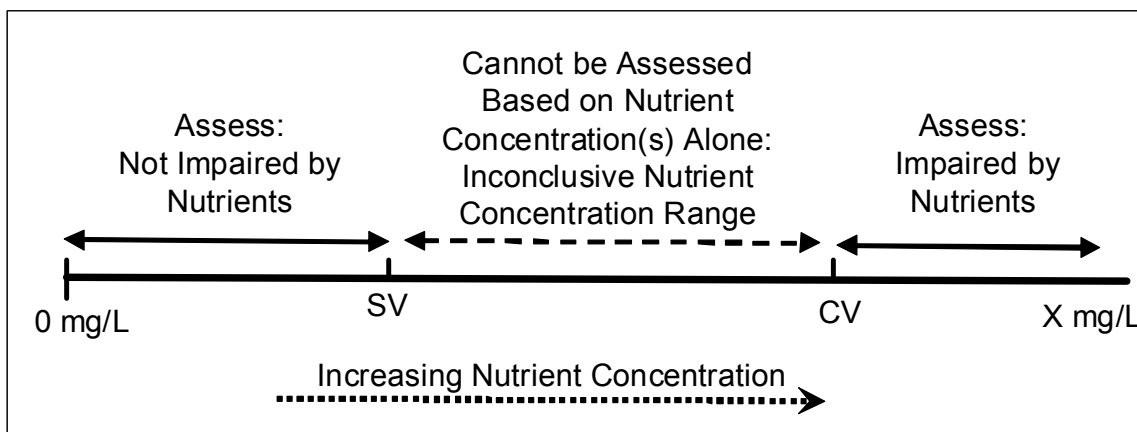
**Figure 2.** Type I and Type II errors. The screening-value approach is being developed with the intention of limiting both Type I and Type II assessment errors.

Application of the screening-value approach requires consideration of trade-offs, given the inherent variability of streams’ responses to nutrient concentrations and the resulting uncertainty of assessment decisions based on fixed thresholds for nutrients.

When applied together, the critical and screening values define a range of nutrient concentrations (termed the “inconclusive-nutrient-concentration range”) for which additional monitoring and assessment resources must be expended for assessment (Figure 3). A conservative approach to establishing these assessment thresholds – setting the critical value at a relatively high concentration and setting the screening value at a relatively low concentration – would result in a high rate of correct assessments. Having a broad distribution of nutrient concentrations within the inconclusive-nutrient -concentration range, however, would increase the monitoring expenditures of DEQ. Given resource limitations that constrain Virginia DEQ (a taxpayer-supported public agency that operates its water-quality protection programs on funds allocated by the state legislature), an expansion of resource expenditures for water-monitoring and assessment would likely require that the agency’s other environmental protection services be reduced. The additional resource expenditures required for a visual assessment of streams that occur within the inconclusive-nutrient-concentration range would be relatively modest, but a visual assessment is expected to be adequate for only a fraction of streams in the inconclusive-nutrient-concentration range. For the remaining streams a benthic-macroinvertebrate assessment would be required. Each benthic-macroinvertebrate assessment requires on the order of one day’s investment of time by regional biologists for sampling and analysis. This level of resource expenditure is considered significant given that DEQ employs a limited number of regional biologists and that these personnel have a range of responsibilities in addition to whatever duties may result from the implementation of nutrient criteria.

The approach described above for defining critical and screening values is conservative. The implementation of a less conservative approach, one with a narrow range of inconclusive

concentrations, could be expected to reduce the agency’s monitoring expenses. The cost savings for monitoring, however, would be accompanied by an increase in the error rate of screening- and critical-value assessments. Thus, the screening-value approach embodies essential trade-offs between public benefits, which require error limitation, and water-monitoring resource expenditures.



**Figure 3.** Graphic representation of nutrient-concentration ranges defined by the screening-value approach to nutrient criteria, as recommended by the AAC. SV = screening value; CV = critical value.

## II. Pilot-Program Description and Results

Working within the context described above, the Virginia DEQ and the AAC conducted a trial run of a screening-value approach for nutrient criteria in wadeable, freshwater streams between March 2007 and June 2009. This study took place in Virginia’s Mountain and Piedmont regions (located within EPA’s Aggregate Nutrient Ecoregions XI and IX, respectively). In the text that follows, we refer to the activity as the “pilot program.”

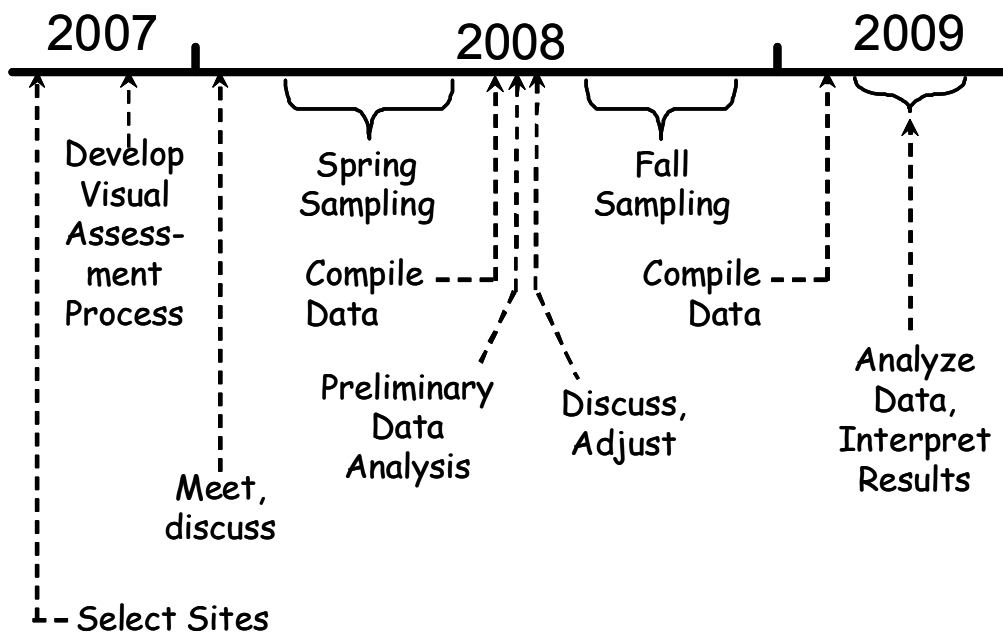
### Project Goals

The goals of the pilot program were to:

- a. Develop a visual-assessment procedure.
- b. Propose visual-assessment levels that may trigger impairment or non-impairment designations (see Figure 1), and determine the levels of uncertainty that would be associated with such designations.
- c. Propose total-nitrogen (TN) and total-phosphorus (TP) values that can serve as screening values and as critical values (see Figure 1), and determine the levels of uncertainty that would be associated with such designations.
- d. Determine the ability of the screening-value approach (Figure 1) to successfully discriminate impaired from non-impaired sites using screening- and critical-values that result with reasonable resource expenditures by DEQ.
- e. Determine the resource requirements of full-scale implementation by DEQ.

## Methods

The pilot program was conducted over a time period extending from mid-2007 through mid-2009, and included site selection, development of the visual-assessment procedure, sampling, and data analysis (Figure 4).



**Figure 4.** Pilot-program timeline for major activities.

### *Site Selection*

Sites included in the pilot program were selected using the following method:

1. All ambient water-quality monitoring sites within Virginia's Mountain and Piedmont regions meeting either of the following two conditions were identified.
  - a.) For sites that have been in operation continuously during the previous 12 months, 5 or more TN and 5 or more TP concentrations recorded during the previous 12 months.
  - b.) For sites that have been in operation continuously only since January 2007, 3 or more TN and 3 or more TP concentrations recorded since January 2007.
2. Median TN and TP values were calculated from monitoring observations collected during the prior 12 months for each station. Using these median values, each monitoring station was placed in a TN category and a TP category (Table 2).
3. DEQ biologists in the Mountain and Piedmont regions were asked to select up to 12 monitoring stations (approximately 6 sites for sampling in the fall and 6 sites for sampling in the spring) for inclusion in the pilot program by applying the following criteria:

- a) Site is represented by recent water-quality data so it can be placed reliably within a nutrient category.
- b) Site is wadeable and suitable for benthic-macroinvertebrate sampling.
- c) Site is not known to be subject to major influence by non-nutrient stressors (urban runoff, toxics, sediments, point source discharges, etc.).
- d) Site is from the list of stations prepared by DEQ's water-monitoring data coordinator, Mr. Roger Stewart.
  - i. At least one station within each of the 6 N-concentration categories and at least one station within each of the 6 P-concentration categories are to be represented. (Note: because each station is placed in both an N-concentration category and a P-concentration category, this condition can be met with fewer than 12 sites).
  - ii. To the extent possible:
    - For the lowest N-concentration category: assure that relatively low, medium, and high P concentrations are represented; and
    - For the lowest P-concentration category: assure that relatively low, medium, and high N concentrations are represented.
- e) Sites are not clustered geographically or fluvially, and thus are distributed throughout the entire region.

**Table 2.** Nutrient-concentration categories used for selection of water-monitoring stations for the pilot program.

<b>Total Nitrogen (mg/L), median</b>	<b>TN Category</b>	<b>Total Phosphorus (mg/L), median</b>	<b>TP Category</b>
<0.5	1	<0.02	1
0.5 - <1.0	2	0.02 - <0.04	2
1.0 - <1.5	3	0.04 - <0.06	3
1.5 - <2.0	4	0.06 - <0.10	4
2.0 - <3.0	5	0.1 - <0.20	5
>=3.0	6	>=0.2	6

*Development of a Visual-Assessment Procedure*

DEQ's biologists, its water quality standards staff, and AAC member Dr. Len Smock collaborated to develop a visual-assessment procedure that can be implemented within the nutrient criteria framework (see Figure 1). The developed visual-assessment field forms are attached to this report as Appendix A (used during Spring 2008) and Appendix B (used during Fall 2008). Site attributes relevant to the potential nutrient effects, such as amount of shading (full shade, partial shade, full sun), estimated surface stream velocity (slow, moderate, fast), stream substrate (sand, gravel, cobble), stream depth and width were included on the field survey forms. The visual-assessment procedure also included a qualitative assessment by the regional biologist regarding whether or not the site is impaired by nutrients.

The visual-assessment procedure was designed to produce numeric results that are both reproducible and independent of the individual who is applying the method. Visual-assessment

components included factors such as an estimated percentage of the visible stream bottom covered with algae or macrophytes, estimated percentage of some number of rocks removed randomly from the stream bottom that are covered with algae, and the type and amount of algae present. The biologists were asked to rate each site by nature and type of algae present. Algal types that were rated included combinations of color (bright green, dark green, brown, and black) and form (film, thin mat, thick mat, short filamentous, and tall filamentous). The types listed above are for the fall rating; a similar but less inclusive set of algal color and form combinations was used for the spring rating. Biologists were asked to rate each site for presence of algal color/form combinations using a scale of 1-10%, 10-40%, 40-70%, and 70-100% coverage categories. We used these ratings to construct the Algal Index for each site by summing the algal color/form combinations that biologists described as being present, weighting each by visually estimated stream bottom coverage on a scale of 1 – 3 – 6 – 10 for the 4 categories; this constructed measure was called the “Algal Index 13610” or “Algal Index” for short.

#### *Development of the Quality Assurance Project Plan (QAPP)*

In collaboration with the AAC, DEQ developed a QAPP, which was submitted to EPA in association with an EPA grant application.

#### *Initiation of Pilot-Program Activities*

The initial schedule called for DEQ biologists to begin sampling in Fall 2007. However, administrative procedures associated with the EPA grant application had not yet been completed by that date so the initial sampling was delayed until Spring 2008. Excessively wet weather in some parts of the state, combined with the study design, which required sampling during baseflow and avoidance of sampling during time periods following scouring rains, interfered with the spring sampling. As a result, some of the sites scheduled for spring sampling were not sampled.

#### *Trial Application Round I: Spring 2008*

DEQ biologists conducted a visual assessment, a benthic-macroinvertebrate assessment, and a habitat assessment at approximately half of the sites selected for study implementation in Spring 2008. All sampling was conducted according to established DEQ protocols as detailed in DEQ Standard Operating Procedures (SOP) manuals and the QAPP prepared in association with this project. Sampling was conducted during baseflow conditions so as to be consistent with DEQ probabilistic-monitoring protocols and to assure lack of algal scouring effects. In addition, sampling took place 14 or more days after the last rain event judged by regional biologists to have caused an algal scouring effect. Benthic-macroinvertebrate sampling results were transformed to a Stream Condition Index score using DEQ standard procedures (Tetra Tech, Inc. 2003).

In-situ water-quality measures were recorded for each sampling site:

- Temperature – In-Situ, YSI or Hydro-Lab multi-probe meter (calibrated with NIST thermometer in lab).



- pH – In-Situ, YSI or Hydro-Lab multi-probe meters (calibrated and post-confirmed checked each field day, using commercially available standards)
- Dissolved oxygen – In-Situ, YSI or Hydro-Lab meter (pre-calibrated and post-confirmed each field day, using (100% RH) air standard)
- Conductivity – In-Situ, YSI or Hydro-Lab meter (calibrated and post-confirmed each field day, using commercially available standards).

In addition to these field measures, water samples were taken as point samples using standard DEQ protocols. Nutrient variables analyzed include nitrate-N ( $\text{NO}_3\text{-N}$ ), nitrite-N ( $\text{NO}_2\text{-N}$ ), total kjeldahl N (TKN), TN, and TP; all are expressed as mg/L as N or P. Other variables measured included suspended solids (Storet 530 – non-filterable residue) and total residue (Storet 500).

Benthic algae (periphyton) were sampled to estimate periphytic biomass. Algal biomass was scraped from 3 randomly selected rocks, and the scraped area was estimated via a tracing. The biomass samples were processed to determine chlorophyll-a (Chl-a) and ash-free dry mass (AFDM) by the Virginia Division of Consolidated Laboratory Services (DCLS) following Standard Methods (APHA 1992) for algal-biomass estimates.

#### *Mid-Course Program Review*

Data from the spring 2008 sampling was assembled and made available to the AAC and to interested parties within DEQ for analysis during the summer of 2008. First-round results were discussed with biologists on a conference call. As a result of this call, several program adjustments were made. The visual-assessment field form was modified (see Appendix B), and several regional biologists decided to move the initially selected sampling stations as needed to better achieve study goals.

#### *Trial Application Round II: Fall 2008*

The trial application protocol, as described above for Spring 2008, was repeated in the fall at the remaining sites, with minor modifications as per the mid-course program review.

#### *Data Analysis and Interpretation: Early 2009*

Data were analyzed using JMP statistical software (SAS Institute, Cary NC), using a variety of statistical procedures including one-way analysis of variance (ANOVA) and linear regression. Most variables were not normally distributed, the primary exception being Stream Condition Index (SCI). When a log transformation was able to transform a non-normally distributed variable to a normal or near-normal distribution, the log-transformed variable was used in data analysis. Otherwise, statistical analysis was performed using non-parametric procedures applied to the ranks.

Preliminary data analysis was completed in March, 2009. Results were presented and discussed at a meeting of the AAC with Virginia DEQ staff in Charlottesville on March 18, 2009.

## Results: Study Process

### Selection of Sites

The goal of the site selection process (described in the Methods section) was to assure that high nutrient concentrations and variable N and P concentration ranges were represented. Past studies had revealed that TN and TP concentrations in Virginia freshwater streams are correlated, and that the distributions of these nutrient concentrations are skewed.

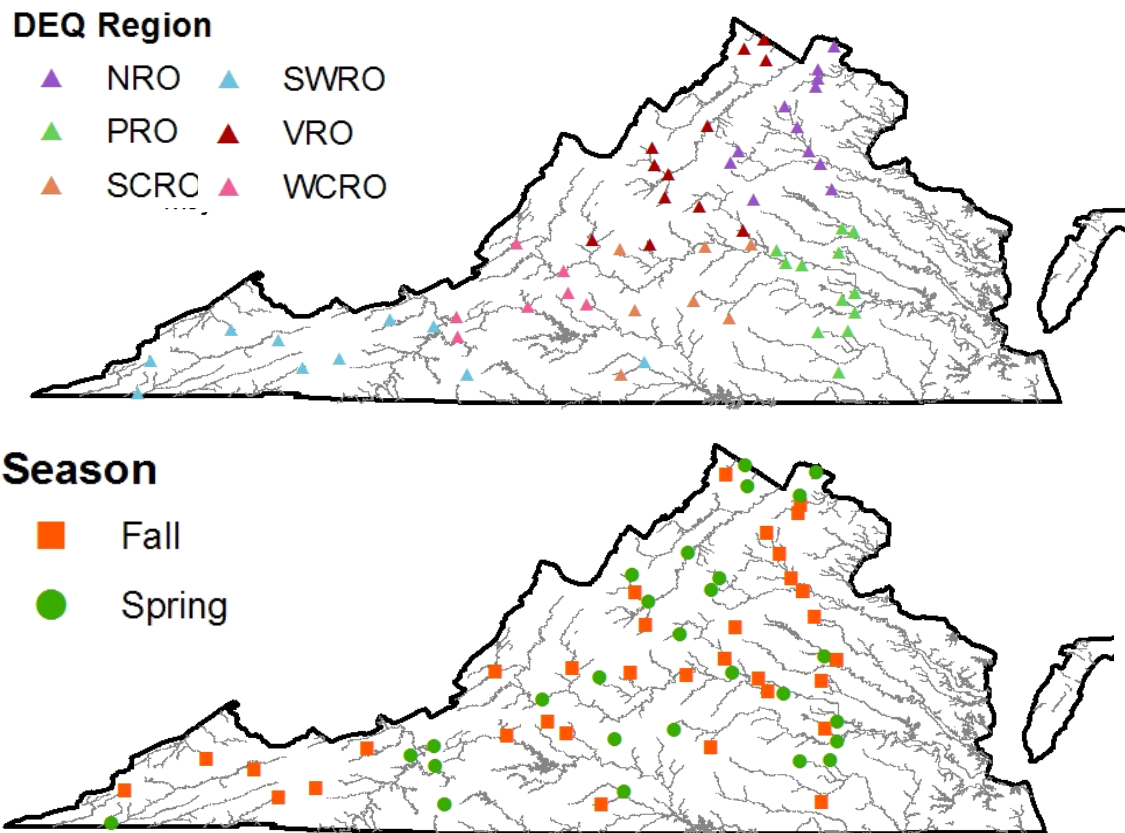
Table 3 shows the distribution of 69 sites among nutrient categories as initially selected (upper table). However, when sampled, some sites had concentrations that differed from the expected concentration. The distribution of the 62 sites actually sampled is provided in Table 3 (lower table). The location of each monitoring site in the pilot program, the DEQ regional office conducting the monitoring, and the season in which monitoring occurred are represented in Figure 5.

**Table 3.** Distribution of stations among TN and TP categories as initially selected (upper table) and as actually measured during the pilot program (lower table).

		TP						
		1	2	3	4	5	6	All
(mg/L)		<0.02	0.02 - <0.04	0.04 - <0.06	0.06 - <0.10	0.1 - <0.20	>=0.2	
TN	1 <0.5	6	8	4	1	2	-	21
	2 0.5 - <1.0	1	4	4	4	3	-	16
	3 1.0 - <1.5	2	2	3	2	-	-	9
	4 1.5 - <2.0	-	2	2	1	3	1	9
	5 2.0 - <3.0	1	2	1	2	2	1	9
	6 >=3.0	-	-	1	3	-	1	5
	All	10	18	15	13	10	3	69

		TP						
		1	2	3	4	5	6	All
(mg/L)		<0.02	0.02 - <0.04	0.04 - <0.06	0.06 - <0.10	0.1 - <0.20	>=0.2	
TN	1 <0.5	5	12	4	1	2	-	24
	2 0.5 - <1.0	-	7	4	2	2	-	15
	3 1.0 - <1.5	-	1	3	3	1	-	8
	4 1.5 - <2.0	-	1	3		1	1	6
	5 2.0 - <3.0	-	-	1	1	-	2	4
	6 >=3.0	-	2	-	-	-	3	5
	All	5	23	15	7	6	6	62



**Figure 5.** The 62 sites monitored and sampled by DEQ biologists during the pilot-program activity, by DEQ region and by season. NRO = Northern Regional Office; PRO = Piedmont Regional Office; SCRO = South Central Regional Office; SWRO = South West Regional Office; VRO = Valley Regional Office; and WCRO = West Central Regional Office.

### *Sampling*

Data from 29 sites were obtained in the spring, and data from 33 sites were obtained in the fall. Benthic-macroinvertebrate assessments were replicated at one site sampled in spring and three sites sampled in fall. Impairment status (i.e., whether or not SCI < 60) for replicate samples did not differ from the primary sample, so only primary sample results are used in the following analysis. Minor adjustments were made in the visual-assessment form after the spring sampling, as several new assessment procedures were added in response to the spring experience. Sites were selected for inclusion in the study based on previously measured TN and TP concentrations, with the intention of ensuring sufficient representation of high-nutrient streams to allow characterization of the high-nutrient effects that are of primary interest in this study. Basic data from streams included in the study are described in Table 4.

**Table 4.** Summary statistics for the 62 water-monitoring sites sampled and characterized through the pilot program.<sup>a</sup>

Parameter	SCI<60 <sup>b</sup>	SCI>60	All
Number of Observations	36	26	62
TN (median, mg/L)	0.85*	0.47	0.61
NO <sub>3</sub> -N (median, mg/L)	0.54*	0.10	0.25
TKN (median, mg/L)	0.4	0.4	0.4
TP (median, mg/L)	0.045	0.03	0.04
Benthic Algae: Ash-free dry mass (AFDM, median, mg/m <sup>2</sup> )	20.8	16.6	17.6
Benthic Algae: Chlorophyll-a (Chl-a, median, mg/m <sup>2</sup> )	56.8	27.0	39.5
SCI (mean)	47.5	68.3	57.3

<sup>a</sup> For replicated sites, only the first replication was used to calculate summary statistics.

<sup>b</sup> SCI = 60 is the impairment threshold. When SCI<60, DEQ considers the site to be impaired for the aquatic-life use.

\* = significantly different ( $P < 0.05$ , one way ANOVA using ranks) vs. SCI > 60 sites. Other water-quality and benthic-algae measures are not significantly different.

## Results: Data Analysis

### *Biochemical Relationships*

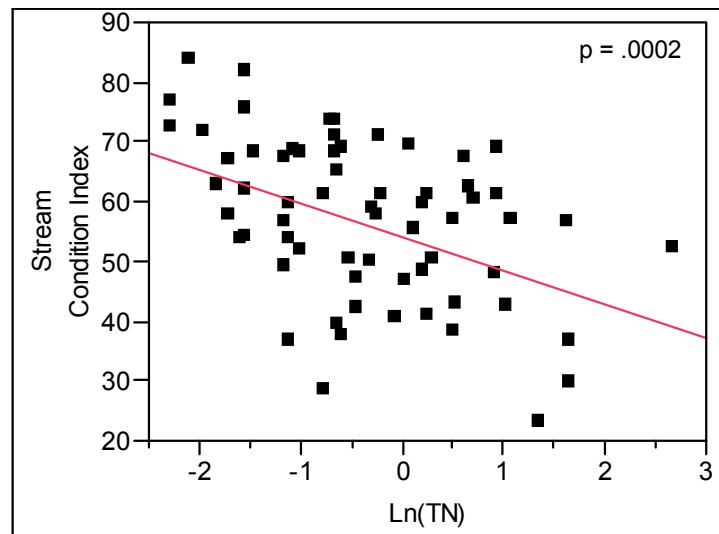
In general, the biochemical relationships occurred as expected: high-nutrient concentrations, high algae/plant densities, and low SCI scores were all correlated. However, those relationships, although often statistically significant and sometimes highly significant, did not provide a basis for development of predictive models. High variance and low coefficients of determination,  $R^2$ , prevented the development of models with the potential for precise application.

Generally speaking, relationships with benthic algae and SCI are stronger for N than for P and are stronger for TN than for either of the two major TN components (TKN, NO<sub>3</sub>-N). Influences of TN, NO<sub>3</sub>-N, and TKN concentrations on the Stream Condition Index (SCI) are all negative and statistically significant ( $P < 0.05$ ). Of the three major nitrogen measures, TN exhibits the strongest relationship ( $P = 0.0002$ ; see Figure 6), but NO<sub>3</sub>-N exhibited a stronger relationship ( $P = 0.0031$ ) than did TKN ( $P = 0.03$ ). The relationship of measured TP values with SCI was not statistically significant. Both measures of benthic algae (AFDM and Chl-a) appeared to influence SCI, with higher benthic-algae levels associated with lower SCI scores, but the relationships were weak (Figure 7).

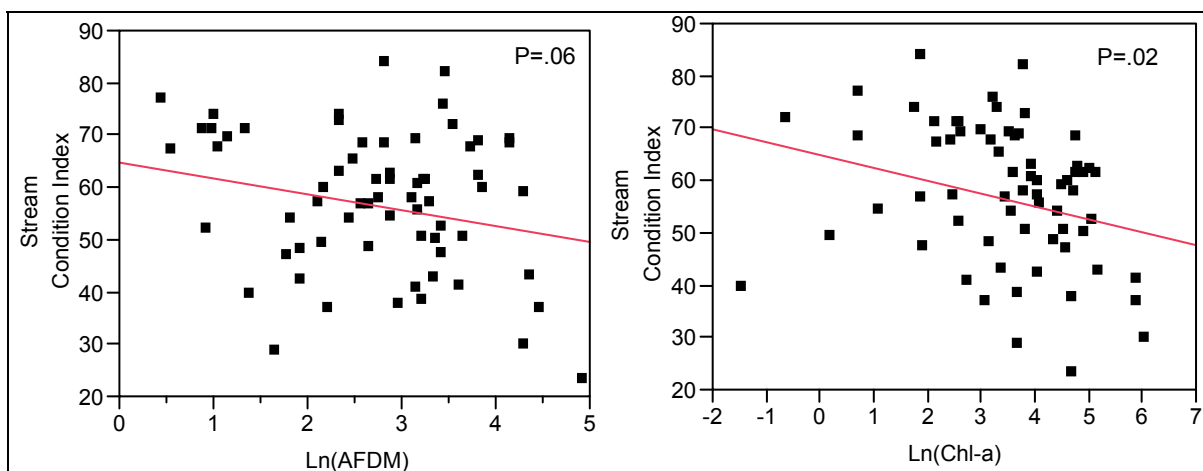
Benthic-algae biomass increased with measured nutrient concentrations. Generally speaking, these relationships were stronger for TN than for TP, and stronger for Chl-a than for AFDM (Figure 8). Only the TN relationships were statistically significant. Of the two major nitrogen components: NO<sub>3</sub>-N exhibited stronger relationships with benthic-algal biomass, especially Chl-a, than did TKN.

Generally speaking, nitrogen exhibited the expected biochemical relationships (i.e., positive relationship with benthic-algal biomass, negative relationship with SCI) more strongly than did TP. This is as expected given that the majority of P in most Virginia streams is generally

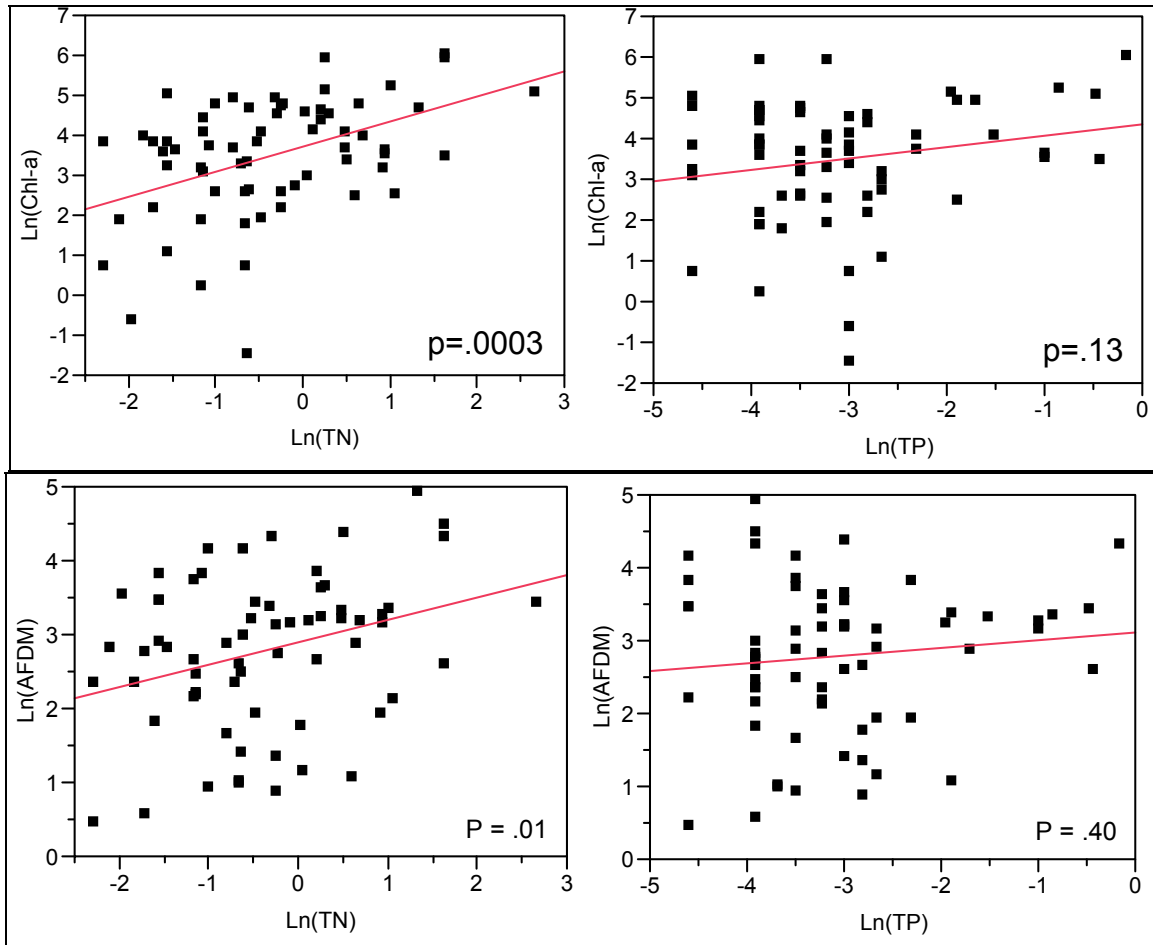
considered to originate from non-point sources and that the streams were sampled under baseflow conditions. Non-point-source P tends to be associated with sediments, the movement of which tends to vary closely with streamflow. Thus, the sampling conditions were not conducive to detection of sediment-associated P movement. TN tended to exhibit stronger biochemical relationships than either  $\text{NO}_3\text{-N}$  or TKN but not consistently. The  $\text{NO}_3\text{-N}$  data exhibited consistently stronger biochemical relationships than did TKN, which supports our interpretation of streamflow conditions as a factor that influenced results. Because  $\text{NO}_3$  occurs only in water-soluble forms, it is easily transported through groundwater systems to the stream under baseflow conditions. In contrast, some TKN components occur as solid-phase forms whose movement tends to be more flow dependent.



**Figure 6.** Linear regression of Log-transformed TN (mg/L) vs. Stream Condition Index (SCI) ( $R^2 = 0.21$ ). The relationship was highly significant ( $p = 0.0002$ ).



**Figure 7.** Linear regression of two measures of benthic-algae biomass – ash-free dry mass (AFDM,  $\text{g/m}^2$ ) and Chlorophyll-a (Chl-a,  $\text{mg/m}^2$ ), both log-transformed – against SCI. The  $R^2$  is 0.06 for the Ln(AFDM) relationship (left), and 0.08 for Ln (Chl-a) (right).



**Figure 8.** Linear regressions of Log-transformed TN (mg/L) and TP (mg/L) against benthic-algae biomass, expressed as Chlorophyll-a (Chl-a,  $\text{mg}/\text{m}^2$ ), log-transformed (above); and ash dry mass (AFDM,  $\text{g}/\text{m}^2$ ), log-transformed (below).  $R^2$  values for these relationships are 0.19 (upper left), 0.04 (upper right), 0.11 (lower left), and 0.01 (lower right).

### Visual Assessments

The visual-assessment procedure required biologists to rate sites for the probability of impairment by *nutrients* during both spring and fall, and to rate sites for a probability of impairment due to *any cause* during fall only.

Sites identified by biologists as having a high probability of being nutrient impaired based on the visual assessment usually were impaired for aquatic life according to the SCI score ( $\text{SCI} < 60$ ) (Of 7 sites rated as high probability for nutrient impairment based on the visual assessment, 6 had  $\text{SCI} < 60$ ) (Table 5). The visual assessments were not as successful at the other end of the spectrum. A number of the sites identified as having a low probability of nutrient impairment based on the visual assessment were identified as impaired according to the SCI (15 sites listed as impaired according to the SCI were among the 31 sites rated as low probability of nutrient impairment based on the visual assessment). Nutrient effects were visually evident at one site rated as non-impaired based on the SCI score. This site had 40-70% of the stream

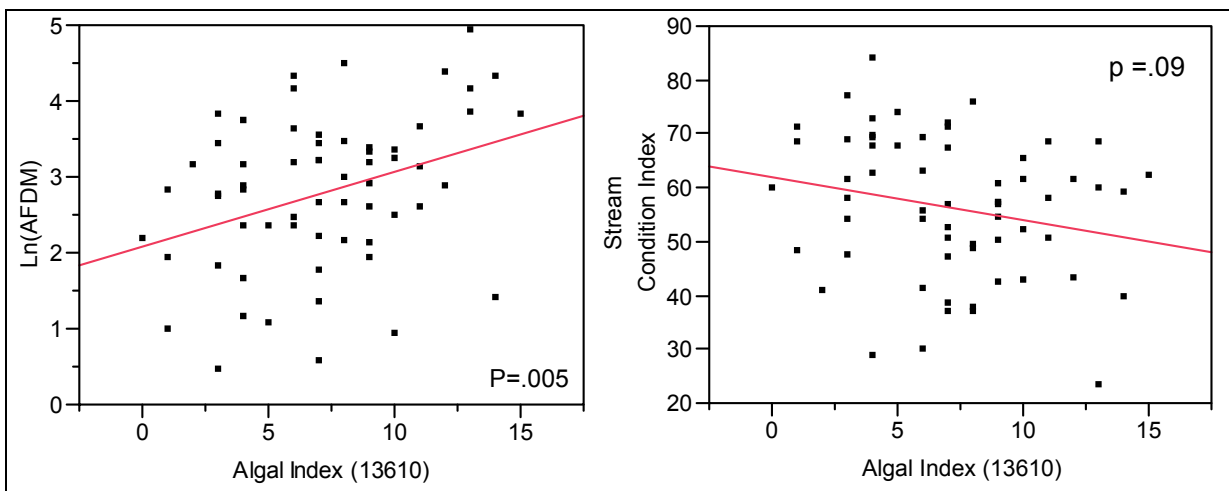
bottom covered by algae (predominantly tall filamentous algae) and plants and thus given a high probability of being impaired according to the visual assessment.

**Table 5.** Impairment status of sites monitored in 2008 as part of the pilot program compared to the rating categories assigned by DEQ biologists.

	Impairment Probability Rating			Total
	Low	Medium	High	
<i>Spring: Nutrient Stressors Only</i>				
Not Impaired (SCI > 60)	8	4	0	12
Impaired (SCI < 60)	8	6	3	17
<i>Fall: Nutrient Stressors Only</i>				
Not Impaired (SCI > 60)	8	5	1	14
Impaired (SCI < 60)	7	9	3	19

One reason for the difficulty in defining sites as “non-impaired for nutrients” based on a visual assessment in comparison to the SCI score is that, most possibly, non-nutrient stressors were also acting at a number of sites. Comments cited by the biologists on the data forms indicated that sediments were by far the most common non-nutrient stressor. The non-nutrient factors may have influenced the SCI score but not the visual assessment, which was based on the visual presence of plants and algae.

The biologists' visual assessments of algae presence tended to agree with in-stream measurements but with high variance. AFDM corresponded more closely with biologists' visual assessments of stream-bottom coverage by algae ( $P = 0.005$ ; Figure 9 left) than did Chl-a (not significant). The Algal Index exhibited a negative relationship with Stream Condition Index, but the relationship was weak ( $P = 0.09$ ; Figure 9 right).

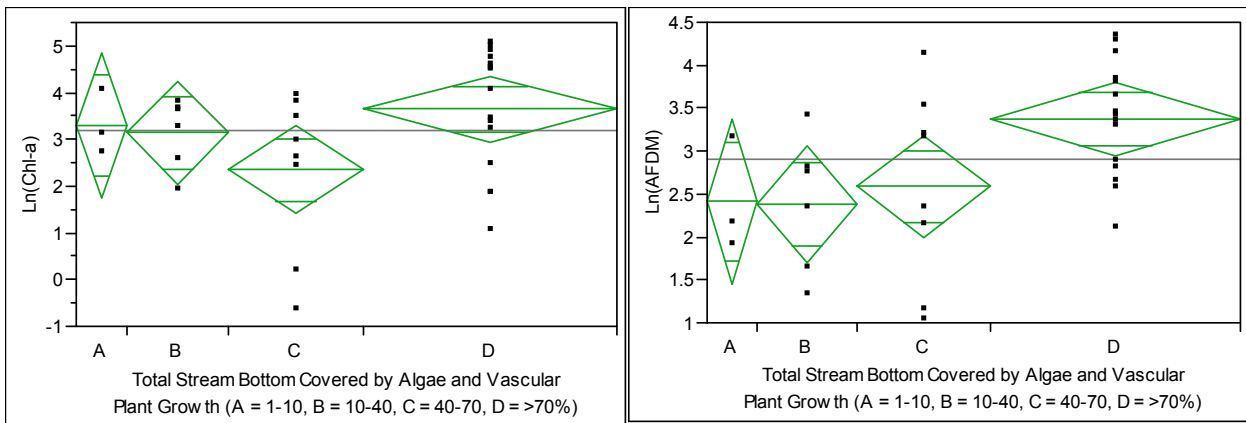


**Figure 9.** Relationship of Algal Index to log-transformed AFDM ( $\text{g}/\text{m}^2$ ) (left) and Stream Condition Index (right).  $R^2$  values for these relationships are 0.12 (left) and 0.05 (right).

In the fall only, regional biologists visually rated each stream for total stream bottom coverage by algae and vascular plants. The biologists' best professional judgment (BPJ) of whether or not the stream was impaired by nutrients was strongly influenced by their perceptions of algae and vascular plant presence (Table 6). The biologists' ratings of 70-100% coverage corresponded with higher levels of algal biomass (Figure 10), measured both as Chl-a and AFDM, although these results were not statistically significant. However, the visual measurement of total stream bottom coverage is meant to include both plants and algae, whereas AFDM and Chl-a are measures of benthic algae only. This difference in what is being measured adds a confounding element to this analysis. Thus, it is not surprising that the biologists' estimates did not correspond more closely with the AFDM and Chl-a values.

**Table 6.** Relationship of regional biologists' best professional judgment of nutrient impairment by visually estimated stream bottom coverage by plants and algae.

<i>Stream Bottom Coverage</i>	<b>Best Professional Judgment Nutrient Impairment Probability Rating</b>			
	<b>Low</b>	<b>Medium</b>	<b>High</b>	<b>Total</b>
A: 0 – 10%	3	0	0	3
B: 10 – 40%	5	1	0	6
C: 40 – 70%	2	5	1	8
D: 70 – 100%	4	8	3	15
Total	14	14	4	32

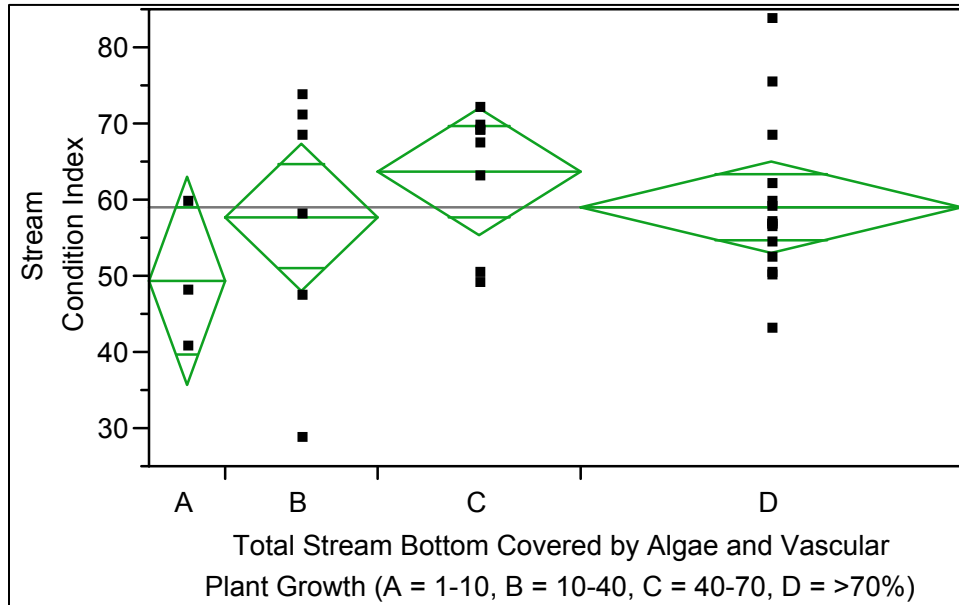


**Figure 10.** Correspondence of biologists' ratings of stream bottom coverage by plants and algae with measured benthic-algae levels. These results were not statistically significant.

The total stream bottom coverage visually assessed (in Fall 2008 only) by estimating algae and vascular plant growth showed no statistically significant relationship with SCI and did not confirm the expected trends. Of the 4 stream-bottom coverage categories (<10%, 10-40%, 40-70%, and >70%), the <10% category showed the highest proportion of SCI-determined



impairments (3 of 3). The 40-70% category showed the lowest proportion of SCI-determined stream impairments (2 of 8) (Figure 11). Eleven (11) of the 15 streams with >70% stream bottom coverage were considered impaired (SCI<60), but the two highest SCI's among fall-sampled streams were also within this (>70%) visual-assessment category.



**Figure 11.** Relationship of Stream Condition Index to biologists' visual ratings of total stream bottom coverage by algae and plants.

### *Potential Critical Values and Screening Values*

“Critical values” and “screening values” are defined in the study plan as in-stream concentrations that allow the stream to be assessed for nutrient impairment. Critical values can be relatively high concentrations that allow sites to be identified as “nutrient impaired,” while screening values are relatively low concentrations that allow sites to be identified as “not nutrient impaired.” Screening values were not evident from this data set, possibly because the data set does not allow discrimination of nutrient from non-nutrient impairment. High-end critical values (i.e., values above which all sites had SCI<60) were evident (Table 7).

**Table 7.** Potential critical values suggested by the results of the pilot program.

<b>Parameter</b>	<b>Critical Value (CV)</b>	<b># sites &gt; CV*</b>
Benthic-Algae Chl-a	170 mg/m <sup>2</sup>	4
Benthic-Algae AFDM	70 g/m <sup>2</sup>	5
TN	2.6 mg/L	6
NO <sub>3</sub> -N	2.3 mg/L	6
TKN	0.9 mg/L	4
TP	0.4 mg/L	4
TN, TP, NO <sub>3</sub> , TKN (WQ)	Combined	10
WQ + Benthic Algae	Combined	13
Best Professional Judgment (BPJ)	High (nutrients only)	7 (6 SCI < 60)
WQ + BPJ	Combined	13
WQ + Benthic Algae + BPJ	Combined	14

\* Out of 62 total sites and 36 impaired (SCI<60) sites in pilot program. At 32 sites, SCI<57.5; of the 4 remaining sites (“borderline impaired”), 1 was caught by the AFDM screen but none were caught by the WQ or BPJ screens.

## **Discussion**

Potential applications of pilot-program results to DEQ’s overall monitoring program must be considered in light of the characteristics of the sites selected and included in the program: sites were selected to include a higher proportion of high-nutrient sites than occurs generally within the population of monitoring sites in DEQ’s program. The relatively high-nutrient levels at the pilot-program sites were a deliberate result of the site-selection process.

Another essential characteristic of the pilot-program data set is that both nutrient and non-nutrient stressors were affecting aquatic resources. Although the study design was intended to isolate nutrient effects by focusing efforts on sites where non-nutrient stressor effects were not evident, this goal was not met despite the best efforts of regional biologists in selecting sites. Sediments were identified as a non-nutrient stressors at 37% of the sites included in the program (Table 8), but 37% should be considered as a lower-bound estimate of the sites where sediments had an effect. Only the field form for the fall visual assessment requested information on non-nutrient stressors. Sedimentation is ubiquitous as a water pollutant in human-inhabited landscapes. Nutrient pollution is often associated with sedimentation, particularly phosphorus because it binds to soil particles.

**Table 8.** Sites where sediments were cited as a non-nutrient stressor by the regional biologists in comments on the visual-assessment forms.<sup>a</sup>

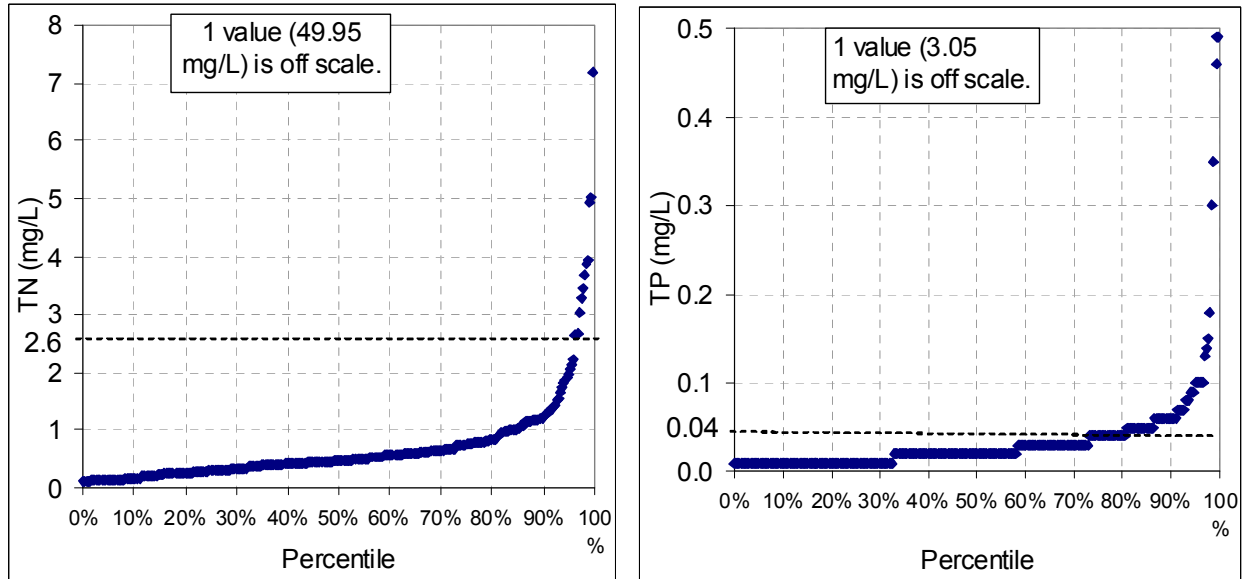
	<b>Sites where sediments cited as an influential non-nutrient stressor</b>	<b>Total sites</b>	<b>% of total sites where sediments were cited.</b>
Spring	5	29	17%
Fall	17	33	52%
Total	23	62	37%

<sup>a</sup> Non-nutrient stressor effects were addressed specifically by the visual-assessment data form during fall only. In spring, sediment effects were noted as general comments.

The pilot-program results indicate that the visual-assessment procedure has the potential for successful identification of some nutrient-impaired sites. Regional biologists were able to successfully identify some sites that were impaired (according to the SCI score) using the visual assessment process. Of the 62 sites included within the study, regional biologists identified seven has having a high probability of being nutrient impaired using the visual assessment; six of these sites were found to have SCI scores of less than 60, indicating impairment. However, regional biologists were not able to classify all sites identified as impaired according to the SCI score by using the visual assessment; of the 36 sites with SCI scores of less than 60, regional biologists visually identified 16% (six) as nutrient impaired.

The pilot-program results provide no indication that a visual assessment will be an adequate mechanism for assessing monitoring sites as “not impaired by nutrients.” Of the 31 sites identified by regional biologists through the visual assessment as having a low probability of being nutrient impaired, 15 were found to have SCI scores of <60, indicating biotic impairment (see Table 5). It may be that the biologists’ success in identifying sites not impaired by nutrients was actually greater than these figures indicate, but these results provide no basis for determining whether impaired sites were primarily affected by nutrients or by non-nutrient stressors.

The pilot program proved to be inadequate as a mechanism for identifying screening values or critical values. Possibly because of the widespread presence of non-nutrient stressor effects (including sediments), no potential screening values were evident. Some impaired sites (SCI<60) had relatively low nutrient concentrations. From a scientific standpoint, the most robust critical values would appear to be TN and TP, since allocation of water-quality N among the TKN and oxidized N forms in Virginia is both seasonally and regionally dependent (Zipper and Holtzman, unpublished). At the upper end of the concentration ranges, nutrient thresholds with a potential to serve as a critical variable were evident (2.6 mg/L TN, 0.4 mg/L TP) (Table 7). However, the TN threshold is very high, relative to the distribution of TN concentrations in Virginia streams and thus would provide little benefit if implemented as a critical value (Figure 12). At first glance, the combination of water-quality data with benthic-algae measurements appears to offer potential; however, benthic-algal biomass is not measured routinely at Virginia DEQ ambient-monitoring sites.



**Figure 12.** Distributions of TN and TP concentrations at Virginia DEQ probabilistic-monitoring sites in the Mountain and Piedmont regions of Virginia where the freshwater nutrient criteria for rivers and streams that are the focus of this report potentially could be applied. The potential critical values suggested by these results are 2.6 mg/L TN and 0.04 mg/L TP.

## Conclusions

Using the visual-assessment procedures, regional biologists were able to successfully identify a subset of sites determined to be impaired ( $SCI < 60$ ). However, efforts to visually identify non-impaired sites were not as successful; a number of the sites identified in the visual assessment as not impaired by nutrients had SCI scores of less than 60, indicating impairment of the benthic-macroinvertebrate community. Although it is possible that many or most of these non-visually evident, but nonetheless, impaired sites were impaired by non-nutrient stressors, the study design did not allow discrimination of impairment sources. Based on this result, we conclude that identification of nutrient-impaired sites has a potential for successful application within a nutrient-criteria program that incorporates a screening-value approach. However, these results do not support the AAC recommendation that a visual-assessment approach be applied to assess sites as non-impaired by nutrients.

Results of the pilot program do not appear as a useful means for identifying nutrient concentrations that can act as critical and screening values. Possibly because non-nutrient stressor effects were evident at a number of the sites selected for study, no potential screening values were evident from these results. Although potential critical values were evident, those suggested by these results are high, relative to the distribution of nutrient concentrations that occur in Virginia streams, especially for TN. A more useful approach in the development of potential critical and screening values would be to analyze water-monitoring data sets that are more representative of the conditions of freshwater rivers and streams in Virginia's Mountain and Piedmont regions. Such an approach could include the probabilistic-monitoring data and a

subset of the ambient-monitoring program sites for which biological-monitoring data are also available.

The pilot-program activity failed to provide the level of support for the screening-value approach to nutrient-criteria development that was anticipated, but the results provided no evidence to suggest that such a program would not be workable. The visual-assessment procedure offers potential to serve as a valid and valuable component of such a program. However, a more in-depth analysis of monitoring data from Virginia's Mountain and Piedmont regions will be required to define and evaluate potential critical values and screening values. Analysis is also needed to evaluate the effect of nutrient criteria developed from the screening-value approach on Virginia DEQ's monitoring resources.

### **III. Development and Application of Screening and Critical Values: Exploratory Analysis**

The AAC's recommended approach to nutrient-criteria development involves the use of critical values and screening values. Nutrient concentrations greater than the set critical values would be defined as "nutrient impaired," while those concentrations less than the screening values would be defined as "not nutrient impaired." Nutrient concentrations in between the critical values and screening values would be assessed using a visual assessment. If the visual-assessment results are not definitive, a benthic-macroinvertebrate assessment would be conducted (see Figure 1).

As a means of illustrating the screening-value approach, we provide the following example. Critical values and screening values in the example are advanced for the purpose of illustrating a possible method for deriving these values from existing data sets. They are intended to stimulate discussion and, as such, should not be considered as actual, suggested, or likely values.

#### **1. Deriving Illustrative Critical Values using a Variant of Paul and McDonald's Conditional-Probability Approach**

Using DEQ probabilistic-monitoring (ProbMon) data (2001-2006) for the Mountain and Piedmont regions of Virginia, TN, TP, NO<sub>3</sub>-N, and TKN were plotted using a [Prob<sub>SCI<60</sub>: X>X<sub>0</sub>] framework derived from Paul and McDonald (2005). This approach is based on the increasing probability that SCI will be <60 as the nutrient concentration increases. For any given concentration, the probability of impairment at that and higher concentrations is calculated as the ratio of impaired sites to total sites within the range of concentrations extending from the given concentration to the maximum. In the graphics that follow, the probability functions, represented as "Prob SCI<60," are overlaid on plots of SCI vs. TN in Figure 13a and SCI vs. TP in Figure 13b.

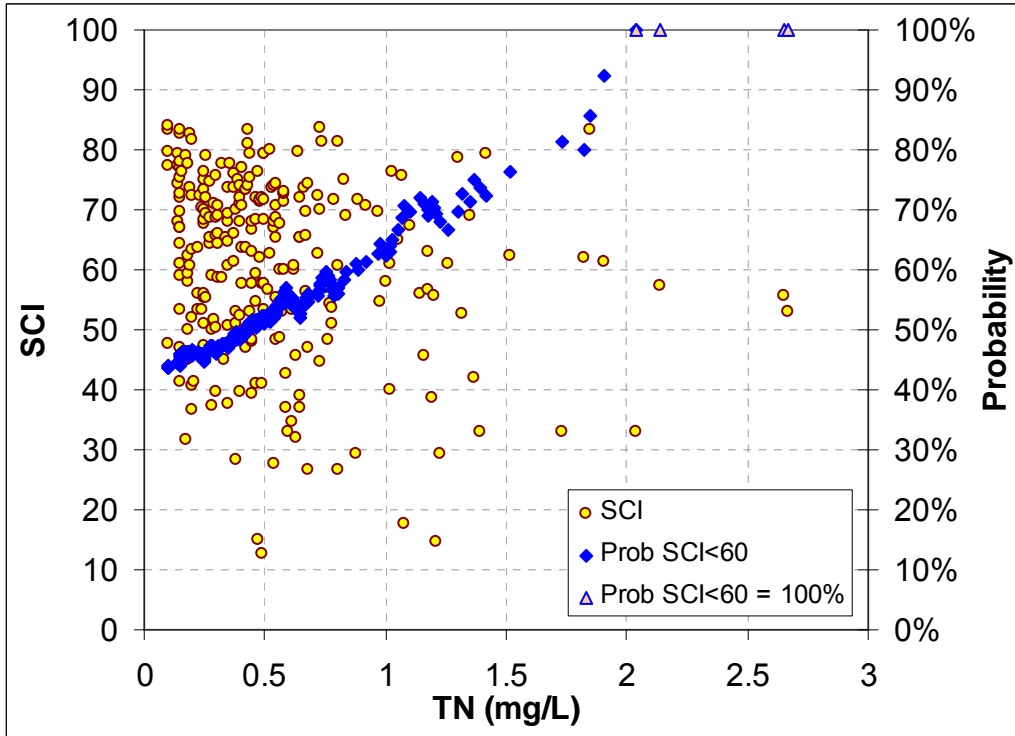
Unlike Paul and McDonald, we included only the threshold concentration for P<sub>SCI<60</sub>=100% (i.e., the lowest concentration at which Prob<sub>SCI<60</sub>=100%) in the data points used to draw a probability trend line (not represented in the figure). Our reasoning is that the nutrient concentrations above the 100% threshold should not influence the general form of a probability

function that is intended to represent biological condition. Furthermore, our goal is to derive critical values, not numeric criteria.

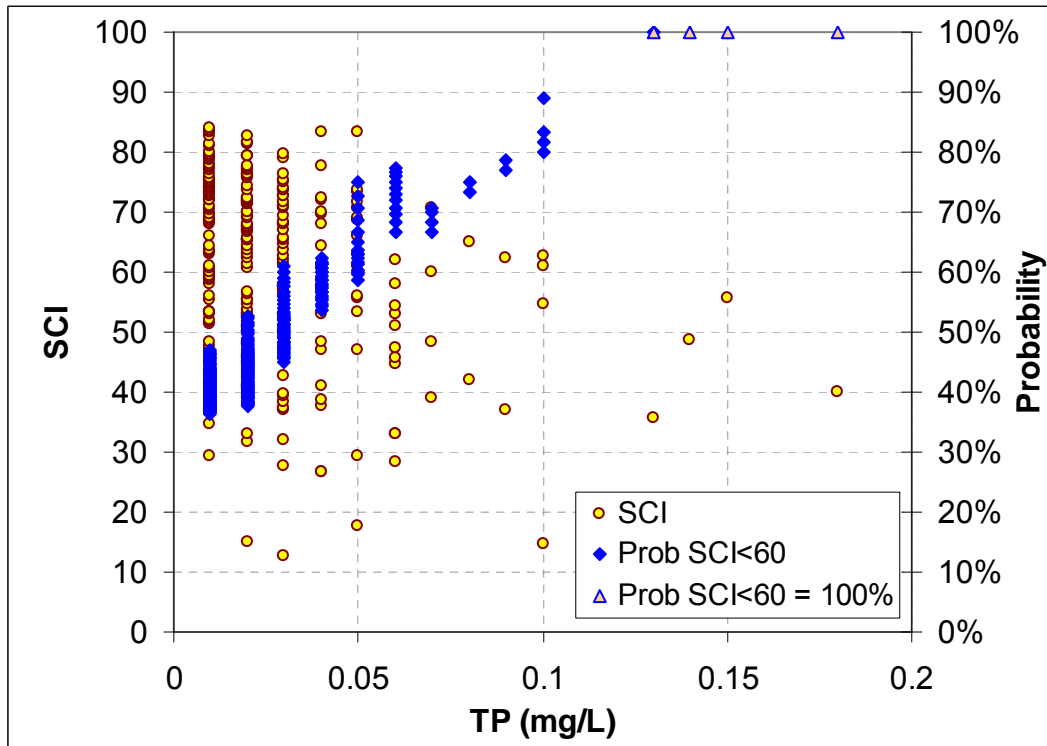
Plotting a line through the “Prob SCI<60” data points on the TN and TP charts (and including only the lowest concentration for which  $\text{Prob}_{\text{SCI}<60} = 100\%$ ) yielded functions that were used to estimate the illustrative critical values (CVs). For this example and for the purpose of discussion, we selected the 90%-probability TN and TP levels as illustrative critical values. The 90%-probability level was selected considering the overall goals of the AAC approach, which seeks to optimize the trade-off between assessment errors and DEQ resource expenditures for conducting benthic-macroinvertebrate assessments. It would also be possible to select CVs at higher or lower probability levels. Table 9 lists the CVs obtained by this method for TN and TP concentrations that indicate a 90% probability of SCI<60.

It would also be possible to derive comparable values for TKN and  $\text{NO}_3\text{-N}$ . We have not done so for two reasons (a) the illustrative CV that results from a trial application of that operation for  $\text{NO}_3\text{-N}$  was greater than the comparable value for TN, and (b) prior investigations revealed that the distribution of TN between TKN and oxidized forms is seasonally and regionally influenced.

The illustrative CVs in Table 9 were applied independently, i.e., if TN or TP exceeded the corresponding CV, the site was defined as “nutrient impaired.” Applying the illustrative CVs to the ProbMon data set revealed that 12 of 15 sites (or 80%) with TN concentrations above the critical value ( $> 1.8 \text{ mg/L}$ ), assessed as nutrient impaired, were also determined to be impaired according to the SCI score (Table 10). Eight sites were identified as impaired for having TP concentrations above the critical value ( $> 0.1 \text{ mg/L}$ ), and all eight sites (100%) were also considered impaired based on the SCI. The combined application of the two CVs yielded an 81% (13 of 16) correct assessment level in comparison to the SCI score. These assessment levels are less than the targeted 90% because the illustrative CV’s were derived from trend lines, not the individual data points. If the two illustrative CVs had been applied in combination (i.e., if an impairment assessment were to require that both conditions be satisfied), eight monitoring locations would have been assessed as impaired. This example is provided for discussion purposes, recognizing that a superior test would have been to apply the illustrative CVs to an independent data set.



**Figure 13a.** SCI vs. TN (left axis) and  $\text{Prob}_{\text{SCI}<60}$  for TN (right axis) plots based on DEQ probabilistic monitoring data, Mountain and Piedmont regions only, 2001-2006.



**Figure 13b.** SCI vs. TP (left axis) and  $\text{Prob}_{\text{SCI}<60}$  for TP (right axis) plots based on DEQ probabilistic-monitoring data, Mountain and Piedmont regions only, 2001-2006.

**Table 9.** Illustrative critical values (CVs) for TN and TP concentrations. These concentrations are for illustrative purposes only and should not be considered as actual, suggested, or likely critical values. TN and TP CVs would be applied independently, i.e., if either TN or TP exceeds the threshold, the site would be assessed as impaired.

<b>Nutrient variable</b>	<b>Critical Value: Concentration where <math>P_{SCI &lt; 60} \geq 90\%</math></b>	<b>Illustrative CV, as Percentile of Probabilistic-monitoring TN Distribution</b>
TN (mg/L)	1.8	94th
TP (mg/L)	0.1	94th

**Table 10.** Results of illustrative critical-value application to probabilistic-monitoring data set.

	<b>Assessment is Correct Based on SCI</b>	<b>Assessment is Incorrect Based on SCI</b>	<b>Sites Below CV so Not Assessed</b>
<b>TN &gt; 1.8</b>	12	3	252
<b>TP &gt; 0.1</b>	8	0	259
<b>Both</b>	13	3	251

## 2. Derive Illustrative Screening Values from Reference Conditions

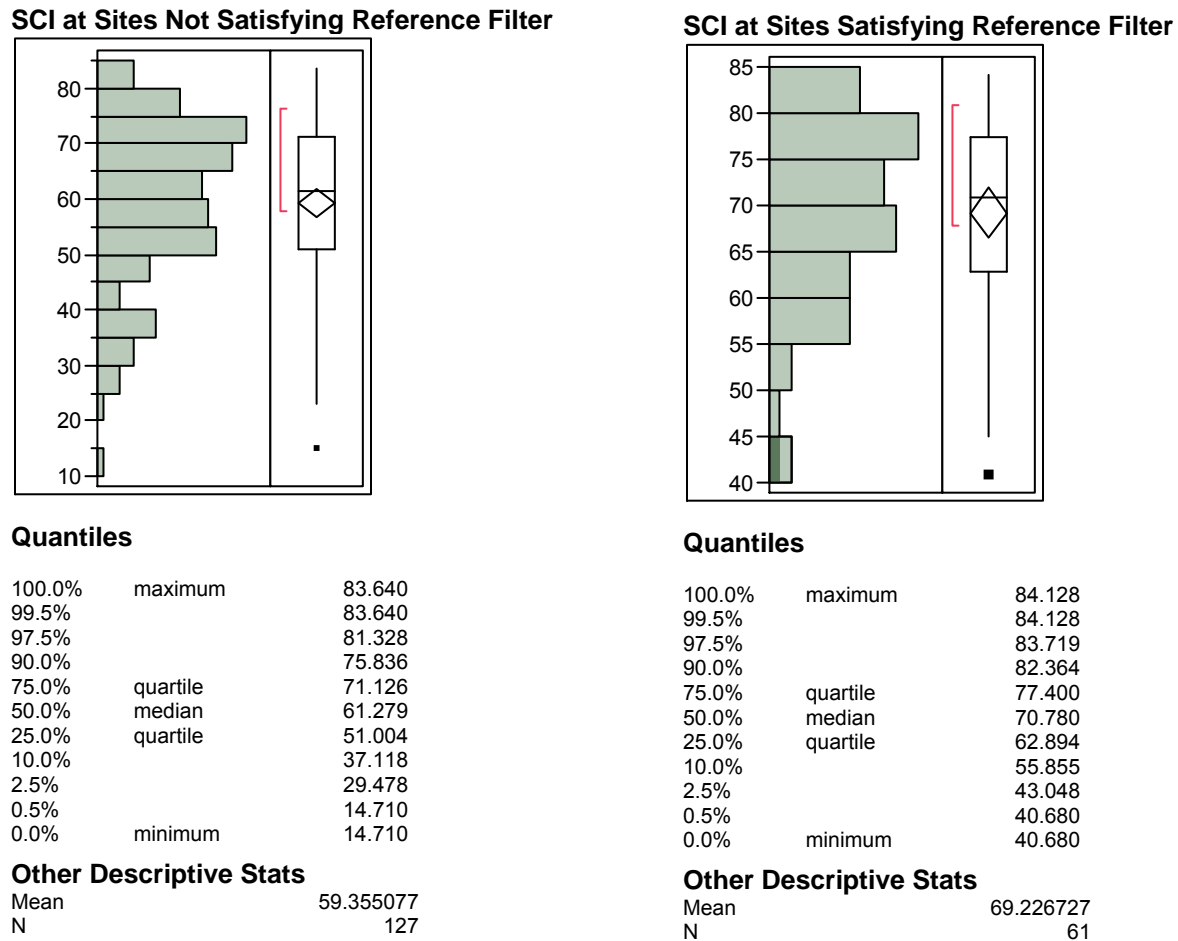
DEQ has used a set of criteria to define reference conditions in various studies. For example, the criteria were used to establish reference conditions in the studies conducted to develop the SCI (Tetra Tech, Inc. 2003), which was approved by EPA. The approach described here uses reference conditions to identify screening values for use in developing nutrient criteria. The following (Table 11) are reference conditions used in the SCI validation study (Virginia DEQ 2006), which were more restrictive than those used by Burton and Gerritsen in the original SCI development.

**Table 11.** Reference filters applied by DEQ for Mountain and Piedmont regions (Virginia DEQ 2006).

	<b>Mountain</b>	<b>Piedmont</b>
% Urban	< 5%	< 5%
Total Nitrogen	< 1.5 mg/L	< 1.5 mg/L
Total Phosphorus	< 0.05 mg/L	< 0.05 mg/L
Specific Conductance	< 250 $\mu$ S/cm	< 250 $\mu$ S/cm
Dissolved Oxygen	> 6 mg/L	> 6 mg/L
pH	> 6 and < 9	> 6 and < 9
Channel Alteration	> 11	> 11
Embeddedness	> 11	
Epifaunal Substrate/Cover	> 11	> 11
Riparian Vegetative Zone	> 11	> 11
Total Habitat Score	> 140	> 140



The Virginia DEQ (2006) reference conditions include TN and TP values. We tested the adequacy of those TN and TP reference-condition values as potential screening values by applying the full set of reference-filter conditions to the probabilistic-monitoring data set (2001-2006, Mountain and Piedmont regions only). Results are listed in Figure 14.



**Figure 14.** Results of applying the reference filters (Table 11) to DEQ probabilistic-monitoring data set (2001-2006, Mountain and Piedmont regions only).

The 10<sup>th</sup> percentile of the SCI distribution at sites satisfying the reference-filter conditions is SCI = 56. If DEQ and the AAC were to decide that screening values (SVs) would be developed with the intent of limiting false negative (Type II) assessment errors to 10 percent or less, the result of this exercise would have been more satisfactory if the 10<sup>th</sup> percentile for the Reference Sites were SCI=60 or above. However, considering that both non-nutrient and nutrient stressors are likely responsible for the observed SCI<60 impairments at the reference-filter sites, we continued the example.

We applied the highest observed TN and TP concentrations derived from the population of sites that satisfied the reference filter as illustrative screening values. The highest observed TN

value within the reference data set was 0.80 mg/L. This value is well below the 1.49 mg/L reference-filter maximum. The highest observed TP value at the reference-filtered sites was 0.04 mg/L, which is the highest possible concentration than can satisfy the reference filter at the analytical precision of these data. Therefore, we describe the screening values in this illustrative example as TN<0.81 mg/L and TP<0.05 mg/L.

Applying these screening values to the probabilistic-monitoring data yields the results in Table 12. These results should be considered while recognizing that both reference and non-reference sites are included within the 267 sites, and that the observed benthic-macroinvertebrate impairments are by both nutrient and non-nutrient stressors.

**Table 12.** Numbers of sites affected by illustrative screening values (SV). The extent to which impairments (SCI<60) occur when TN and TP are below the screening values cannot be used to determine the adequacy of the screening values because the SCI<60 values can occur due to the effects of non-nutrient stressors.

<b>Illustrative Screening Value</b>	<b>SCI&gt;60</b>	<b>SCI&lt;60</b>	<b>Total</b>	<b>Illustrative SV as Percentile of ProbMon TN/TP Distributions</b>
TN<0.81 mg/L	130	89	219	76 <sup>th</sup>
TP<0.05 mg/L	133	87	220	77 <sup>th</sup>
TN<0.81 mg/L and TP<0.05 mg/L	121	78	199	
Total Sites	150	117	267	

### **Hypothetical Applications of the AAC Recommended Approach**

The illustrative CVs and SVs were applied to the probabilistic-monitoring data set and pilot-program data set. Sites were hypothetically considered “not impaired by nutrients” when the TN concentration was below 0.81 mg/L and the TP concentration was below 0.05 mg/L. Sites were listed as “impaired by nutrients” if either the TN concentration was above 1.8 mg/L or TP concentration was above 0.1 mg/L.

When applied to the probabilistic-monitoring data set, the illustrative CVs and SVs were sufficient to assess 81% of the observations (Table 13). The remaining 19% of observations were not classified. Extending this result to a real-world context and assuming the AAC recommended procedure were in place, this would mean that 19% of the total number of sites would need to be assessed visually by regional biologists. Additionally, a percentage of the visually assessed sites would need to be further evaluated using the benthic-macroinvertebrate community.

**Table 13.** Results of hypothetical combined application of illustrative critical values (CVs) and screening values (SVs) to ProbMon (2001-2006), Mountain and Piedmont regions.

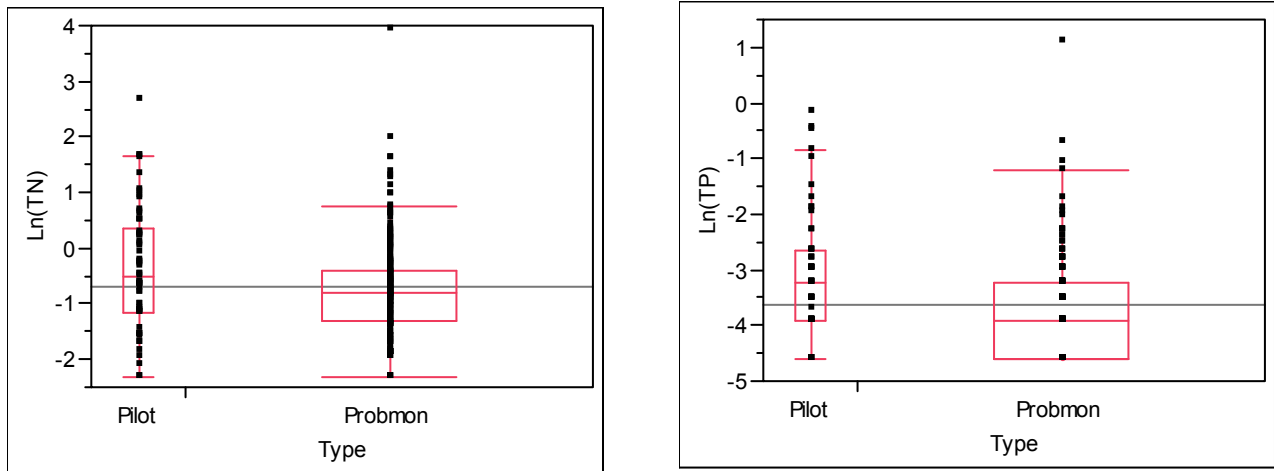
	<b>Number of sites</b>	<b>% of total sites</b>
All sites	267	100%
“Assessed” by SV	199	75%
“Assessed” by CV	16	6%
“Assessed” by either SV or CV	215	81%
Not “Assessed”	52	19%

It is possible to apply the illustrative SVs and CVs to the pilot-program data to generate a second hypothetical example. For this data set, if the status of the site was not determined by the nutrient concentrations, it was evaluated based on the results of the visual assessment. The results were generated assuming a visual assessment that indicated “high probability of nutrient impairment” would result in a designation of “assessed as nutrient impaired.” The results of the visual assessment were only used to determine if a site would be considered “impaired by nutrients” (The visual assessment was not used to define a site as “not impaired by nutrients.”). All sites in the pilot-program that were not assessed using the SV, CV, or visual assessment would need a benthic-macroinvertebrate assessment. Results of this hypothetical application are summarized in Table 14; station-specific results are reviewed in Appendix C.

**Table 14.** Outcome of hypothetical application of illustrative CVs and SVs, in combination with regional biologists’ visual assessments, to the pilot-program data set.

<b>Outcome</b>	<b>SCI&gt;60</b>	<b>SCI&lt;60</b>	<b>Total</b>
	<b>--- Number of sites ---</b>		
All sites	26	36	62
“Assessed” by SV (Not Impaired by Nutrients)	15	13	28
“Assessed” by CV (Impaired by Nutrients)	6	10	16
“Assessed” Visually as Impaired by Nutrients	-	3	3
Not “Assessed”– Benthic-Macroinvertebrate Assessment Needed	5	10	15

In evaluating the results, readers should consider the limitations of the pilot-program data set as a basis for inferring potential results if these procedures were to be applied more generally. Monitoring locations used in the pilot program were characterized by higher nutrient concentrations than those in the probabilistic-monitoring data set (Figure 15). This high-nutrient-level characteristic was by design because the procedure to select stations for the pilot program was intended to assure that high-nutrient locations (of primary interest in nutrient criteria development) were adequately represented. In contrast, the probabilistic-monitoring locations are selected with the intention of representing the population of Virginia streams. Non-parametric comparisons of the pilot-program and probabilistic-monitoring data sets reveal that both nutrient distributions differ significantly ( $p < 0.01$  for TN,  $p < 0.0001$  for TP) (Figure 15).



**Figure 15.** Distributions of Ln-transformed TN (left) and TP (right) concentrations for the pilot-program and probabilistic-monitoring data sets.

#### IV. Analysis of Nutrient Concentration Stability in Time

An essential question in evaluating how nutrients might be applied by the Virginia DEQ in water-quality assessments concerns the stability in time of measured-nutrient concentrations. Whereas both the pilot-program and probabilistic-monitoring data analyses were conducted using the nutrient concentrations of one water sample per site, Virginia DEQ would be applying nutrient criteria to assess water quality using data containing multiple observations collected over extended periods of time by its ambient water-monitoring program. Thus, it is reasonable to ask how conclusions derived from the pilot-program and probabilistic-monitoring data analyses might be applied within a nutrient-criteria program that is implemented as an assessment of the ambient-monitoring data.

The analysis of the pilot-program data was conducted for the purpose of aiding the process to develop nutrient criteria. Here, we conduct an additional analysis to investigate the effect of using values derived from the pilot program as opposed to values derived from monitoring data collected over prior-time periods. Understanding this relationship is important because monitoring data collected over prior-time periods will likely be used to determine a stream's impairment or non-impairment by nutrients once nutrient criteria are fully developed and implemented.

##### Methods

The ambient-monitoring database was queried by DEQ's water-monitoring coordinator to extract water-monitoring observations for each of the pilot-program sites over a three-year period extending from 1/1/2006 through 12/31/2008. For each location, the coordinator isolated water-monitoring observations occurring within 183 days, 365 days, and 730 days prior to the sampling period of the pilot program. Median TN and TP were calculated for each of these periods for those locations where >2 observations (i.e., 3 or more) were in the database for the 183-day prior

period, >4 observations were available for the 365-day prior period, and >6 observations were available for the 730-day prior period.

The TN and TP prior-period medians were analyzed for correspondence with observed values obtained from the pilot program. For each prior-period median, the difference from the corresponding pilot-program value was calculated, and the distribution of those differences was tested for equivalence to 0.0 using the non-parametric, Wilcoxon Rank Sum procedure. Ratios of TN and TP pilot-program values to period medians were calculated, and the distribution of those ratios was tested for difference from 1.0 using the Wilcoxon Rank Sum procedure. Log-transformed, prior-period medians were regressed against log-transformed, pilot-program values.

Relationships of prior-period medians to benthic-algae metrics and the SCI were compared to corresponding relationships for the pilot-program observations. Log-transformed TN and TP concentrations – as measured by the pilot program, and prior 183-day, 365-day, and 730-day medians – were regressed against four benthic-algae measures and the SCI. The four algae measures included two algal indices (Algal Index 1234 and Algal Index 13610), benthic chlorophyll-a (Chl-a), and ash-free dry mass (AFDM). The algal indices were constructed for each site by summing the algal color/form combinations that biologists described as being present in the visual analysis procedure, weighting each by visually estimated stream bottom coverage on a scale of 1 – 2 – 3 – 4 to construct the “Algal Index 1234,” and using a weighting of 1 – 3 – 6 – 10 to construct “Algal Index 13610.” Medians were calculated only when the number of prior-period observations exceeded a minimum threshold (> 2 for 183 days, > 4 for 365 days, and > 6 for 730 days) as described above. The monitoring locations included in this analysis were defined separately for TN and TP, and only those locations with sufficient prior-period observations to enable calculation of at least one prior-period median were used.

Critical-value thresholds were derived using the prior-period medians and compared to those derived using the pilot-program observations.

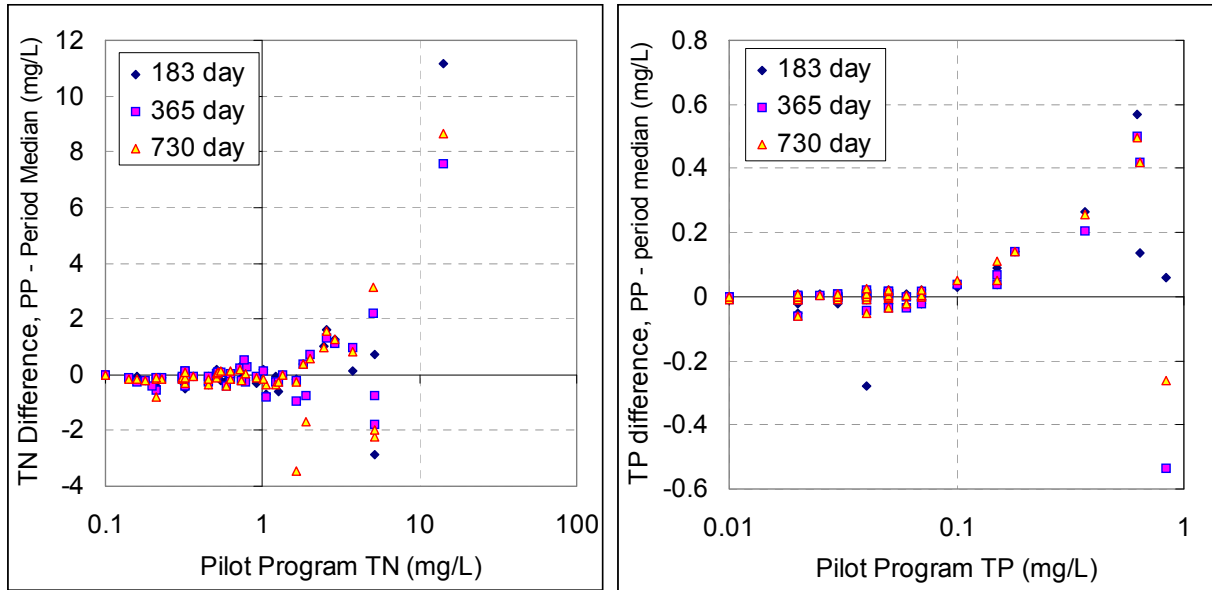
## **Results**

### *Pilot-Program Results vs. Period Medians*

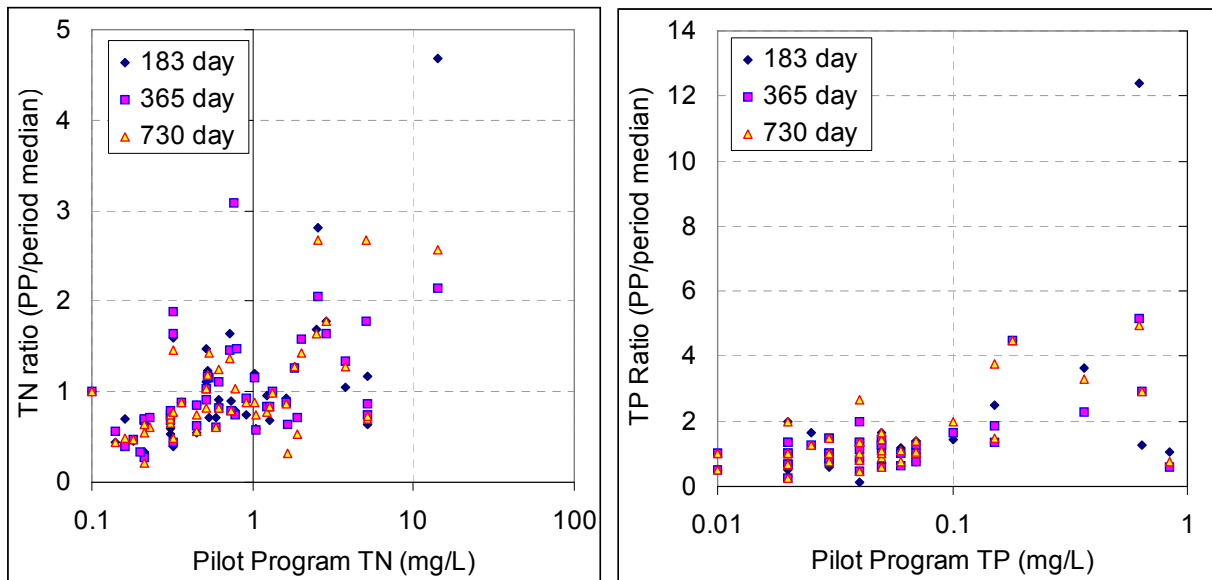
The non-parametric analyses found no pilot-program measured concentrations minus period-median concentrations to be significantly different from zero. Likewise, no ratios of the pilot-program concentrations to the period-median concentrations were significantly different from 1.0. Both measures, however, exhibited substantial variability around measures of central tendency.

As expected, the magnitude of TN and TP differences (pilot-program concentration minus period-median concentration) increased with concentration (Figure 16); larger magnitude differences were mostly positive for both TN and TP. Thus, the highest concentrations observed during the pilot program tended to be unusually high values, suggesting that concentration deviation from the median is primarily on the positive side at such sites. Concentration ratios also increased with pilot-program concentration for both TN and TP, and for all period medians ( $p < 0.0001$  for TN;  $p < 0.05$  for TP) (Figure 17).

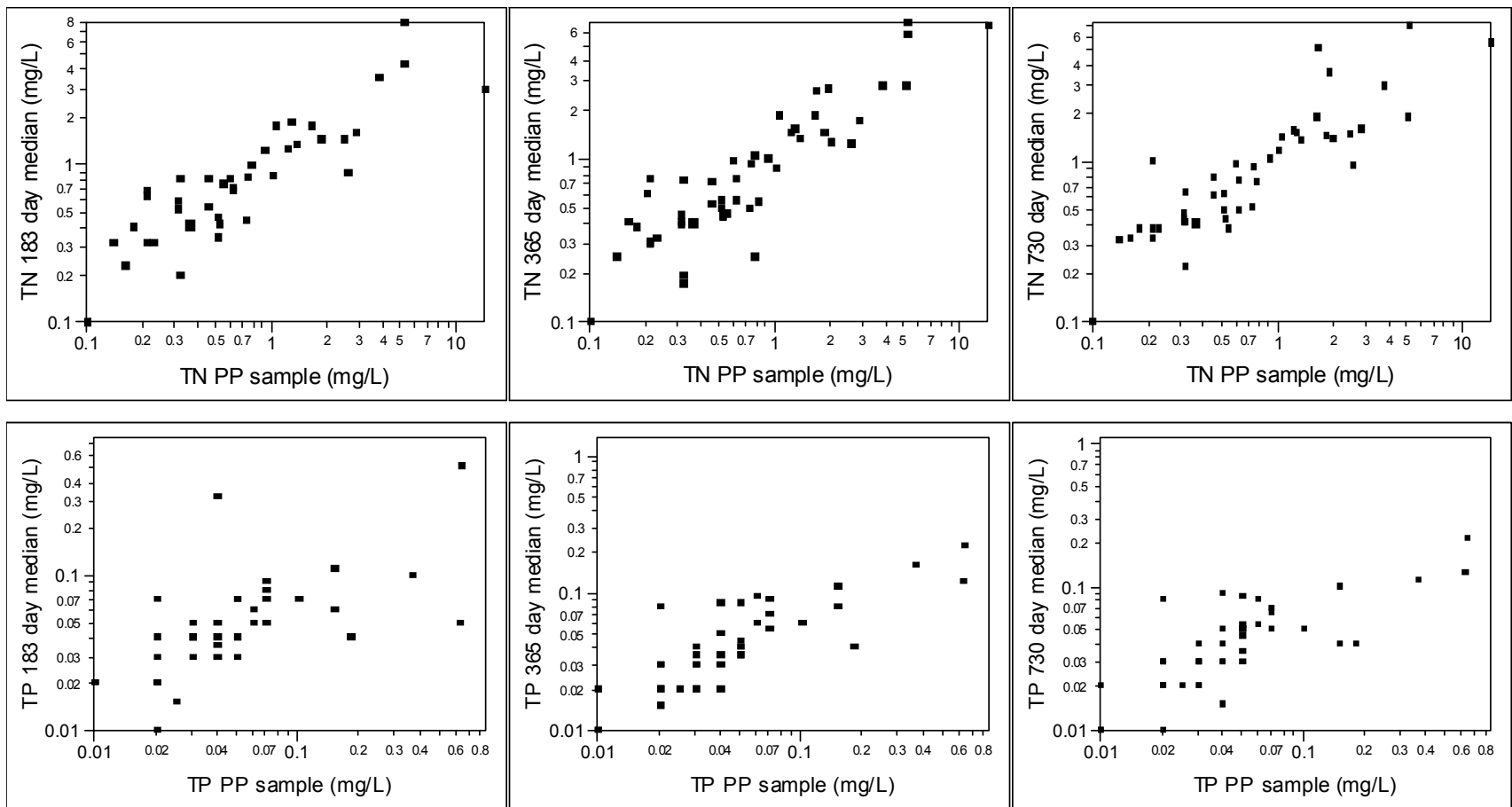
Both measures can be interpreted to indicate that nutrient concentrations in streams with low concentrations tend to remain stable, whereas high-concentration streams exhibit greater variability on both a concentration-magnitude and on a proportionate basis. However, the pilot-program values and all period medians were highly correlated for TN and TP (Figure 18).



**Figure 16.** Concentration differences (pilot-program concentrations minus period-median concentration) as a function of pilot-program concentrations.



**Figure 17.** Concentration ratios (pilot-program concentration / period-median concentration) as a function of pilot-program concentration.



**Figure 18.** The 183-day, 365-day, and 730-day median TN and TP concentrations as a function of pilot-program (PP) concentrations. All relationships are statistically significant ( $p < 0.0001$ ).

*Relationships with Benthic- Algae and Benthic- Macroinvertebrate Measures*

Results of the comparative analysis of nutrient concentrations against benthic-algae and benthic-macroinvertebrate measures are listed in Table 15. In general, use of the prior-period medians resulted in tighter regressions (higher R<sup>2</sup> values, lower p values) for TN relative to pilot-program values. Longer period medians (e.g., 730-day median) were responsible for the highest R<sup>2</sup> values. The degree of improvement, however, was not sufficient to alter the basic conclusions derived from the pilot-program analysis. For the TP analysis, no systematic change in outcomes was apparent as due to use of the prior-period medians.

**Table 15.** Results of comparative linear-regression analyses for pilot-program TN and TP concentrations and prior-period median TN and TP concentrations against benthic-algae indices and the Stream Condition Index (SCI). All nutrient concentrations were Ln-transformed.

		Algal Index (1234)	Algal Index (13610)	Ln (Chla)	Ln (AFDM)	SCI
TN	n	50	50	50	50	50
Pilot	R <sup>2</sup>	0.014	0.015	0.141	0.071	0.144
Program	p	0.66	0.71	0.0072	0.061	0.0066
TN 183	n	40	40	40	40	40
Day	R <sup>2</sup>	0.020	0.009	0.157	0.116	0.295
Median	p	0.6523	0.5541	0.011	0.018	0.0003
TN 365	n	49	49	49	49	49
Day	R <sup>2</sup>	0.019	0.016	0.171	0.107	0.144
Median	p	0.3335	0.38	0.0032	0.0212	0.0071
TN 730	n	47	47	47	47	47
Day	R <sup>2</sup>	0.023	0.023	0.226	0.133	0.210
Median	p	0.031	0.031	0.0007	0.011	0.0012
TP	n	47	47	47	47	47
Pilot	R <sup>2</sup>	0.0000	0.005	0.017	0.0002	0.008
Program	p	0.963	0.615	0.394	0.946	0.544
TP 183	n	41	41	41	41	41
Day	R <sup>2</sup>	0.033	0.003	0.003	0.0000	0.034
Median	p	0.247	0.733	0.733	0.956	0.247
TP 365	n	45	45	45	45	45
Day	R <sup>2</sup>	0.017	0.032	0.012	0.0000	0.018
Median	p	0.399	0.237	0.477	0.998	0.383
TP 730	n	47	47	47	47	47
Day	R <sup>2</sup>	0.019	0.043	0.002	0.0000	0.021
Median	p	0.35	0.056	0.797	0.949	0.331



*Potential Screening-Value and Critical-Value Thresholds*

As with the analysis of the pilot-program data set, the analysis of the prior-period data set offered little in the way of useful thresholds. Potential screening values derived from the prior-period medians are quite low (0.1 mg/L for the three TN prior-period medians, and 0.01 mg/L for the three TP prior-period medians). Potential critical values derived from the prior-period medians tend to be at very high levels relative to the distribution of TN and TP values from the 2001-2006 probabilistic-monitoring locations (Table 16).

**Table 16.** Comparison of potential critical values<sup>a</sup> (CV) for TN and TP derived from prior-period medians to those derived from the pilot-program observations, and corresponding percentiles within DEQ’s probabilistic-monitoring observations (2001-2006, Virginia’s Mountain and Piedmont regions).

	CV Concentrations (mg/L)		ProbMon Percentile	
	TN	TP	TN	TP
Pilot-program observations	2.6	0.4	96	99
Prior-period medians:				
183-day medians	1.8	0.2	94	98
365-day medians	2.75	0.2	97	98
730-day medians	4	0.12	99	97

<sup>a</sup> Potential critical values are set at approximate midpoint of range between the highest concentration at a non-impaired site and the next-highest concentration.

**Conclusions**

This analysis should be considered as an initial effort to address questions regarding the operational aspects of applying the screening-value approach within DEQ’s water-quality monitoring and assessment framework.

Nutrient concentrations at any given location in a stream are variable in time. The TN and TP concentrations of water samples collected during the pilot program were good estimates, in a statistical sense and on average, of median values for water-monitoring samples collected during 183 days, 365 days, and 730 days prior to the pilot program sampling event (prior-period medians). When comparing the measured concentrations from the pilot program to the concentration medians of the prior-period data, the variability increased in both measured (mg/L) and relative terms at the higher concentrations. Substitution of prior-period-median values for pilot-program-measured values affected results of several analyses, but the differences were minor and inconsequential to the conclusions drawn from the pilot-program data analysis.

One would expect that the additional information in the historical record would provide better results than a single nutrient-concentration measurement obtained during the pilot program. However, questions remain about how the historical data should be analyzed in order to provide an improved result.

Reducing the set of available observations to the median might not be the best way to utilize the historical record. Diminution of the SCI would be caused by a history of high-nutrient concentrations over a period of time, i.e., by an accumulation of high-concentration events over a period of time. If such events were to occur frequently, although less than 50% of the time, they would not be reflected by a median value. Thus, an alternative approach would be to use a mean or a weighted, moving average of the historical record.

## **V. Summary and Future Plans**

The AAC has recommended that Virginia DEQ apply a screening value approach for developing nutrient criteria (Figure 1). The proposed approach employs N and P screening values (nutrient-concentration thresholds below which monitoring sites are determined to be unimpaired by nutrients) and critical values (nutrient-concentration thresholds above which sites are considered impaired by nutrients). Streams with nutrient concentrations that do not allow assessment using the screening or critical values would be visually assessed. If the visual assessment is inconclusive, a benthic-macroinvertebrate assessment would be employed to assess the stream.

During calendar year 2008, Virginia DEQ biologists executed the pilot program, enhanced monitoring activities to test the efficacy of the screening-value approach. Program results did not suggest screening values. In addition, the critical values suggested by the program results would be sufficient to assess only a very small number of monitoring sites because they are at the extreme upper end of the distribution of nutrient concentrations that occur in Virginia streams. Using a visual procedure, regional biologists were able to identify a subset of sites as impaired by nutrients, but they could not apply the visual-assessment method to prove that a stream was not impaired by nutrients.

An exploratory analysis was conducted in an attempt to develop an alternative procedure for identifying screening and critical values. In developing this procedure, we recognized the trade-offs embodied by the screening-value approach and sought to limit assessment errors to 10% or less. We applied “reference conditions” used by DEQ for other analyses (including the development of the Stream Condition Index) to derive screening values. The 2001-2006 probabilistic-monitoring data were used for the exploratory analysis, and its results are considered for illustrative purposes only. The results of the analysis indicate that the technique employed shows promise as a potential mechanism for deriving screening values. However, as with the pilot program, the critical values suggested would be sufficient to assess only a very small number of sites because they are at the extreme upper end of the distribution of nutrient concentrations found in Virginia streams.

The analyses described above utilized data from nutrient concentrations measured from single-point-in-time water samples to characterize each monitoring site’s nutrient status. The Virginia DEQ, however, is expected to assess water-quality nutrient data collected over extended periods of time. A third analysis, therefore, was conducted for the purpose of exploring the stability in time of TN and TP concentrations in Virginia streams. The results indicate that sites

with high concentrations of nutrients had more variability with regard to nutrient concentrations than did sites with low concentrations of nutrients.

Several additional activities are planned for fiscal year 2010 (July 2009-June 2010). These activities include an analysis of the 2001-2008 probabilistic-monitoring data using a more rigorous application of the exploratory data analysis procedure. The planned analysis, which uses an extended data set that includes a larger number of monitoring sites with benthic-algae measurements, is considered desirable and necessary to derive more robust results.

Also during FY2010, the AAC will continue to explore mechanisms for deriving critical values. Downstream-loading issues will be considered in this activity, given the fact that all of the coastal waters that receive Virginia's surface-water streams (Chesapeake Bay, Pamlico Sound, Gulf of Mexico) suffer from nutrient overenrichment. Furthermore, the distribution of nutrient concentrations in Virginia streams is upwardly skewed (see Figure 12), suggesting that a small number of Virginia's surface water streams with excessively high-nutrient concentrations are responsible for a disproportionate share of the nutrients carried by surface waters into the coastal water bodies.

An additional activity planned for FY2010 is an analysis of DEQ's ambient-monitoring data to determine how a screening-value approach would be expected to affect DEQ resource allocations. This analysis would consider regional biologists' time as a critical resource that must be applied to implement a screening-value approach successfully.

Also during FY2010, regional biologists have stated an intent to continue developing the visual-assessment procedure that was employed on a trial and developmental basis during the pilot-program activity. The AAC is willing to continue working with the DEQ's biological-monitoring staff in this activity, as per DEQ and staff preferences.

## **VI. Acknowledgements**

Roger Stewart, Jason Hill, and David Whitehurst of Virginia DEQ prepared data sets used in these analyses. DEQ's regional biologists assisted in developing and conducting the pilot program, especially with the visual-assessment component. David Whitehurst, Alex Barron, and Amy Genung provided oversight and coordination of AAC activities.

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## VII. Disclaimer

The contents of this document are solely the responsibility of the authors and do not necessarily represent the official views of the U.S. EPA Region 3, USGS, Virginia DEQ, or Virginia Water Resources Research Center at Virginia Tech.

## VIII. References

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# Appendix A: Nutrient Criteria Visual Assessment Field Form (Spring)

Station ID: \_\_\_\_\_ Field Crew: \_\_\_\_\_  
 Stream Name: \_\_\_\_\_ Ecoregion: \_\_\_\_\_  
 DEQ Region: \_\_\_\_\_ **TP Category** \_\_\_\_\_  
 Location: \_\_\_\_\_ **TN Category** \_\_\_\_\_

DATE \_\_\_\_\_

Start Time \_\_\_\_\_ Finish Time \_\_\_\_\_

LATITUDE  
(Decimal degrees) \_\_\_\_\_

LONGITUDE  
(Decimal degrees) \_\_\_\_\_

## Stream Physicochemical Measurements

TEMPERATURE: \_\_\_\_\_ °C CONDUCTIVITY: \_\_\_\_\_ μS/cm

DISSOLVED OXYGEN: \_\_\_\_\_ mg/L pH: \_\_\_\_\_

## Benthic Macroinvertebrate Collection

Method used (circle one) **Single habitat** **Multi-habitat**

Riffle quality (circle one) **Good** **Marginal** **Poor** **None**

Habitats sampled **Riffle** **Snags** **Banks** **Vegetation**  
 # jabs \_\_\_\_\_

## Algae Community

Algae community growth (% of stream bottom) **Categories; 1-10; 10-40; 40-70; >70**

Type of growth	bright green	dark green	brown	black	other
Film					
Thin mat					
Thick mat					
Filamentous					

## Vascular Plant Growth

Vascular plant growth (% of stream bottom) **Categories; 1-10; 10-40; 40-70; >70**

Submerged macrophytes	
Emergent macrophytes	
Other	

## Observations

Stream substrate type **Categories; 1-10; 10-40; 40-70; >70**  
**sand gravel cobble bedrock mud**

Estimated average stream width (Meters): \_\_\_\_\_

Estimated average stream depth (Meters): \_\_\_\_\_

Stream shading: (circle one)

**Categories; 1-10; 10-40; 40-70; >70**

Stream flow (circle one)

**Low      Normal      Above Normal**

Estimated stream velocity (Meters/sec): \_\_\_\_\_

Days since last potentially scouring rain: \_\_\_\_\_

Photo documentation taken? **YES / NO**

BPJ based on observations of algae and macrophyte biomass; probability of impairment to macroinvertebrate community (circle one)

**Low      Medium      High**

Provide a brief explanation for rating: \_\_\_\_\_

---

### **Watershed features**

#### Land Use

(Indicate the predominant surrounding land use with a "1". . If applicable, indicate a secondary land use with a "2".)

\_\_\_ Forest      \_\_\_ Commercial  
\_\_\_ Field/Pasture      \_\_\_ Industrial  
\_\_\_ Agricultural      \_\_\_ Residential  
\_\_\_ Livestock      \_\_\_ Other \_\_\_\_\_

#### Local Watershed Pollution (circle one)

No evidence      Some potential sources  
Obvious sources

#### Local Watershed Erosion (circle one)

None      Moderate  
**Low**      Heavy

# Appendix B: Nutrient Criteria Visual Assessment Field Form (Fall)

Station ID: \_\_\_\_\_ Field Crew: \_\_\_\_\_  
 Stream Name: \_\_\_\_\_ Ecoregion: \_\_\_\_\_  
 DEQ Region: \_\_\_\_\_ **TP Category** \_\_\_\_\_  
 Location: \_\_\_\_\_ **TN Category** \_\_\_\_\_

DATE \_\_\_\_\_

Start Time \_\_\_\_\_ Finish Time \_\_\_\_\_

LATITUDE  
(Decimal degrees) \_\_\_\_\_

LONGITUDE  
(Decimal degrees) \_\_\_\_\_

## Stream Physicochemical Measurements

TEMPERATURE: \_\_\_\_\_ °C CONDUCTIVITY: \_\_\_\_\_ μS/cm

DISSOLVED OXYGEN: \_\_\_\_\_ mg/L pH: \_\_\_\_\_

## Benthic Macroinvertebrate Collection

Method used (circle one) **Single habitat** **Multi-habitat**

Riffle quality (circle one) **Good** **Marginal** **Poor** **None**

Habitats sampled **Riffle** **Snags** **Banks** **Vegetation**  
 # jabs \_\_\_\_\_

## Algae Community and Vascular Plant Growth

Algae community growth (% of stream bottom) **Categories; 1-10; 10-40; 40-70; >70**

Type of growth	bright green	dark green	brown	black	other
Film					
Thin mat					
Thick mat					
Short Filamentous					
Tall Filamentous					

Vascular plant growth (% of stream bottom) **Categories; 1-10; 10-40; 40-70; >70**

Submerged macrophytes	
Emergent macrophytes	
Mosses	
Other	

Total stream bottom coverage by algae and vascular plant growth \_\_\_\_\_  
**(Categories; 1-10; 10-40; 40-70; >70)**





## Appendix C. Pilot Program Data

Hypothetical application to sites in the pilot program for illustrative screening and critical values within AAC recommended approach.

StationID	Sea- son	TN (mg/ L)	TP (mg/L)	BPJ: Prob Nutrient Impair- ment	BPJ: Prob Non- Nutrient Impair- ment	CV: TN>1.8	CV: TP>0.1	SV: TN<0.81 & TP<0.05	BPJ	Outcome	Stream Con- dition Index
6BPLU002.15	Spr	1.27	0.04	MEDIUM	-	-	-	-	-	BenMac Assess	40.94
2-PCT002.46	Spr	0.62	0.10	MEDIUM	-	-	-	-	-	BenMac Assess	42.12
1ANOG005.69	Spr	1.02	0.06	MEDIUM	-	-	-	-	-	BenMac Assess	46.81
1BSSF053.09	Spr	1.22	0.06	LOW	-	-	-	-	-	BenMac Assess	48.28
4ATKR000.69	Fal	1.34	0.05	MEDIUM	MEDIUM	-	-	-	-	BenMac Assess	50.34
6CMFH055.88	Fal	0.59	0.05	MEDIUM	MEDIUM	-	-	-	-	BenMac Assess	50.35
4ASEE003.16	Fal	0.21	0.07	MEDIUM	LOW	-	-	-	-	BenMac Assess	54.37
2-CNE000.96	Spr	1.11	0.05	LOW	-	-	-	-	-	BenMac Assess	55.31
6BIDN000.69	Fal	1.22	0.03	MEDIUM	LOW	-	-	-	-	BenMac Assess	59.81
5AGRV000.08	Spr	0.51	0.05	LOW	-	-	-	-	-	BenMac Assess	68.20
2-NOR000.20	Spr	0.34	0.10	LOW	-	-	-	-	-	BenMac Assess	68.62
3-MTN000.59	Fal	1.05	0.07	MEDIUM	MEDIUM	-	-	-	-	BenMac Assess	69.51
3-RAP006.53 (S1)	Fal	0.78	0.06	LOW	MEDIUM	-	-	-	-	BenMac Assess	70.99
2-HAT000.14	Fal	0.14	0.05	LOW	MEDIUM	-	-	-	-	BenMac Assess	71.86
1ASYL000.02	Spr	3.77	0.02	MEDIUM	-	imp	-	-	-	Impaired	23.01
1AOPE036.13	Spr	5.13	0.84	LOW	-	imp	Imp	-	-	Impaired	29.78
1BMDD005.81	Spr	5.13	0.02	MEDIUM	-	imp	-	-	-	Impaired	36.66
9-STE007.29	Spr	1.63	0.05	HIGH	-	-	-	-	Imp	Impaired	38.18
5ABTR002.80	Spr	0.52	0.05	HIGH	-	-	-	-	Imp	Impaired	39.41
2-CHK079.23	Fal	0.92	0.07	LOW	HIGH	-	-	-	Imp	Impaired	40.62

4AMEY016.00	Spr	2.76	0.43	HIGH	-	imp	Imp	-	Imp	Impaired	42.66
1BCKS001.03	Fal	1.66	0.05	HIGH	HIGH	-	-	-	Imp	Impaired	42.98
3-THM001.40	Fal	2.48	0.07	LOW	MEDIUM	imp	-	-	-	Impaired	47.94
2-JKS018.68	Fal	0.72	0.15	HIGH	LOW	-	Imp	-	Imp	Impaired	50.13
3-GRT001.70	Fal	14.2	0.62	HIGH	LOW	imp	Imp	-	Imp	Impaired	52.43
4ALOR008.64	Fal	5.04	0.64	MEDIUM	MEDIUM	imp	Imp	-	-	Impaired	56.52
1BSTH019.52	Fal	1.62	0.22	LOW	LOW	-	Imp	-	-	Impaired	56.78
2-SOL001.00	Fal	2.86	0.04	LOW	MEDIUM	imp	-	-	-	Impaired	57.11
6BPOW179.20	Fal	0.74	0.02	MEDIUM	HIGH	-	-	NotNI	Imp	Impaired	59.03
9-DEN000.03	Spr	1.99	0.04	MEDIUM	-	imp	-	-	-	Impaired	60.44
1BSTH002.14	Spr	1.28	0.14	MEDIUM	-	-	Imp	-	-	Impaired	61.15
2-APP012.79	Spr	0.45	0.18	MEDIUM	-	-	Imp	-	-	Impaired	61.29
9-MLC005.44	Spr	1.91	0.03	LOW	-	imp	-	-	-	Impaired	62.34
6CMFH033.40	Fal	1.83	0.15	LOW	LOW	imp	Imp	-	-	Impaired	67.39
2-RVN015.97 (S1)*	Fal	2.54	0.37	HIGH	MEDIUM	imp	Imp	-	Imp	Impaired	69.06
1ALIV012.12	Fal	0.45	0.03	LOW	LOW	-	-	NotNI	-	Not Nut Imp	28.56
6ASAT000.26	Spr	0.32	0.01	LOW	-	-	-	NotNI	-	Not Nut Imp	36.70
2-IVC010.20	Spr	0.54	0.02	LOW	-	-	-	NotNI	-	Not Nut Imp	37.37
2-MTC001.24	Fal	0.62	0.04	LOW	MEDIUM	-	-	NotNI	-	Not Nut Imp	47.17
2-LIH005.28	Fal	0.31	0.02	MEDIUM	MEDIUM	-	-	NotNI	-	Not Nut Imp	49.07
8-LTL009.54	Spr	0.36	0.03	LOW	-	-	-	NotNI	-	Not Nut Imp	52.02
3-RAP077.28	Spr	0.32	0.02	LOW	-	-	-	NotNI	-	Not Nut Imp	53.66
9-LTL001.22	Spr	0.20	0.02	LOW	-	-	-	NotNI	-	Not Nut Imp	54.01
2-LIA000.50	Fal	0.31	0.02	MEDIUM	MEDIUM	-	-	NotNI	-	Not Nut Imp	56.40
6BWAL005.97	Spr	0.77	0.03	MEDIUM	-	-	-	NotNI	-	Not Nut Imp	57.56
6AIND000.52	Fal	0.18	0.02	MEDIUM	MEDIUM	-	-	NotNI	-	Not Nut Imp	57.88
8-SAR097.82	Fal	0.32	0.04	LOW	NO	-	-	NotNI	-	Not Nut Imp	59.81
1ACAX004.57	Spr	0.80	0.02	LOW	-	-	-	NotNI	-	Not Nut Imp	61.39
2-MIS000.04	Fal	0.21	0.01	LOW	MEDIUM	-	-	NotNI	-	Not Nut Imp	62.16
9-NBS000.70	Fal	0.16	0.02	MEDIUM	LOW	-	-	NotNI	-	Not Nut Imp	62.93

2-FIN000.81	Spr	0.52	0.03	MEDIUM	-	-	-	NotNI	-	Not Nut Imp	65.18
3-ROB023.06	Spr	0.18	0.02	LOW	-	-	-	NotNI	-	Not Nut Imp	66.98
4ASNA015.30	Fal	0.31	0.03	LOW	-	-	-	NotNI	-	Not Nut Imp	67.42
1AHOC006.23	Fal	0.36	0.01	MEDIUM	MEDIUM	-	-	NotNI	-	Not Nut Imp	68.21
1AGOO022.44	Fal	0.23	0.04	LOW	LOW	-	-	NotNI	-	Not Nut Imp	68.38
5ATRE038.07	Fal	0.54	0.03	MEDIUM	MEDIUM	-	-	NotNI	-	Not Nut Imp	68.91
6CSFH097.42 (S1)	Spr	0.52	0.03	LOW	-	-	-	NotNI	-	Not Nut Imp	71.04
2-JES000.80	Spr	0.10	0.02	LOW	-	-	-	NotNI	-	Not Nut Imp	72.61
8-POR008.97	Fal	0.49	0.04	LOW	LOW	-	-	NotNI	-	Not Nut Imp	73.77
8-NAR005.42 (S1)	Fal	0.21	0.01	MEDIUM	LOW	-	-	NotNI	-	Not Nut Imp	75.48
2-BNF003.52	Spr	0.10	0.01	LOW	-	-	-	NotNI	-	Not Nut Imp	76.91
2-RKI003.40	Fal	0.12	0.02	LOW	LOW	-	-	NotNI	-	Not Nut Imp	83.64

\* SCI for 2-RVN015.97 (S2) was 61.22.