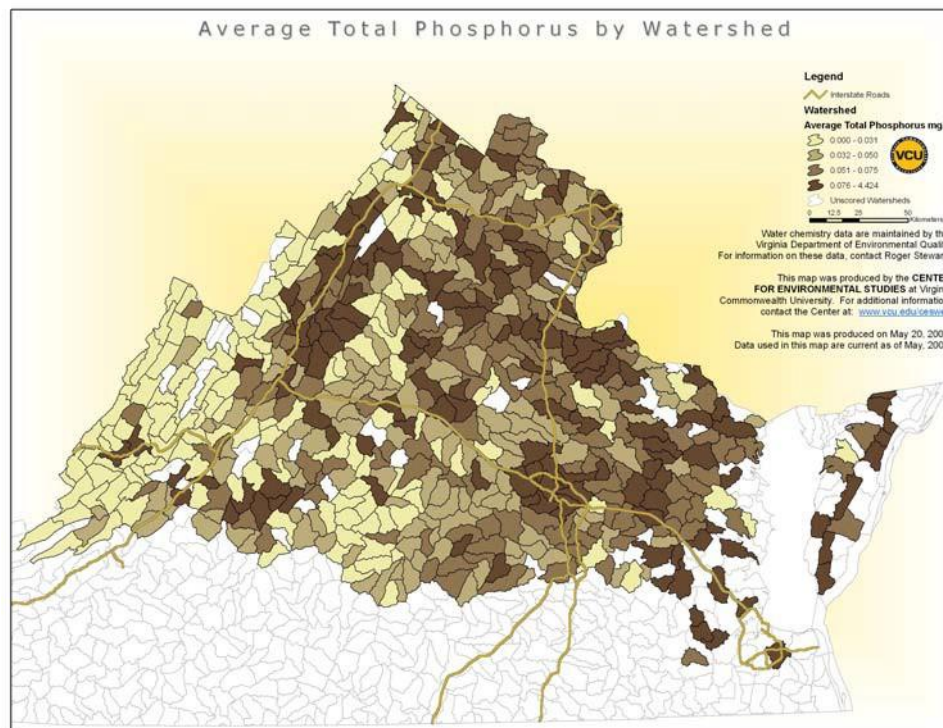


Report to the Academic Advisory Committee, Virginia Department of Environmental Quality and Virginia Tech

Development of Freshwater Nutrient Criteria for Non-wadeable Streams in Virginia: Fish Community Assessment, Phase III



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## Introduction

The complex ecological and biological relationships among nutrient concentrations and fishes in freshwater systems, including streams, lakes, and reservoirs, are documented by a large and diverse literature. Many such studies focus on the role of nutrients in determining rates of secondary production (and, therefore, potential yields) of higher trophic levels, including fishes (e.g. Dodds, et al. 2002), nutrient cycling and spiraling (e.g. Griffiths 2006), and the effects of nutrient releases from aquaculture facilities (e.g. Dalsgaard and Krause-Jensen 2006). The impact of nutrient enrichment (eutrophication) from anthropogenic sources on aquatic systems has also been widely-studied and is considered a serious threat to aquatic ecosystem health and function (EPA 1998). In response, many U.S. jurisdictions have moved to develop and implement regional nutrient criteria, with the goal of protecting aquatic living resources, including fishes. Frequently, measures (indices) of biotic assemblages (fish and macroinvertebrates) are used to assess stream health, integrity, and, indirectly, water quality. However, only a limited number of published studies (e.g. Wang, et al. 2006) have examined *directly* the putative effects of cultural eutrophication on fish community structure and function in streams, and only a few of these reports (e.g. Morgan, et al. 2007) have focused on the mid-Atlantic region.

At a 2006 meeting of an Academic Advisory Committee working group focused on establishing numeric nutrient criteria for Virginia's streams, participants discussed several potential approaches for linking nutrient concentrations and criteria to aquatic life use standards in larger (i.e., non-wadeable) streams and rivers. Specifically, the subcommittee reasoned that fish community structure may be a useful diagnostic of nutrient-related effects in such systems, which are typically too large for standard benthic macroinvertebrate sampling protocols. The subcommittee proposed a preliminary analysis, using existing data, to determine whether statistically significant relationship(s) exist among a limited suite of variables representing nutrient conditions and fish community structure, and at broad geospatial scales. If such a relationship can be demonstrated, based on analyses with archival data alone, additional future analyses and targeted database development may support the establishment and validation of ecologically-based, and scientifically defensible, numeric nutrient criteria for larger (i.e., non-wadeable) lotic ecosystems.

Previous studies for the AAC (Garman, et al. 2007 & 2008) completed preliminary analyses of archived fish community and nutrient data for streams and rivers in the Virginia Coastal Zone. These analyses were based on an extensive database of fish community metrics for Chesapeake Bay freshwater systems and DEQ's nutrient concentration data (TP, TN) and algal biomass data (Chl-a) from that agency's ambient monitoring program. These earlier studies had the following objectives: 1.) create a working database by combining and distilling large amounts of archival data representing nutrient concentrations and fish community structure from multiple sources, and 2.) conduct simple correlation analyses to test the hypothesis that derived measures of nutrient conditions and stream health (fish communities) may be related statistically and could, therefore, be the basis for future predictive models and nutrient criteria thresholds. Previous reports (Garman, et al. 2007 & 2008) demonstrated that statistical relationships among fish community indices (modified Index of Biotic Integrity, mIBI) and nutrient concentrations (DEQ ambient monitoring) may be useful in developing nutrient criteria related to both localized and downstream effects. Unfortunately, these preliminary analyses were constrained limited by several factors, including the lack of temporally and spatially synoptic data for nutrients and fish

community health, representation by only a few basins, and the inability to separate wadeable and non-wadeable ambient monitoring stations within the DEQ/STORET database. The current (2009) study, described below, expands and improves the earlier analyses and includes specific analyses of putative non-wadeable locations.

## 2009 Objectives

- 1.) Attempt to document statistically significant relationships among TN, TP, and Chlorophyll-*a* concentrations and fish community-based stream health metrics based on an expanded database (*cp.* 2008) that represents all Chesapeake basin watersheds (6<sup>th</sup>-order hydrologic units) in Virginia.
- 2.) Evaluate differences, if they exist, between responses of coastal *versus* non-coastal stream fish assemblages to nutrients and trophic status.
- 3.) Confirm statistically significant, watershed-based patterns for a subset of paired data representing putative non-wadeable streams.
- 4.) Propose draft nutrient criteria for the identification (and assumed protection) of ecologically healthy, non-wadeable streams, based on fish community assessment.

## Approach and Methods

DEQ monitoring data representing ambient nutrient concentrations (total nitrogen, TN; total phosphorus, TP; mg/l) and algal biomass (as Chlorophyll *a*, Chl-*a*; ug/l) at georeferenced stream locations were downloaded to a VCU server for post-processing in April, 2009. These data (provided by Mr. Roger Stewart, Virginia DEQ) were ‘filtered’ by location (Chesapeake Bay drainages), content (availability of all three nutrient parameters and minimum n=10 per station) and other criteria (e.g. stream characteristics, date range), producing a working database of approximately 32,000 records. The final DEQ data were joined to a subset of the fish community database maintained by VCU’s INSTAR stream assessment program (<http://INSTAR.vcu.edu>), which generates stream health (i.e., biotic integrity) scores at stream reach and watershed spatial scales, based on empirical data and established models for fish community structure and function (described below). Data ranges for TN, TP, and Chl-*a* in the final dataset were divided into equal categories based on quartiles, i.e., TN category 1 represents the lowest concentrations of the range, while category 4 represents the highest concentrations. Nutrient data were not distributed normally.

Because no objective criteria exist to identify streams as non-wadeable and quantitative and large-river data for fish communities in Virginia are limited, nutrient data and fish community metrics were combined (pooled) to generate descriptive statistics (means and percentiles) for 6<sup>th</sup>-order watersheds (hydrologic units, HUCs) in the Chesapeake Bay basin for each selected parameter and all stream reaches. Some HUCs did not have sufficient data (nutrients and/or fish) and were eliminated from further analysis. Preliminary analysis suggested that stream fish assemblages in the Coastal Zone may respond differently to nutrient and trophic status. Coastal HUCs were, therefore, separated from non-coastal regions (i.e., Piedmont and Ridge and Valley) for subsequent, watershed-scale analyses. The fall-zone (inferred from Interstate 95) was used as the line of separation for coastal *versus* non-coastal watersheds. Analyses conducted at the watershed scale included fish community data from wadeable and non-wadeable streams.

We also conducted analyses on a very limited (n=57) *paired* dataset of spatially co-incident nutrient values and fish health metrics for putative *non-wadeable* (> 3<sup>rd</sup> order) streams and rivers

within the Chesapeake basin. This analysis assumed that DEQ ambient monitoring station

within 500 meters of an INSTAR location represented the same stream reach. The small size of this reach-specific dataset for non-wadeable streams reflects, in part, the lack of relevant, archival data for large streams and rivers in Virginia.

Geospatial analyses were conducted using ESRI's ArcGIS version 9.3. Statistical comparisons across nutrient categories and between 'healthy' and 'compromised' stream fish assemblages were based on nonparametric Chi-square tests ( $\alpha=0.05$ ). More detailed methods and data descriptions are provided below:

*Stream Nutrient Concentrations and Trophic Status:* The following nutrient parameters were selected from the DEQ ambient monitoring database and developed for further analysis: total nitrogen concentration (TN, mg/l; Figure 1), total phosphorus concentration (TP, mg/l; Figure 2) and chlorophyll-*a* concentration (Chl-*a*, ug/l; Figure 3). Chlorophyll-*a* concentration is indicative of the trophic status of a water body and high Chl-*a* values generally indicate eutrophication. A detailed description of DEQ's ambient monitoring program for nutrients is provided at: <http://www.deq.virginia.gov/watermonitoring/aqm.html>.

*Stream Fish Community Assessment:* The **INSTAR** application (<http://instar.vcu.edu>) and the extensive aquatic resource database on which it runs, were developed to support a variety of stream assessment and planning activities aimed at restoring and protecting water quality and aquatic living resources throughout the Commonwealth. In addition, regional reference stream models (i.e., *virtual* streams) for both non-tidal and small to medium-sized tidal tributaries are developed as criteria for prioritization of candidate streams and watersheds for protection and restoration, objective and quantitative performance measures, and as a decision support tool for environmental planning and implementation. Currently, **INSTAR** has compiled information on approximately 2,200 Virginia streams and **INSTAR** databases comprise over 245,000 records. Probabilistic study reaches for **INSTAR** sampling were selected through a statistically powerful, stratified (by stream order) random design. Although **INSTAR** compiles data for both aquatic macroinvertebrates and fishes, only fish community data were included in this analysis. Within each geo-referenced reach (150-500 m), fishes are sampled quantitatively using electrofishing equipment (backpacks, tote barge units, boats) and EPA QAPP methods. Backpack and tote barge sampling is performed throughout the entire reach in a single pass. Boat electrofishing may include additional sampling effort depending on stream width and habitat variability. Data are compiled in Access® databases and application macros within **INSTAR** calculate over 50 separate metrics and ecological variables, including those typically generated for the Index of Biotic Integrity (IBI), Rapid Bioassessment Protocol (RBP), and Rapid Habitat Assessment (RHA). Variables and metrics are then subjected to ordination and cluster analysis using unimodal models (e.g. correspondence analysis (CA), detrended correspondence analysis (DCA), and canonical correspondence analysis (CCA)) and linear response models (e.g. principal components analysis (PCA), multiple regression techniques). The site scores (i.e., coefficients from the final response model) are entered as the response variable and significant ( $P<0.05$ ) biotic and abiotic variables and metrics are entered as explanatory variables, and used to develop a series of reference stream models (i.e., *virtual* streams). We used Gower's similarity index to compare empirical scores obtained from sampled stream reaches to the appropriate virtual reference stream, generating an index of stream health (VSA score) as a measure of percent comparability to the appropriate (*virtual*) reference condition model. Fish community data collected as part of DEQ's ProbMon program were included, where appropriate.

Fish assemblages with high percent comparability scores (VSA scores > 71%) were assumed to represent streams with high ecological integrity (i.e., healthy and exceptional categories).

Conversely, fish assemblages with low VSA scores (< 57%) were assumed to represent biologically degraded streams (i.e., compromised category). These 'healthy' and 'degraded' VSA categories generally represented +/- 1 standard error of the mean VSA score from the distribution of all VSA scores in the database. Only those INSTAR stream locations in upper and lower categories were included in 2009 analyses, based on the assumption that streams representing mid-range VSA scores (58-70%) are less likely to be influenced by ambient nutrient concentrations.

## Findings

Stream nutrient concentrations (mg/l) averaged 1.57 and 0.98 and ranged up to 47.22 and 4.42 for TN and TP, respectively. Chlorophyll *a* concentrations averaged 2.97 ug/l and ranged up to 52.58 ug/l). Coastal zone watersheds classified as 'degraded' based on stream fish community assessments were strongly associated (Chi-square test,  $p < 0.01$ ; Table 1). For both TN and Chl-*a*, the relationship was positive, i.e., there were significantly more degraded streams in HUCs with the highest nutrient values (Figure 5). In non-coastal watersheds, only the association between stream health and Chl-*a* values was significant ( $p < 0.01$ ), suggesting that trophic status as inferred from Chl-*a* concentrations is the best predictor of compromised stream health in both coastal and noncoastal regions. Stream nutrient concentrations and trophic status were also associated statistically (Chi-square test,  $p < 0.05$ ; Table 1) with high ecological integrity ('healthy') streams. For example, there were significantly more healthy streams in coastal and noncoastal watersheds with the lowest Chl-*a* values (Figure 6). In contrast, the relationship between TN concentrations and high biotic integrity was unimodal, with the greatest representation of healthy streams at intermediate TN concentrations. These findings suggest that Chl-*a* and TN may be better predictors of stream health than TP; the associations between Chl-*a* and the incidence of healthy or degraded streams in a given HUC were statistically significant for both coastal and noncoastal regions.

Analysis of paired, reach-level data for nonwadeable streams and rivers (Figures 9-11) generally mirrored the statistically significant relationships demonstrated by watershed-scale analyses of wadeable and nonwadeable streams combined (Table 1, Figures 6-8). Specifically, Chl-*a* mean concentrations were strongly and negatively correlated (Figure 11) and no stream reaches classified as biologically healthy were observed at paired Chl-*a* values above 0.25 ug/l. The relationship between fish community healthy and TN concentrations was also negative (Figure 9) but depended on a single observation. No streams classified as healthy were observed at paired TN values above 2.0 mg/l. There was no obvious relationship between stream health and TP concentrations (Figure 10).

The analyses suggest that nutrient criteria for the protection of biologically healthy streams and rivers are supported by simple, but statistically significant, models of relationships among TN, Chl-*a*, and VSA scores. For the watershed scale analysis, the proposed 'protection' criteria are as follows: TN < 0.66 mg/l and Chl-*a* < 0.88 ug/l for coastal and noncoastal streams. The paired, reach-level analysis of nonwadeable streams, based on a much smaller sample size, suggests the following criteria for healthy stream protection: TN < 2.0, Chl-*a* < 0.25 ug/l. Criteria based on TP concentrations are not supported by this analysis.

## Summary

- 1.) Statistically significant relationships were documented among TN, Chlorophyll-a, and to a lesser degree TP, and fish community-based (INSTAR) stream health metrics using an expanded database (n=35,000 records, DEQ ambient monitoring) of all Chesapeake basin watersheds (6<sup>th</sup>-order HUCs) in Virginia. Some of these relationships (e.g. Chl-a and VSA score) were relatively strong predictors of both healthy and degraded stream assemblages and might reasonably serve as the basis for establishing biologically valid nutrient criteria. Some of the strong associations between nutrients and trophic status and fish community structure at watershed scales were corroborated by analysis of a much smaller database of paired, nonwadeable streams and rivers. Specifically, fish community metrics were strongly and negatively correlated with TN and Chl-a concentrations in 77 putative nonwadeable streams.
- 2.) Proposed, *conservative* criteria for the protection of high quality nonwadeable streams are as follows: TN < 2.0 mg/l and Chl-a < 0.88 ug/l. At this time, criteria based on TP may not be warranted.
- 3.) Differences did exist between responses of coastal *versus* non-coastal stream fish assemblages to nutrient and trophic status, but the geographic differentiation may not warrant separate nutrient criteria for streams. However, this issue should be explored in more detail.
- 4.) Chlorophyll-*a* concentration appears to be the most promising predictor of ecological health in nonwadeable streams, and therefore the most likely basis for establishing nutrient criteria based on fish community structure; however, the availability of Chl-a data is limited, compared to other parameters including TP and TN.
- 5.) Future efforts should focus on: a.) expanding the paired database for nonwadeable streams and rivers through additional data mining and GIS analysis, b.) refining the proposed nutrient criteria for TN and Chl-a based on this expanded coverage, c.) leverage ongoing fieldwork (e.g. DEQ's ProbMon Program) to develop a separate and synoptic database of nutrient and fish community metrics that can be used to formally validate proposed nutrient criteria for nonwadeable streams in Virginia and d.) expand the discussion statewide into non-Bay drainages.

Table 1. Summary of statistical comparisons across nutrient (TN, TP, Chl-a) categories for watersheds classified as ‘healthy’ or ‘degraded’ based on INSTAR assessment of fish communities in coastal and non-coastal streams and rivers (Chesapeake Bay basin). The analyses tested the null hypothesis that classified streams were distributed uniformly or randomly among nutrient categories. Rejection of the null suggests that stream biological health is significantly associated with nutrient or trophic status. All data were pooled by watershed (HUC). Statistically significant relationships are described as ‘positive,’ ‘negative,’ or ‘unimodal.’ Refer to Figures 5-8 for specific comparisons.

	TN		TP		Chl-a	
	Coastal	Noncoastal	Coastal	Noncoastal	Coastal	Noncoastal
<b>Degraded Streams</b>	** positive	n.s.	** unimodal	n.s.	** positive	** positive
<b>Healthy Streams</b>	* unimodal	* unimodal	* positive	n.s.	* negative	* negative

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\*\* alpha <0.01, \* alpha <0.05, n.s.=not significant

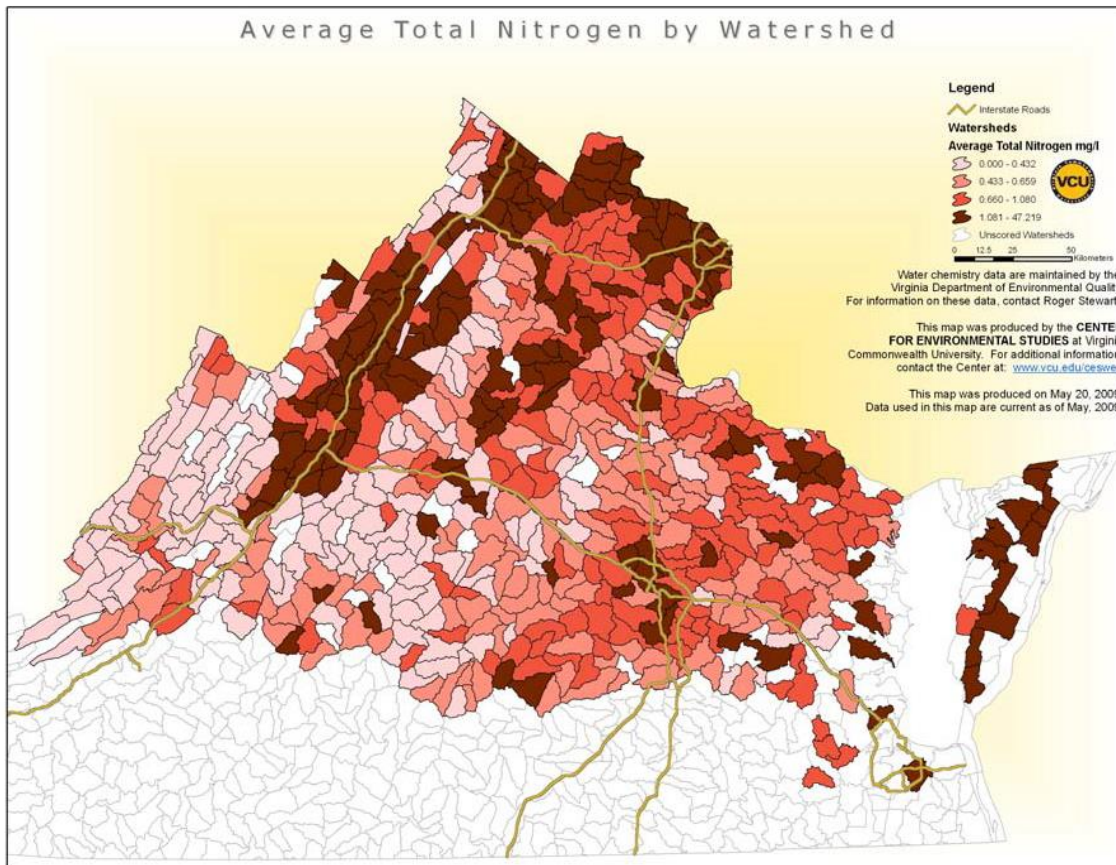


Figure 1. Distribution of total nitrogen concentrations (TN, mg/l) for streams in 6<sup>th</sup>-order hydrological units in the Chesapeake Bay basin, Virginia. Data provided by DEQ ambient monitoring program.

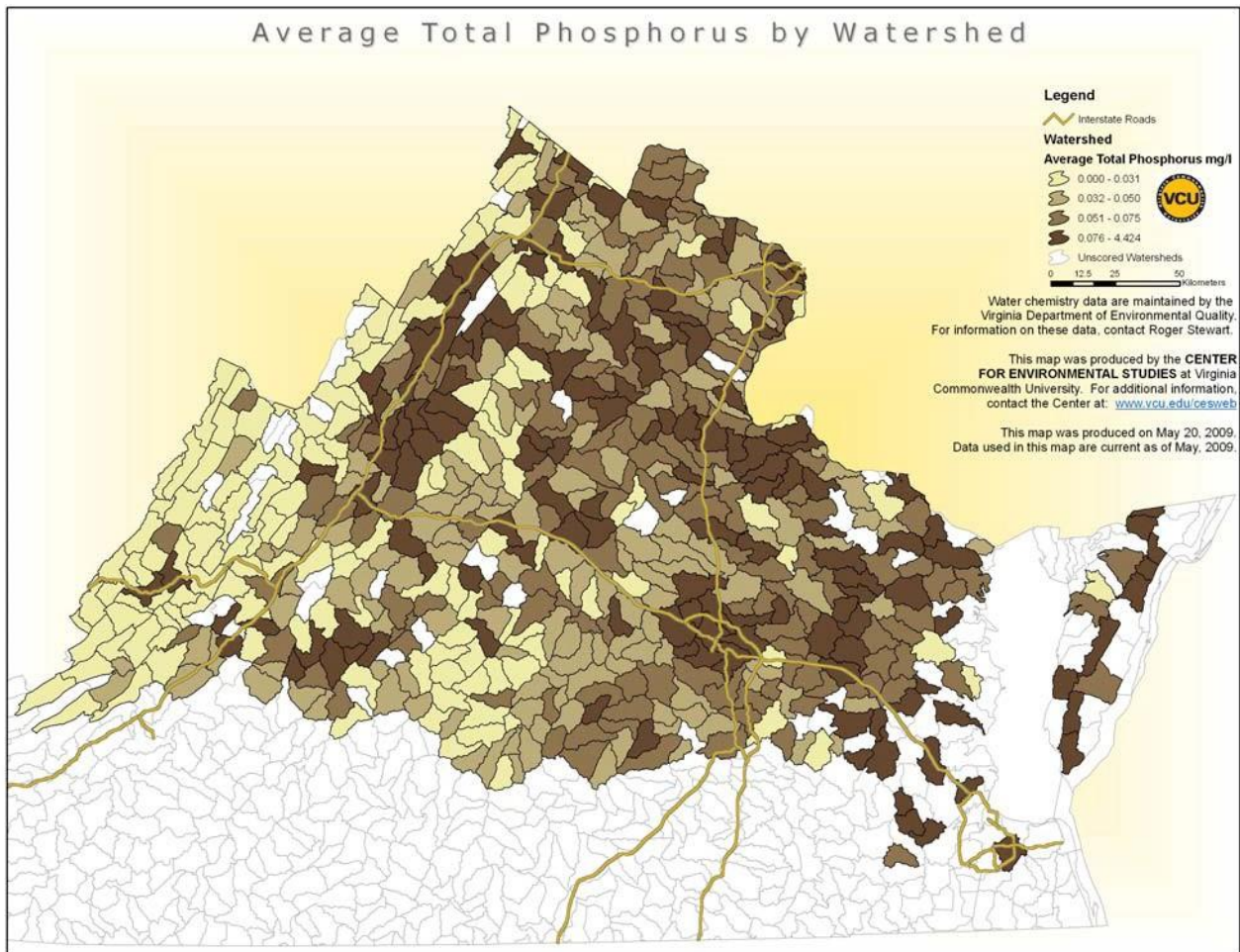


Figure 2. Distribution of total phosphorous concentrations (TP, mg/l) for streams in 6<sup>th</sup>-order hydrological units in the Chesapeake Bay basin, Virginia. Data provided by DEQ ambient monitoring program.

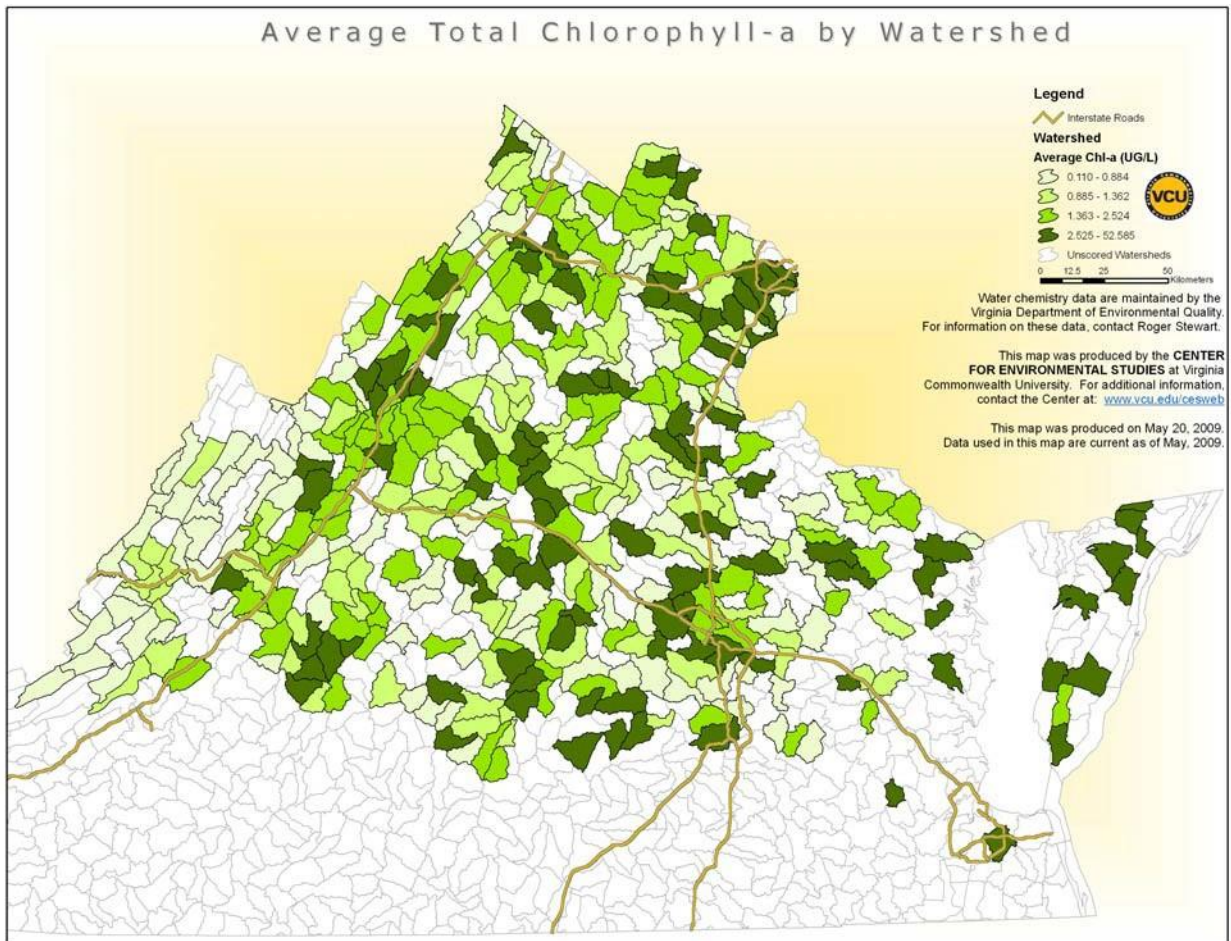


Figure 3. Distribution of Chlorophyll-*a* concentrations (Chl-*a*, ug/l) for streams in 6<sup>th</sup>-order hydrological units in the Chesapeake Bay basin, Virginia. Data provided by DEQ ambient monitoring program.

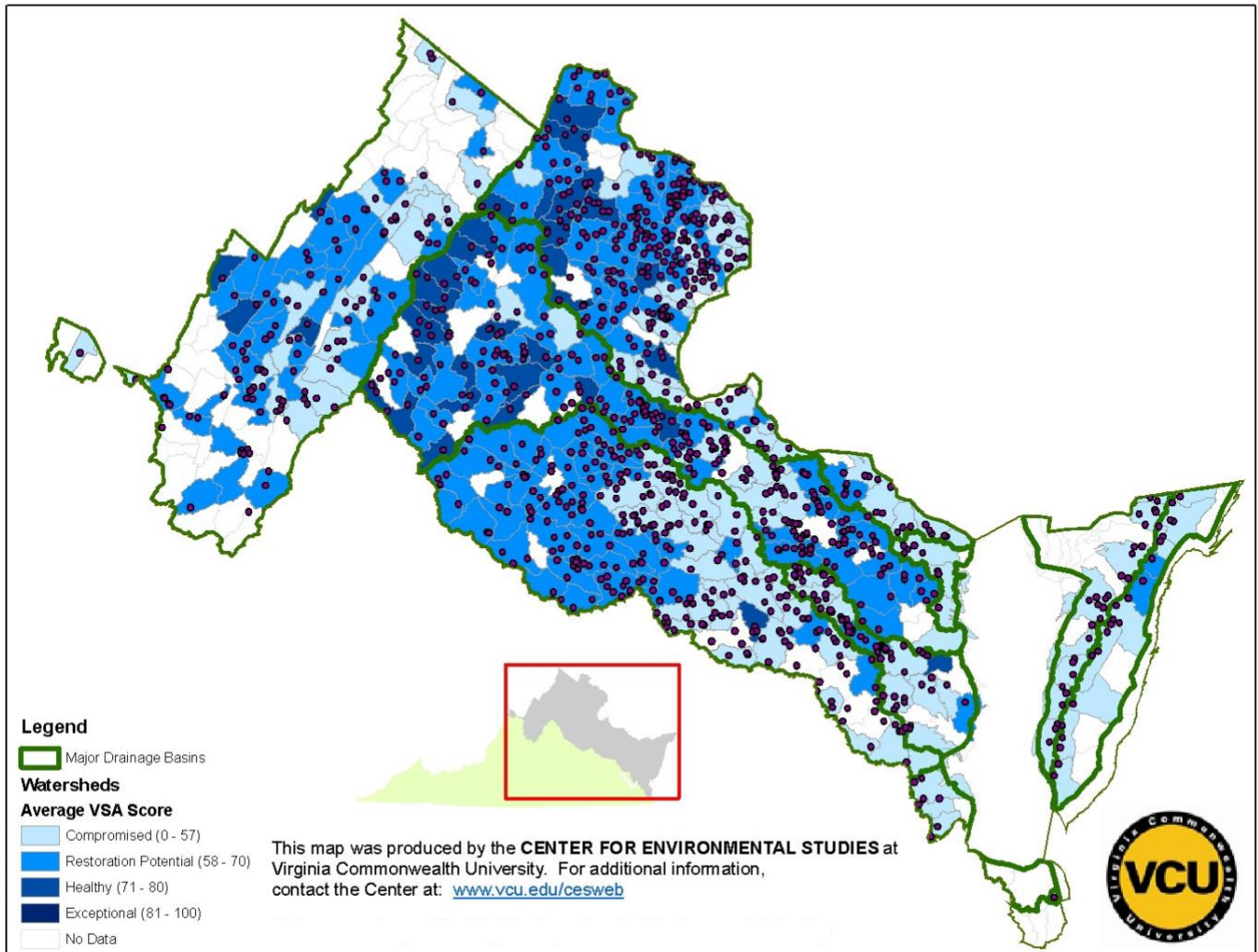


Figure 4. Stream health classification (INSTAR fish assemblage models) for streams in 6<sup>th</sup>-order hydrologic units in the Chesapeake Bay basin, Virginia. Categories are based on the mean VSA score (percent comparability to appropriate virtual reference condition). Breakpoints for stream health categories are based on the mean, +/-1 standard error, and + 2 standard errors of the distribution of n=1,033 randomly selected VSA scores for INSTAR stream reaches. HUCs in the 'exceptional' and 'healthy' categories are dominated by streams exhibiting high ecological integrity. Points represent individual quantitative (electrofishing) collections for selected HUCs.

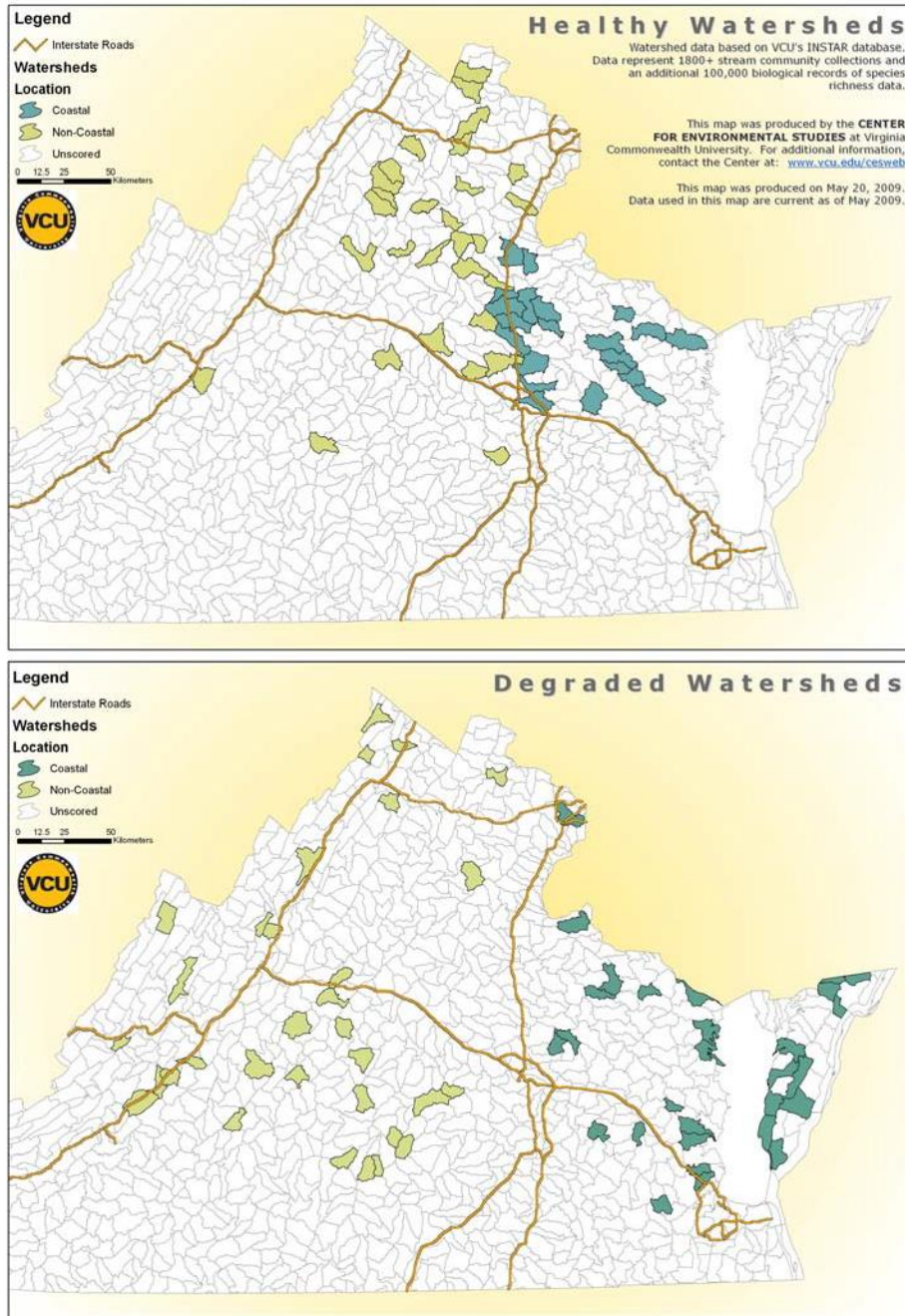


Figure 5. Watersheds (6<sup>th</sup>-order HUCs) classified as 'healthy' or 'degraded' based on INSTAR assessment of fish community data. Refer to the text for a more detailed explanation.

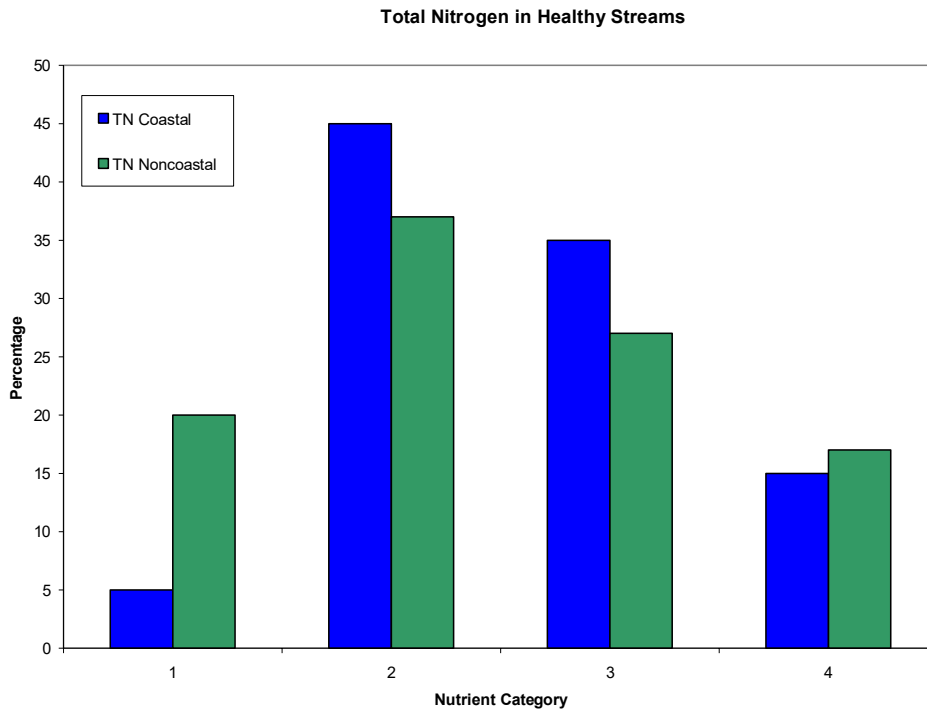
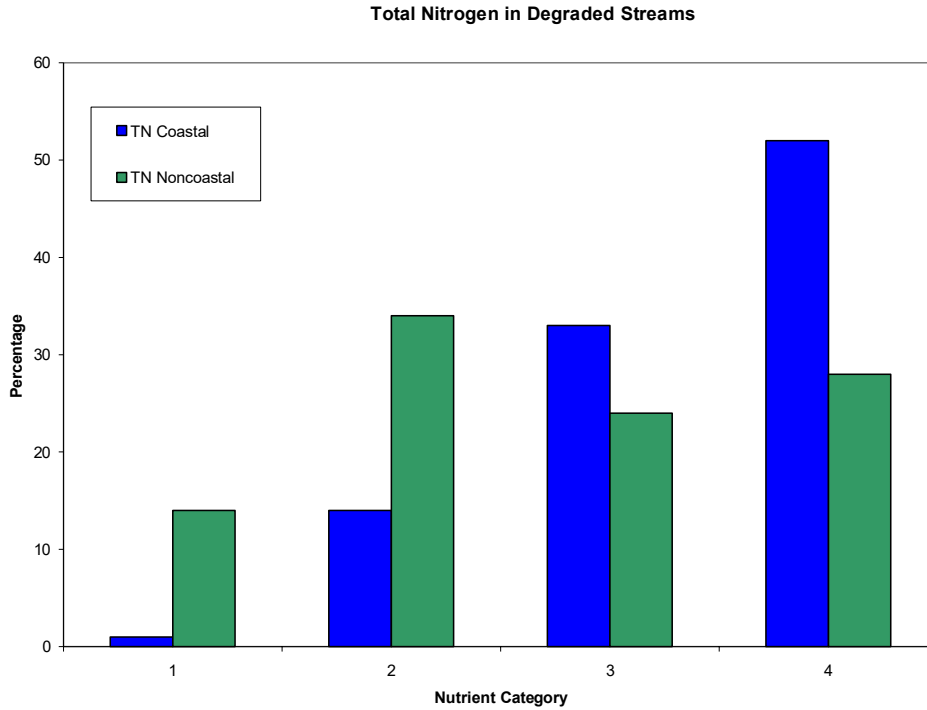


Figure 6. Percent occurrence of biologically degraded (upper plot) and healthy (lower plot) watersheds as a function of TN concentration, where category 1 represents the lowest nutrient concentrations in mg/l. Please refer to Figure 1 for category breakpoints and to Table 1 for results of statistical comparisons.

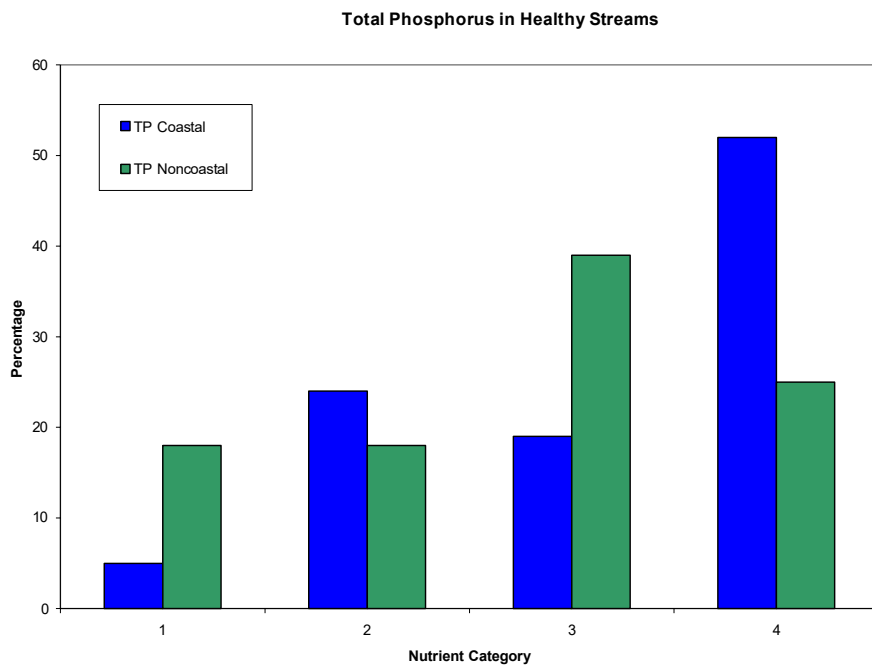
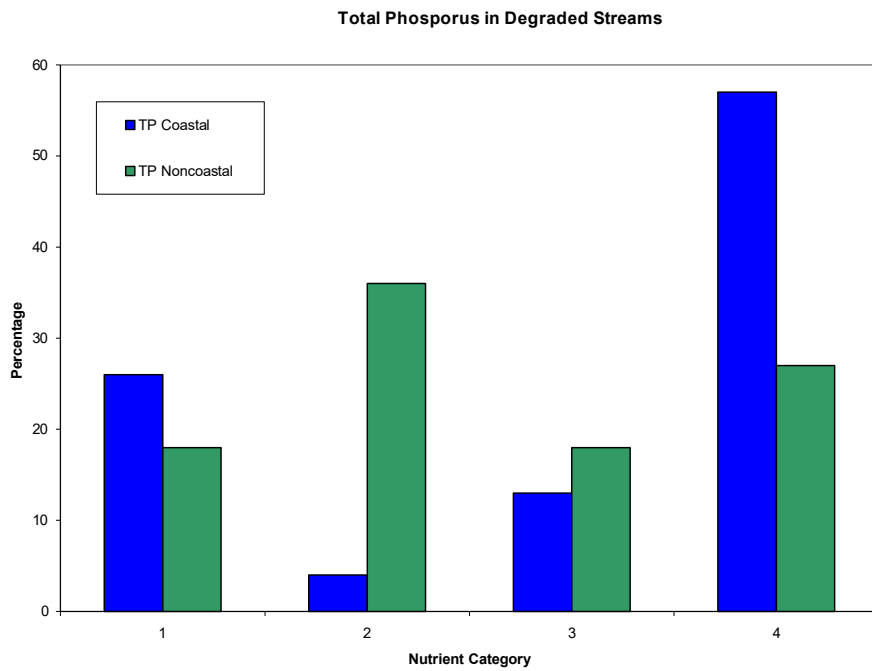


Figure 7. Percent occurrence of biologically degraded (upper plot) and healthy (lower plot) watersheds as a function of TP concentration, where category 1 represents the lowest nutrient concentrations in mg/l. Please refer to Figure 2 for category breakpoints and to Table 1 for results of statistical comparisons.

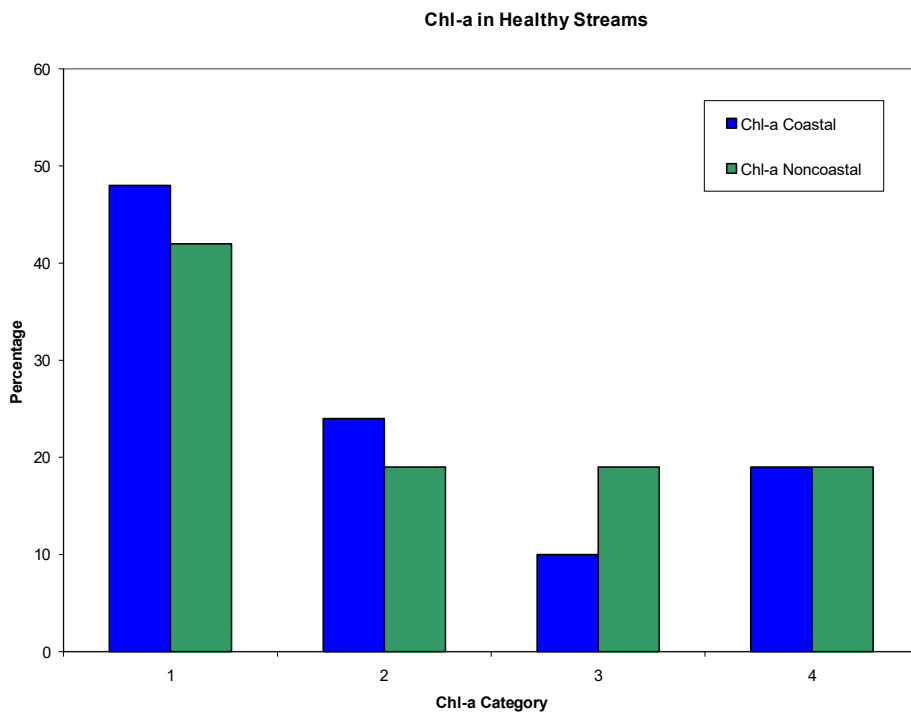
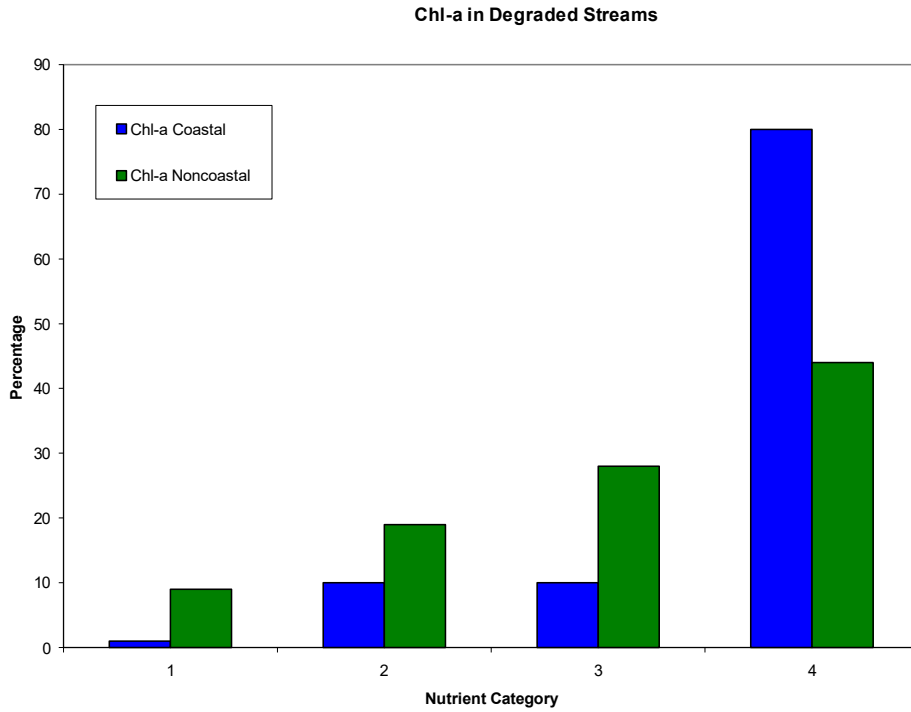


Figure 8. Percent occurrence of biologically degraded (upper plot) and healthy (lower plot) watersheds as a function of Chl-a concentration, where category 1 represents the lowest nutrient concentrations in ug/l. Please refer to Figure 3 for category breakpoints and to Table 1 for results of statistical comparisons.

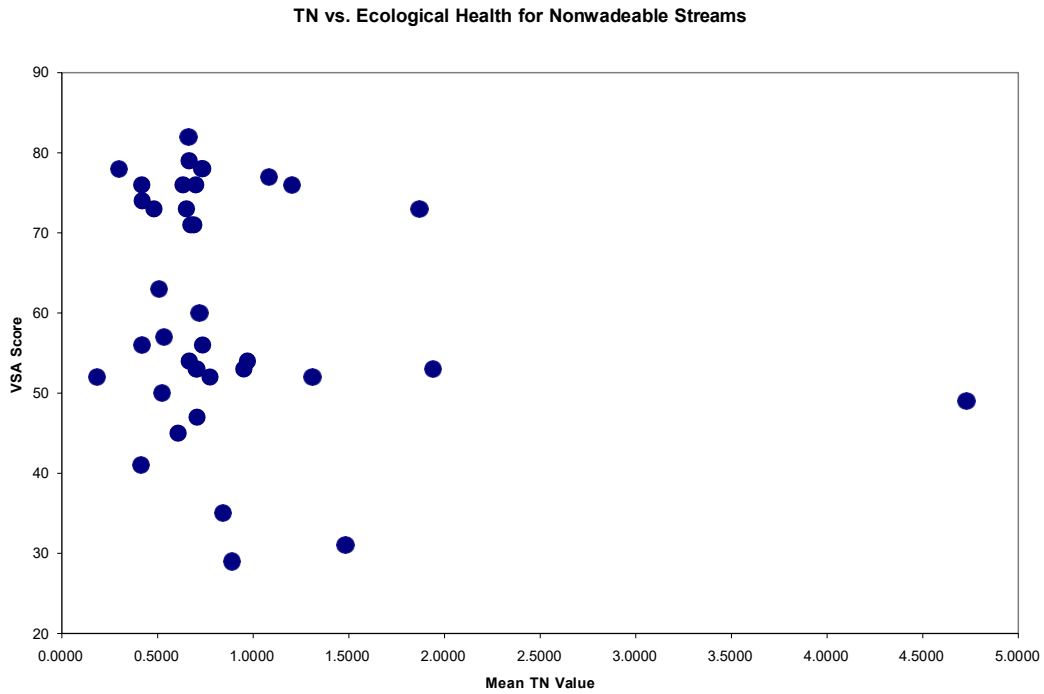


Figure 9. Scatterplot of fish community health score (VSA, % comparability to reference) and TN concentration (mean, mg/l) for paired, non-wadeable stream and river reaches, Chesapeake Bay basin, Virginia.

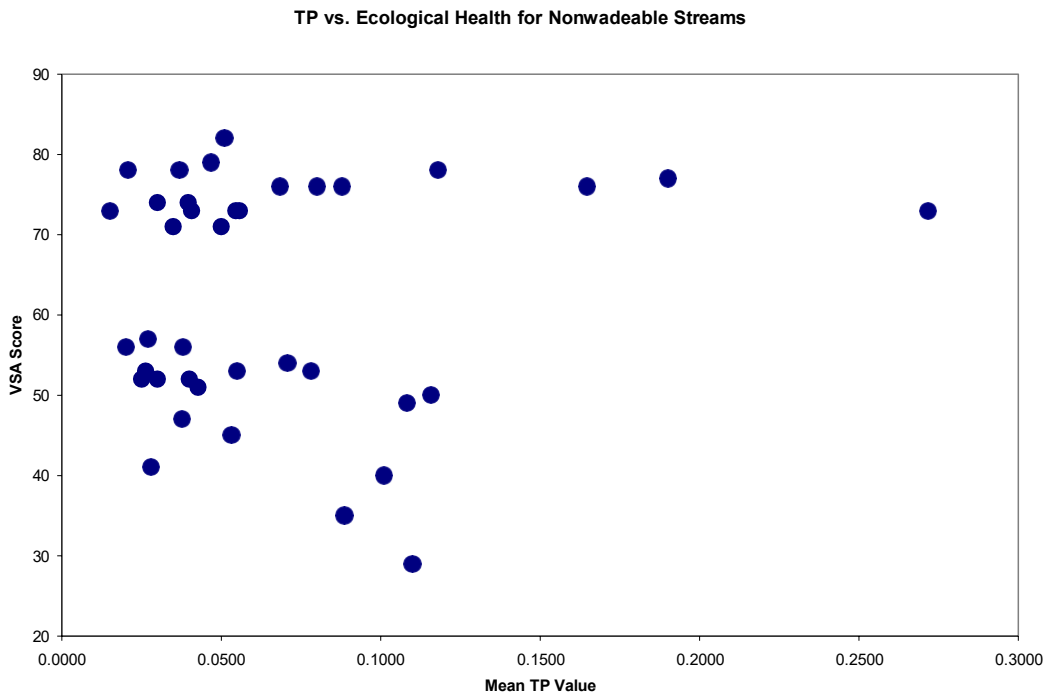


Figure 10. Scatterplot of fish community health score (VSA, % comparability to reference) and TP concentration (mean, mg/l) for paired, non-wadeable stream and river reaches, Chesapeake Bay basin, Virginia.

Paired Chl-a vs. Ecological Health for Non-wadeable Streams

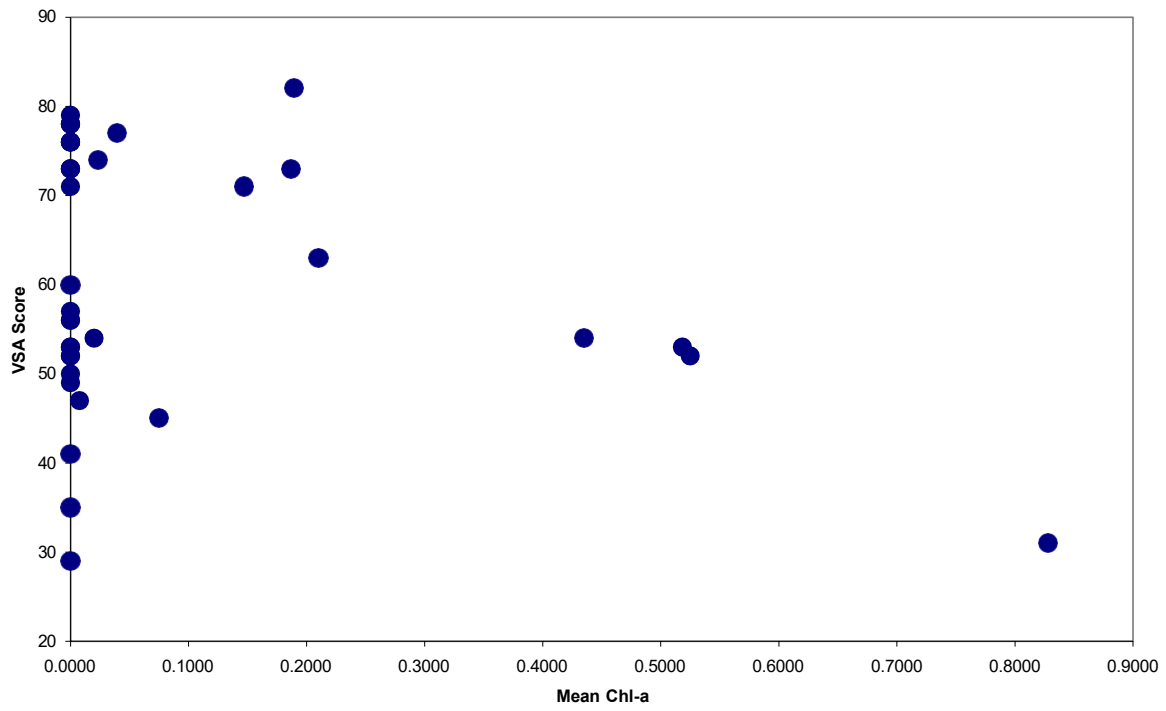


Figure 11. Scatterplot of fish community health score (VSA, % comparability to reference) and Chl-a concentration (mean, ug/l) for paired, non-wadeable stream and river reaches, Chesapeake Bay basin, Virginia.

