Ecology and Management of Filamentous Green Algae

Charles A. Dean and Gordon M. Selckmann

Interstate Commission on the Potomac River Basin

30 West Gude Dr, Suite 450

Rockville, MD 20850

301-984-1908

www.potomacriver.org

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Interstate Commission on the Potomac River
Basin 30 West Gude Drive, Suite 450
Rockville, MD
20850

Or call,
301-984-1908.

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Introduction

Anthropogenic nutrient pollution is ubiquitous in North American freshwater systems, often leading to increased primary production and subsequent declines in ecosystem function. Nutrient pollution in streams and rivers often stems from agricultural or urban land uses within the watershed introducing excess nitrogen (N) and phosphorous (P) (McDowell et al. 2004, Alexander and Smith 2006). In a process termed eutrophication, these excess nutrients promote algal and microbial primary production, which at nuisance levels decrease water quality, alter habitat, and harm the stream biota (Stevenson et al. 2012, Pavagadhi and Balasubramanian 2013, Mebane et al. 2014). The negative impacts of this eutrophication can be both biological (e.g., declines in biodiversity, shifts in dominance), and a function of how the system serves a specifically human purpose (e.g., declines in fishery yields, prevention of recreation). Consequently nutrient pollution and eutrophication has long been a primary concern of freshwater ecologists and management agencies.

To catalyze research and management efforts, we review here the response of algae to nutrient pollution and the impacts of nuisance blooms on recreational use and ecosystem functioning in freshwater systems. The objectives of this review are to (1) summarize the biogeochemical and ecological processes governing nutrient sources and algal responses to nutrient pollution, especially in the Mid-Atlantic region, (2) discuss methods to describe blooms and measure ecological impact, and (3) provide suggestions to managers regarding study areas and relevant methods. Summaries of studies utilizing these methods are also provided. Summarized articles were obtained through searches using Google Scholar. All articles are available online and do not require journal subscriptions. We extracted information most pertinent to Mid-Atlantic management issues to provide a brief overview of each study. Our goal is that this will serve as a tool for managers to become familiar with the literature of algal ecology and guide further research.

Biogeochemical and ecological processes

Phosphorous generally limits primary production in fresh waters, and this section will focus primarily on the actions of P, rather than N, in catchments. However it is important that water analysis include N and other nutrients in addition to P, as nearly half of published bioassay results show a maximum periphyton growth response when N and P are added simultaneously (Dodds and Welch 2000).

Agriculture as a source of phosphorous

Fertilizers and manure applied to agricultural land often contain more nutrients than crops are able to assimilate. Excess nutrients are exported from fields and flow into streams and rivers, mostly in overland or subsurface runoff following precipitation events (McDowell et al. 2004). Farm management practices generally aim to retain nutrients in terrestrial soil (e.g., applying fertilizer when precipitation events are least likely and the use of riparian buffers). However many of these practices are either insufficient or not well-regulated, and agricultural land use remains one of the primary causes of eutrophication in freshwater systems (McDowell et al. 2004, Alexander and Smith 2006).

Nutrients in fertilizers and manure are highly labile and mobile, and easily assimilated by primary producers. Phosphorous is absorbed by plants and bacteria primarily in the form of inorganic orthophosphates (containing a single phosphate molecule). Although fertilizers contain mostly polyphosphates, these are readily converted to orthophosphates and associate with calcium and magnesium ions (Ca^{+2}, Mg^{2+}) in crop-soil conditions (Rehm et al. 2002, McDowell et al. 2004). Manure contains a mixture of orthophosphates and organic phosphates. Organic phosphates are not directly available to most primary producers, yet microbial catabolism of these molecules can indirectly promote primary production. Following precipitation, most P release from soil occurs at the start of surface and subsurface flow events, where P can release in dissolved (DP) and particulate (PP) form. Factors influencing the amount and form of P released during flow events include antecedent soil moisture, timing and method of fertilizer or manure application, and soil chemical conditions (McDowell et al. 2004).

Poultry production is a common agricultural source of nutrient pollution in the eastern United States. Manure from poultry operations are frequently applied to nearby crop fields and subsequently increase P concentrations in runoff water. In the Illinois River Watershed (IRW) in Arkansas and Oklahoma, poultry house density was explicitly observed to increase in-stream P concentrations and stream cover by filamentous green algae (FGA; Stevenson et al. 2012). This inherent increase of benthic production ultimately led to diurnal dissolved oxygen (DO) swings that reached levels harmful to stream biodiversity. In the Lost River basin of West Virginia, poultry house production has been linked to an increase of P in runoff water (Elrashidi et al. 2008). Phosphorus concentrations in runoff from cropland was nearly 10 times higher than runoff from forested land and over twice that from pastureland (Elrashidi et al. 2008). Low in-stream P concentrations and a high abundance of aquatic algae downstream of the poultry operations suggests that excess P is assimilated by algae and other aquatic weeds in the Lost River basin. Furthermore, the P concentrations in runoff water also exceeded the EPA's recommended limits, and the authors assert that this could cause water quality issues and environmental problems in the Lost River (Elrashidi et al. 2008). The eutrophication in the IRW and Lost River Basin are typical responses of riverine systems to agricultural activity in the watershed.

Other Sources of Phosphorous

Phosphorous pollution can stem from a variety of other sources, including non-agriculture fertilizer use and sewage outfalls. For example, golf course land-use and sewage outfalls were identified in West Virginia to stimulate FGA blooms (Summers 2008). The sewage outfall in particular contributed 80-90% of the phosphorous loading in a tributary of the Greenbrier River, while the golf courses contributed about 3% (Summers 2008). Interestingly, many areas in the Greenbrier basin where algae was expected to thrive did not produce blooms. It was determined that aspects other than nutrient concentrations were limiting algal growth, including hardness, alkalinity, and pH. The importance of these confounding factors cannot be overstated when analyzing freshwater algae and their relation to stream nutrient concentrations.

Ecological impacts of eutrophication

Eutrophication generally includes increased production by plants, algae and bacteria in freshwater systems. Attached algae include FGA, cyanobacteria (also known as blue-green algae or BGA), and other colony forming algae. Excess growth of attached algae can impair the recreational use of waterways. Phytoplankton (algae floating in the water column) also can reach harmful levels in response to nutrient pollution, however this usually occurs in wide and well-lit water bodies with low flow, where they can accumulate into harmful blooms. Although this review primarily focuses on FGA and other attached algae, it should be noted that the effects of nutrient pollution cascade down-stream from the source of pollution as explained by the River Continuum Hypothesis (Vannote et al. 1980). For example, nutrient input in headwater streams promotes localized growth of attached algae, and nutrients will "spiral" downstream (McDowell et al. 2004) as dead algae detach and enter the water column. This can potentially stimulate phytoplankton blooms or macrophyte growth far downstream in deeper water.

Filamentous green algae can cause large diurnal swings in pH and DO during periods of rapid production, as well as other habitat alterations that stress ecosystem health. During daylight hours, these algae will increase DO and decrease CO_2 concentrations in the water column through photosynthesis. Decreased CO_2 concentrations in turn raise the pH of stream water. Conversely, algae are only respiring at night, decreasing DO in the water column and producing CO_2 that lowers pH levels. These swings in DO and pH can cause acute stress as seen in large-scale fish kills (Stevenson et al. 2012), and also chronic stress that lowers an organism's fitness over longer periods (Winter et al. 1996). Filamentous green algae blooms can also effect streams and rivers by physical habitat alteration, and compete for space with other macrophytes and invertebrates that require holding space on rocky substrates (Dudley et al. 1986). In excluding other macrophytes or periphytic algae, FGA can decrease food availability to some macroinvertebrate grazers (Dudley et al. 1986). However, some grazers benefit from the food provided by these blooms, and dense epiphyte colonies (plants that grow on other plants) can also form on FGA filaments and provide food (Dudley et al. 1986). These changes cascade through trophic levels and alter vertebrate community composition as well.

Variables controlling algal response to nutrient input

The development of accurate classification methods to determine impairment by nuisance algae is a significant problem faced by managing agencies because of the confounding interactions of physical characteristics (e.g., river hydrology, light, temperature, pH, TN, TP) and biotic processes (e.g., species interactions, primary production, microbial respiration; Dodds and Welch 2000, Summers 2008). For example, it is important to recognize that nutrient concentrations are not necessarily the best predictor of algal growth since many other factors can be limiting. Summers (2008) observed many streams in West Virginia to not produce algal blooms even with sufficient nutrients, and found that hardness, alkalinity, and light availability were limiting growth in many of these cases. Sufficiently high hardness, a measure of Ca and Mg levels, can limit algal growth because these cations interact with phosphates to form precipitates, making P unavailable for assimilation by algae. Sufficiently low alkalinity will also cause precipitation of phosphate species. High turbidity or low light levels will decrease photosynthetic activity. Species interactions must also be taken into account since algae is a key food source for many grazers. For example, water column nutrients may correspond more to grazing invertebrates than macrophyte biomass if grazers exert enough pressure to limit macrophyte biomass (Bourassa and Cattaneo 1998).

Monitoring and classification methods

Effective classification and management of impaired waterways requires system-specific knowledge of ecological and biogeochemical processes. Many state and federal agencies already have established protocols that involve a combination of macrophyte standing crop or biotic assemblage estimates coupled with water quality or nutrient analysis to set criteria (Dodds and Welch 2000, reviewed in Griggs et al. 2015). However these protocols and the criteria they produce are rarely applicable across ecoregions or even watersheds (Dodds and Welch 2000). Furthermore, comprehensive and labor-intensive sampling is required to accurately identify management criteria based on traditional measures of water quality, habitat characterization, nutrient concentrations, or biological indices. It is evident that the dynamic processes governing nutrient pollution and eutrophication necessitate a holistic approach and modern methods to accurately and efficiently assess impairment and establish management criteria. This section describes critical aspects of management of nuisance algae, from identification of bloom sites to estimation of ecological impact. Methods are summarized also in Appendix A, Table 1.

Locating algal blooms is a key step in the management or identification of polluted waterways, however visual observation of an entire watershed is labor intensive and usually not realistic. Promising methods to identify blooms across basins involve remote sensing that can be performed by unmanned aircraft. Remote sensing techniques have been in development for over four decades and vary from basic digital photography from planes to hyperspectral imaging from satellites. Although technically demanding, these methods have the potential to be of great use to management agencies and can be scaled to the extent of specific projects and budgets. Anker et al. (2014) tested the efficacy of aerial digital photography-spectral analysis (ADP-SA) as a more cost-effective alternative to large scale satellite hyperspectral analysis and small-scale ground level visual surveys. They tested species detection and relative cover estimation of the filamentous green algae *Cladophora glomerata* and the watercress *Nasturtium officinale*. The ADP-SA method exhibited similar accuracy at both scales, and better spatial resolution (4 cm) than both the hyperspectral and visual methods (1 m and 10 cm, respectively). The ADP-SA method also could differentiate between submerged and emergent plants. Catteneo et al. (2013) produced a statistical model using climate and hydrological data coupled with remote sensing to assess the hydrological and meteorological determinants of FGA (*Cladophora* sp., *Oedogonium* sp., *Hydrodictyon* sp., and *Spirogyra* sp.) in a fluvial lake associated with the St. Lawrence River. They found water depth and water level change to be the most accurate hydrological predictors of FGA occurrence, and hours of sunshine and wind velocity were the most accurate meteorological predictors. No water chemistry variables were significant predictors. The authors validated model predictions with remote sensing images and found a 74% success rate in predicting FGA occurrence with these methods.

Once algal blooms are located, study sites must be characterized to inform development of watershed-specific management or restoration plans. A comprehensive approach is required, as discussed above, however this can be labor-intensive and time-consuming. Sampling can be especially difficult in deep waters, streams and rivers with poor access, or watersheds with high variation in pollution response across streams and rivers. A traditional yet comprehensive approach was employed by Stevenson et al. (2012) in the Illinois River Watershed, who combined estimates of algal biomass and percent cover with habitat characterization, water quality parameters, nutrient analysis, and land-use GIS analysis. The authors were able to determine the limiting nutrient, threshold responses of FGA to P, and estimate habitat response and degradation. Phosphorous thresholds corresponding to nuisance FGA biomass and percent cover were determined using classification and regression tree (CART) statistical methods. At TP concentrations past these thresholds, nuisance FGA would grow at a significantly greater rate than at lower concentrations. Importantly, the addition of land-use GIS analysis allowed these observations to be definitively linked to poultry operations in the watershed and provide managers with a specific source of nutrient pollution.

Unlike the study above, Mebane et al. (2014) did not find P concentrations in the water to be positively associated with macrophyte biomass in the agriculture-dominated Snake River basin (ID). This study examined several traditional measures of habitat quality and nutrient concentrations and tested how strongly each factor determined macrophyte abundance. Nitrogen in the sediment and water was positively correlated to biomass, as was loosely sorbed P in sediment. This study cautions against relying solely on water column nutrients and supports the use of sediment P analysis in management strategies. When sediment P was normalized to Al or Fe levels, which partly control P release through association, the strength of this nutrient analysis in determining macrophyte abundance increased further. Loosely sorbed P was extracted from sediment using $MgCl_2$ and the authors suggest this as a good method by which to measure bioavailable P in sediments. Furthermore, visual estimates of macrophyte percent cover were strongly correlated with biomass measurements. This is supported by Kilroy et al. (2013), who found that visual estimates of macrophytes distinguished sites and temporal events as effectively as chl *a* measurements. These estimates also did not differ across operators or rivers with sufficient training (Kilroy et al. 2013), and are a less laborious method to estimate relative macrophyte abundance in survey studies.

Sonar has the potential to greatly decrease the labor required to estimate macrophyte abundance in deeper bodies of water. Depew et al. (2009) successfully described the spatial distribution and stand height of *Cladophora* sp. in the littoral zone (1-10 m depths) of Lake Ontario using a high-frequency echosounder. This study utilized a single-beam echosounder which emits short bursts of sound and analyzes the intensity level of reflected sound (backscatter intensity), which can characterize the benthos based on known backscatter intensity signatures of species or taxa. The authors of this study were able to accurately estimate stand height and percent cover of *Cladophora* sp., but only on stands >7.5 cm. An accurate estimate of biomass was not able to be obtained. As suggested by Mebane et al. (2014), however, a biomass estimate is not necessarily required to obtain a relative measure of macrophyte standing crop. The efficacy of this method in rivers has yet to be demonstrated, but this is a promising tool that requires little effort in deep waters compared to scuba-based quadrat sampling.

Once an algal bloom site has been identified and well characterized, it is essential to understand ecological impact. Nutrient concentrations and algal abundance measures are indicative of stream health and often used as indicators. Macroinvertebrate or fish assemblages can also be used as indicators of stream health. These indices estimate biodiversity and can be reliable indicators of eutrophication, but not always (Wang et al. 2007). The simultaneous analysis of multiple indices, referred to as multi-metric indices (MMIs), have emerged as one of the most accurate and widely applicable methods by which to estimate ecosystem health (reviewed in Stevenson 2014). Impact as a function of biodiversity can also be determined by comparing the biota of near-pristine reaches (also termed reference reaches) and those suspected to be impaired or experimentally manipulated (Rosemond et al. 2015). By pairing this with analysis of water chemistry analysis and nutrient concentrations, and other variables that may be stream or watershed specific (Cattaneo et al. 2013), managers can develop informed methods by which to measure ecosystem health.

Suggestions for managers

In-stream monitoring would be spatially, temporally, and biogeochemically comprehensive in ideal studies. However limited resources always preclude such an exhaustive monitoring effort, and researchers should utilize methods that analyze processes most likely to control nutrients and macrophyte growth. Analysis of nutrient sources should include bioavailable nutrients in sediment as well as water column concentrations, since nutrients can leach from sediment and become available to macrophytes (Mebane et al. 2014). For example, loosely-sorbed P in sediment as measured by MgCl extraction can be a more accurate predictor of macrophyte production than water column nutrients in some systems (Mebane et al. 2014). Continuous monitoring of water quality measures such as pH and DO also are valuable, because large macrophyte blooms can cause harmful pH and oxygen swings overnight. In-stream light levels are an important consideration in any study of FGA blooms since light can often limit growth. Light, pH, and DO levels can be continuously monitored with fairly inexpensive sondes or light-logging pendants. If available, USGS monitoring stations should be utilized since they often measure these parameters, and may also monitor stream height, discharge, temperature, and specific conductivity.

Because algal response to nutrient input can be highly variable across basins, it is important that researchers comprehensively sample or monitor many reaches within watersheds. Expanding the scale of study to an entire basin would be ideal, and methods that require less effort and time than traditional methods will be highly valuable. ImageJ image analysis software and handheld GPS mapping computers are two promising tools that will decrease effort and increase the spatial scale of monitoring programs. ImageJ is an image analysis software that has been successfully tested as a means to measure benthic algal cover from images taken in the field (Griggs et al. 2015). This method could possibly be used to train observers and validate visual estimates taken in the field. Handheld GPS mappers make it possible to map the extent of bloom events with relatively little effort, and these have the potential to be incorporated into large-scale rapid assessment protocols.

Article Summaries

Title: The relative importance of local conditions and regional processes in structuring aquatic plant communities

Authors: Capers, R. S., R. Selsky, and G. J. Bugbee

Journal: Freshwater Biology 55:952–966, 2010

Questions Asked:

1. Can regional variation in plant distribution be explained entirely by environmental conditions, or do spatial patterns in distribution still exist when environmental variation has been eliminated?
2. Does community similarity among lakes decline with distance after accounting for variation in environmental conditions, indicating dispersal-limited distribution?
3. Can dispersal-related functional traits explain geographic distribution of species?

Abstract: (Capers et al. 2010)

1. The structure of biological communities reflects the influence of both local environmental conditions and processes such as dispersal that create patterns in species' distribution across a region.

2. We extend explicit tests of the relative importance of local environmental conditions and regional spatial processes to aquatic plants, a group traditionally thought to be little limited by dispersal. We used partial canonical correspondence analysis and partial Mantel tests to analyse data from 98 lakes and ponds across Connecticut (northeastern United States).

3. We found that aquatic plant community structure reflects the influence of local conditions (pH, conductivity, water clarity, lake area, maximum depth) as well as regional processes.

4. Only 27% of variation in a presence/absence matrix was explained by environmental conditions and spatial processes such as dispersal. Of the total explained, 45% was related to environmental conditions and 40% to spatial processes.

5. Jaccard similarity declined with Euclidean distance between lakes, even after accounting for the increasing difference in environmental conditions, suggesting that dispersal limitation may influence community composition in the region.

6. The distribution of distances among lakes where species occurred was associated with dispersal-related functional traits, providing additional evidence that dispersal ability varies among species in ways that affect community composition.

7. Although environmental and spatial variables explained a significant amount of variation in community structure, a substantial amount of stochasticity also affects these communities, probably associated with unpredictable colonisation and persistence of the plants.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. The distribution of organisms in space generally results from dispersal limitations and regional variation in environmental conditions
    b. Aquatic plants have traditionally been thought to be limited little by dispersal because many species are widely distributed.
        i. However the relative influence of dispersal limitation and variation in environmental conditions on small-scale plant community structure (within lakes and ponds) has not been tested.
    c. Because aquatic plants are used as indicator species, representing an integration of many environmental characteristics, it is important to understand the effects of dispersal limitation and environmental variation on the spatial structure of these communities.
    d. This study assessed the relative importance of environmental conditions and dispersal in aquatic plant community structure in lakes and ponds by investigating the following:
        i. Can regional variation in plant distribution be explained entirely by environmental conditions, or do spatial patterns in distribution still exist when environmental variation has been eliminated?
        ii. Does community similarity among lakes decline with distance after accounting for variation in environmental conditions, indicating dispersal-limited distribution?
        iii. Can dispersal-related functional traits explain geographic distribution of species?
2. **Methods**
    a. Sites
        i. 98 lakes and ponds in Connecticut; late June and late September in 2004 and 2005.
        ii. Selected non-randomly, but in all areas of the state with varying levels of human perturbation and environmental conditions.
    b. Sampling
        i. Surveys from small boats in all areas shallow enough to support plants, recording all submerged and floating-leaved species, based on visuals and collections with a long-handled rake.
            1. Used a grapple to sample in areas too deep to be reached with the rake.
            2. Also established line transects (80m length) extending perpendicular from shoreline. These recorded species were combined with those from surveys to compile full species list for each lake.
        ii. Environmental Conditions: water clarity, temperature, DO, pH, alkalinity, conductivity, total phosphorus
            1. Sampled water column at deepest point in each lake
            2. Water Clarity measured by Secchi depth
            3. Water temperature and DO measured at 0.5m below surface and 0.5m above bottom using YSI 58 meter

4. **4.** Water samples taken at same depths and stored at 3°C until analyzed for pH, alkalinity, conductivity, and total phosphorus
    - **a.** Conductivity and pH measured with Fisher-Accumet AR20 m.
    - **b.** Alkalinity quantified by titration with 0.16 N H_2SO_4 with an endpoint of pH 4.5.
    - **c.** Total phosphorus analysis on samples acidified with three drops of concentrated H_2SO_4 performed using ascorbic acid method and potassium persulphate digestion

- **c.** Statistical Analyses
    - **i.** Regional variation in environmental conditions
        - **1.** Canonical Correspondence Analysis executed with Canoco
    - **ii.** Aquatic plant community similarity with distance
        - **1.** Mantel tests
    - **iii.** Link of functional traits to dispersal
        - **1.** Fisher's exact test to assess independence of inter-lake distance distribution of species and their dispersal-related functional traits
        - **2.** Traits identified through a search of aquatic plant literature: reproducing asexually, producing large numbers of reproductive structures, dispersing readily with vegetative fragments, spreading on stolons or rhizomes, producing other kinds of propagules, growing unrooted, and remaining viable during transport by birds and/or boats

3. Results

- **a.** Regional variation in environmental conditions
    - **i.** "Overall, environmental and spatial variables together explained 27.5% of variation in species occurrences, with 72.5% unexplained… Of the total variation, 16.7% was associated with environmental variables, and 15.0% was associated with spatial structure."
    - **ii.** Most strongly correlated variables were pH, conductivity, area, depth, and water clarity
- **b.** Aquatic plant community similarity with distance
    - **i.** As distance between lakes increased, plant communities became less similar
    - **ii.** Distance between lakes correlated positively with pH, conductivity, and total phosphorus
        - **1.** Community differences increase with distance even when these effects are removed
- **c.** Link of functional traits to dispersal
    - **i.** Dispersal traits are not independent from inter-lake distance distributions of species, however these traits are not correlated with overall frequency.

4. Discussion

- **a.** Both environmental conditions and dispersal ability appear to influence aquatic plant community structure

b. Community similarity decreased with distance and was associated with dispersal-related plant traits, indicating that spatial variance among plant communities is in part caused by dispersal limitation

   i. "… runs counter to traditional thinking about aquatic plant communities."

c. Chance establishment and extinction may have resulted in the unexplained variation in community structure observed in this study.

   i. "For macrophytes, the questions of importance relate to how species vary in dispersal and colonization abilities, and how these differences affect the spatial pattern in species' occurrence and community composition."

Title: Species richness of both native and invasive aquatic plants influenced by environmental conditions and human activity

Authors: Capers, R. S., R. Selsky, G. J. Bugbee, and J. C. White

Journal: Botany 87:306–314, 2009

Questions Asked:

1. Which environmental conditions, including human activities, are associated with species richness of native and invasive aquatic plants?
2. Are invasives more likely than natives to become dominant, and do the dominant species of lakes reflect that?

Abstract: (Capers et al. 2009)

Invasive species richness often is negatively correlated with native species richness at the small spatial scale of sampling plots, but positively correlated in larger areas. The pattern at small scales has been interpreted as evidence that native plants can competitively exclude invasive species. Large-scale patterns have been understood to result from environmental heterogeneity, among other causes. We investigated species richness patterns among submerged and floating-leaved aquatic plants (87 native species and eight invasives) in 103 temperate lakes in Connecticut (northeastern USA) and found neither a consistently negative relationship at small (3-m2) scales, nor a positive relationship at large scales. Native species richness at sampling locations was uncorrelated with invasive species richness in 37 of the 60 lakes where invasive plants occurred; richness was negatively correlated in 16 lakes and positively correlated in seven. No correlation between native and invasive species richness was found at larger spatial scales (whole lakes and counties). Increases in richness with area were uncorrelated with abiotic heterogeneity. Logistic regression showed that the probability of occurrence of five invasive species increased in sampling locations (3 m2, n = 2980 samples) where native plants occurred, indicating that native plant species richness provided no resistance against invasion. However, the probability of three invasive species' occurrence declined as native plant density increased, indicating that density, if not species richness, provided some resistance with these species. Density had no effect on occurrence of three other invasive species. Based on these results, native species may resist invasion at small spatial scales only in communities where density is high (i.e., in communities where competition among individuals contributes to community structure). Most hydrophyte communities, however, appear to be maintained in a non-equilibrial condition by stress and/or disturbance. Therefore, most aquatic plant communities in temperate lakes are likely to be vulnerable to invasion.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**

a. It has traditionally been thought that community species richness is negatively correlated with invasion risk by transients. However observational studies under natural conditions have reported a positive correlation between native and invasive species richness. Furthermore, dominant species can influence the occurrence of other community members, yet it is unknown whether native or invasive species are more likely to exhibit dominance. "[This study] investigated patterns of species richness and dominance among native and invasive aquatic plants in 99 temperate lakes."

2. **Methods**
   - **a.** Sites
     - **i.** 99 lakes and ponds in Connecticut; late June to late September in 2004 and 2005.
     - **ii.** Selected non-randomly, but in all areas of the state with varying levels of human perturbation and environmental conditions.
   - **b.** Sampling
     - **i.** Surveys from small boats in all areas shallow enough to support plants, recording all submerged and floating-leaved species, based on visuals and collections with a long-handled rake.
       1. Used a grapple to sample in areas too deep to be reached with the rake.
     - **ii.** Also established line transects (80m length) extending perpendicular from shoreline. These recorded species were combined with those from surveys to compile full species list for each lake.
   - **c.** Environmental Conditions: water clarity, temperature, DO, pH, alkalinity, conductivity, total phosphorus
     - **i.** Sampled water column at deepest point in each lake
     - **ii.** Water Clarity measured by Secchi depth
     - **iii.** Water temperature and DO measured at 0.5m below surface and 0.5m above bottom using YSI 58 meter
     - **iv.** Water samples taken at same depths and analyzed for pH, alkalinity, conductivity, and total phosphorus
       1. Conductivity and pH measured with Fisher-Accumet AR20 m.
       2. Alkalinity quantified by titration with 0.16 N H_2SO_4 with an endpoint of pH 4.5.
       3. Total phosphorus analysis on samples acidified with three drops of concentrated H_2SO_4 performed using ascorbic acid method and potassium persulfate digestion
   - **d.** Human Activity
     - **i.** Categorical assignments based on boat accessibility and motor restrictions from Connecticut DEP
   - **e.** Dominance
     - **i.** Used a 5-point abundance scale, and calculated mean abundance across all sampling sites in each lake. A species was declared dominant if it

achieved a mean abundance value 50% or greater of the 5.0 maximum value.

- **ii.** Productivity was estimated by multiplying the abundance of a species by a categorical estimate of biomass

f. Statistical Analyses

- **i.** Linear regression used to determine which measured variables had the greatest influence on species richness for native and invasive plants
- **ii.** Linear regression used to determine which environmental conditions contributed most to dominance
- **iii.** "… also used logistic regression with the full data set to determine which environmental conditions affected the probability of invasive species' occurrence."

3. Results

a. Species Richness

- **i.** Native richness correlated with water clarity, productivity, human activity, and lake area
- **ii.** Invasive species richness correlated with alkalinity, pH, and human activity
- **iii.** "… the probability of invasive species' occurrence increased with human activity… and surface water alkalinity, but not with other environmental variables."

b. Dominance

- **i.** 29 of 99 lakes had high dominance
- **ii.** invasive species were dominant in 11% of the lakes where they occurred; native plants were dominant in 22% of the lakes where they occurred
- **iii.** Dominance was correlated with productivity, lake area, and maximum depth

4. Discussion

a. Species richness

- **i.** Native and invasive plants responded similarly to human activity, yet differently to the other environmental variables
- **ii.** Alkalinity was negatively correlated to native species richness and positively correlated to invasive species richness and probability of occurrence. Invasive species may have stronger affinities for alkaline waters, and therefore areas with alkaline waters may be at greater risk for invasion.
- **iii.** Human activity positively affected native and invasive species richness
- **iv.** Nutrient levels appear to have no effect on species richness overall, but have greater importance in eutrophic lakes

b. Species richness as a buffer against invasion

- **i.** Native species richness did not affect invasive richness, and vice versa
  - **1.** "These results suggest that environmental conditions, not biotic interactions, determine community membership among aquatic

plants. They suggest that the number of native species does not provide resistance against invasion of aquatic plant communities, as predicted by the community resistance hypothesis."

2. "The relationship between native and invasive species richness is scale-dependent. Negative correlation is most likely to be observed at the smallest spatial scales, at which neighboring plants interact, and evidence has been found in the studied lakes that high plant density (and not species richness) provides richness against invasion by some species in individual sampling locations."

Title: Phosphorus regulates stream injury by filamentous green algae, DO, and pH with thresholds in responses

Authors: Stevenson, R. J., B. Bennett, D. Jordan, and R. French

Journal: Hydrobiologia 695:25–42, 2012

Hypotheses Tested:

1. "Poultry house operations, as well as other human activities in the [Illinois River Watershed (IRW)], increased phosphorous concentrations in IRW streams"
2. "Phosphorous concentrations in IRW streams stimulated algal biomass accumulation"
3. "Algal biomass accumulation affected DO concentrations and pH in streams"

Abstract: (Stevenson et al. 2012)

Nutrient concentrations, benthic algal biomass, dissolved oxygen (DO), and pH were measured in 70 or more streams during spring and summer in the Illinois River Watershed (IRW), which crosses the Oklahoma and Arkansas (USA) border, to determine whether injury to streams occurred and if that injury was related to spreading poultry waste on fields. Definitions of injury were based on Oklahoma water quality regulations and scientific literature. Phosphorus and nitrogen concentrations were each independently related to poultry house density (PHD) in watersheds and percent urban land use in watersheds. In addition, phosphorus and nitrogen concentrations were unusually high compared to regions with similar geology and hydrology. Molar N:P ratios were high and indicated that phosphorus was the most likely limiting nutrient. Phosphorus concentrations, as well as PHD and urban land use, were related to algal biomass during spring, but were less related during summer. A threshold response in cover of stream bottoms by nuisance filamentous green algae (NFGA: Cladophora, Rhizoclonium, and Oedogonium) during spring was observed at 27 μg TP l−1 using regression tree analysis. Great increases in average NFGA cover (from 4 to 36% cover) occurred with relatively small increases in TP concentration at the 27 μg TP l−1 threshold. Average concentrations of DO, variability in DO, and pH during spring were positively related to TP, chlorophyll a, and NFGA cover. Minimum DO during spring and early morning DO during summer were negatively related to TP concentration. Spring pH and summer DO frequently violated water quality requirements for protecting biodiversity that were established by the state of Oklahoma. We conclude that poultry house operations as well as urban activities, independently and interactively, pollute IRW streams with phosphorus, which resulted in injury to aesthetic condition and the potential for injury of biodiversity.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**

   a. Study a result of lawsuit filed by Oklahoma Attorney General against poultry companies to control application of poultry manure to fields in the Illinois River Watershed (IRW)
   b. Study was "designed to quantitatively relate nutrient pollution to decreases in ecosystem services and evaluate whether poultry waste application to fields was a significant contributor to decreases in ecosystem services."
   c. "Thresholds in ecological responses along environmental gradients help develop consensus among stakeholders for management actions. We use the term threshold in this article to refer to an unusually great change in a dependent variable (e.g., algal biomass) within a small range of the independent variable [e.g., total phosphorous (TP) concentration] when compared to the magnitude of change across the entire range of the independent variable."

2. **Methods**
   a. IRW Study Area: 4,328 km^2 watershed in Ozark Highlands ecoregion
      i. 8.8% Urban, 43.7% agricultural, 46.0% forested land
         1. 99.7% of agricultural land is pasture
      ii. 3,226 poultry houses, of which 60% are active
         1. 26% inactive, 11% abandoned, 3% removed, 9% "suspected to be poultry houses"
   b. Site Selection
      i. Land use (% Urban, % Agricultural) and poultry house density (PHD) of accessible site watersheds determined
         1. Range in PHD used to split sites into quintiles, and ~70 sites semi-randomly selected with even numbers from each quintile
   c. Sampling
      i. Summer 2006 and Spring 2007 to account for high algal biomass associated with cooler Spring water temps and low DO in low-flow, warm-water summer
         1. "… sampled streams in haphazard order from week to week during the spring 2007 campaign to eliminate bias that could be cause by diurnal variation in temperature, light, and nutrient concentrations."
      ii. Water Analysis
         1. pH, conductivity, and temperature measured with Oakton model 300 multimeter
         2. DO measured in the field calorimetrically with Chemetrics V-2000 photometer
         3. Water samples collected for TP, TP, SRP, NO_x, and stored on ice until analysis using standard APHA assay methods
      iii. Chl *a* per unit area of stream bottom
         1. Fifteen rocks randomly selected from five riffle regions in each stream
         2. Algae scraped from pre-determined areas

a. Filtered and frozen day of sampling and until chl *a* was measured fluorometrically and used to determine chl *a* per unit area of stream bottom

iv. Nuisance filamentous green algae (NFGA) percent cover

1. Viewing frame with 50 point grid to quantify percent cover

2. 20 observations along 5-10 transects in riffles

d. Statistical Analyses

i. "… grouped variables into four categories according to their causes and effects in the conceptual model. These categories were: (1) land use and land cover, (2) nutrients, (3) algal biomass, and (4) chemical stressors."

1. Tested direct and indirect effects among variables using regression analysis

2. Used logarithmic, square root, and positive power transformations to normalize distributions of variables

ii. Threshold values identified using classification and regression tree analyses (CART)

3. Results

a. N:P ratios indicate P is limiting

b. TP and TN both positively related to PHD and also percent urbanized land use in Spring

c. Benthic algal chl *a* and NFGA significantly and positively related to TP, PHD and percent urban land use

i. "Thresholds, [or change points,] were indicated in [both] chl *a* and NFGA cover along the TP gradient, but only the NFGA CART model explained substantially more variation than the linear model."

1. Change points in TP:

a. Benthic chl *a*: 0.029 mg/l

b. NFGA cover (spring): 0.027 mg/l

d. DO

i. Average Spring DO positively related to chl *a*, NFGA cover, TP, and PHD and urban land use

ii. Summer DO levels negatively related to TP, but only when analysis was limited to "streams with a low percentage of urban land use in their watershed..."

iii. DO positively related to urban land use, but not PHD

1. "… statistical masking of poultry house effects by urban effects became evident."

2. When analysis again limited to streams with low urban land use in their watersheds, DO was negatively related to PHD

e. pH: average pH increased with increasing benthic algal biomass, NFGA cover, TP, urban land use, "and probably PHD"

4. Discussion

a. "Many watersheds in the IRW have high PHD, high phosphorus, and low urban land use, leaving poultry waste as the only reasonable source of phosphorous in those streams."

b. "NFGA densities were low and unrelated to TP during summer, probably because optimal temperatures for NFGA growth occur during spring.

c. Benthic algal biomass generally becomes saturated at ~0.030 mg/l in streams throughout the world (Dodds et al. 1997a)

d. "Estimating NFGA cover by visual assessment is relatively error proof compared to assessing benthic algal chl *a* in the laboratory…"

- **i.** NFGA believed to be more accurate because chl *a* were likely under-sampled. Use of a small-bore pipette to collect subsamples during assays which could not accurately sample the filamentous algae.
- **ii.** Significant relationships observed with chl *a* likely due to micro algae on NFGA that fell off during processing

e. A fish kill occurred in spring 2006, wherein 1,343 fish (stonerollers) were estimated to have died in a 2 km segment of the Illinois River.

- **i.** Extensive growths of NFGA observed at this site
- **ii.** DO monitored 24 hours after site, and dropped to 0.9 mg/l the following morning
- **iii.** "It seems highly likely that the low DO concentrations killed the fish and were caused by NFGA, which in turn resulted from high TP concentrations."

f. "Increases in pH with algal biomass are probably related to algal uptake of CO_2 and a shift in the carbonate equilibrium…"

- **i.** Elevated pH related to high algal biomass can have a negative effect on biodiversity based on spring values (>9.0)

g. Overall threats to biodiversity caused by poultry industry activity and NFGA

- **i.** Punctuated DO and pH stress
- **ii.** Habitat Alteration

Title: Phosphorus in runoff from two watersheds in lost river basin, West Virginia

Authors: Elrashidi, M. A., C. A. Seybold, D. A. Wysocki, S. D. Peaslee, R. Ferguson, and L. T. West.

Journal: Soil Science 173, 2008

Study Objective:

"… to estimate the loss of water and P by runoff from two watersheds in the Lost River basin (CR/UCR watersheds) and to predict the impact on water quality."

Abstract: (Elrashidi et al. 2008)

The loss of nutrients in runoff from soils treated with heavy manure application is a major cause of poor surface water quality in the United States. Poultry production in the Hardy County, West Virginia, has increased considerably since the early 1990s. The Lost River basin contains the highest density of poultry houses in the county. Most of phosphorus (P)-rich manure produced is land applied, and concerns over water quality impacts are widespread. The objectives of this study were to apply the Natural Resources Conservation Service technique on two watersheds (Cullers Run and Upper Cove Run) in the Lost River basin to predict the loss of water and P from soils by runoff and to estimate the impact on water quality. The predicted average runoff was 4374 m3/ha per year, and agreed with the observed average runoff of 4267 m3/ha per year. This gives an annual runoff of 74.6 million m3 for the two watersheds. The average P loss by runoff was 0.57, 1.98, and 5.51 kg/ha per year from soils under forest, pasture, and crop, respectively. The high P loss by runoff was probably associated with application of P fertilizer or poultry manure to cropped soils. The total annual loss of P from soils by runoff was estimated at 16,435 kg. The predicted P concentration varied widely in runoff water generated from different soils and land covers. The average P concentration in runoff water was 133, 432, and 1146 µg/L for forestland, pastureland, and cropland, respectively. The predicted average P concentration in runoff was 224 µg/L for the two watersheds. However, the observed P concentration was very low (1.3-13.3 µg/L) in the monthly water samples (January-December 2006) collected from the Lost River, where the pH ranged between 7.6 and 8.4. The average pH in soils was 4.22, 5.42, and 6.15 for forestland, pastureland, and cropland, respectively. Changing the pH of runoff water from acidic (soils) to the alkaline range in the Lost River could precipitate calcium phosphates and decrease P concentration in water. The technique predicted P concentration in runoff at the edge of field. The increase in water pH as well as P removal by aquatic weeds and algae could be the cause of the low P concentration observed in the Lost River.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. Hardy County (containing the Lost River) ranks first in West Virginia poultry production

b. County is 73% forest, 19% pasture, 6% crops, 1% urban, 1% recreational
c. Only level land in the county is that which runs along the major rivers of the Potomac basin
d. The poultry industry “has increased considerably” in the 20 years prior to 2008
    i. “The Lost River basin was identified by these agencies [state and federal] as producing twice as much poultry litter, or manure, as the available agricultural land [(U.S. Department of Agriculture, National Resources Conservation Services (USDA/NRCS) 1996)].”
    ii. “Both private and government programs are in place to move poultry litter out of the Lost River watersheds; however, it is generally accepted that considerable amounts of manure are still applied to soil.”
e. This study specifically investigated Cullers Run and the Upper Cove Run watersheds

2. **Methods**
    a. See article for soil sampling and analysis
    b. Water samples and analysis
        i. Samples taken monthly (January-December 2006) from 2 sites (see article for map and locations)
            1. One in the CR and representative of that watershed,
            2. One in the UCR, which was representative of the UC/UCR watersheds combined
        ii. Taken mid-stream with poly bottles, following 2 rinses with stream water; refrigerated at 4 °C until analysis
        iii. Filtration: glass syringe and Whatman GD/X disposable nylon filter, 0.45 µm pore size
        iv. pH: “measured with a combination glass electrode and digital pH/ion meter…” (reference in article)
        v. P concentration: modified phosphomolybdate/ascorbic acid method (reference in article)
        vi. Stream flow and total discharge data from USGS stations in 6 other watersheds (there were none in the CR/UCR watersheds) used to test accuracy of runoff model
    c. P runoff model technique (references in article) to “estimate runoff P for agricultural watersheds”
        i. “the AER [anion exchange resin] method is used to determine phosphorus (P) release characteristics (PRC) for soils”
            1. A way to account for different soil types in the watershed and their different propensities to release P
        ii. “the runoff model is applied to estimate run-off from soil by an annual rainfall”
        iii. “an energy conversion factor that relates soil-water suspension (AER method) to rainfall energy is used to estimate runoff P”

3. **Results**

a. Condensed Results
    i. Cropland contributes the most runoff P to the watershed despite a lower runoff water rate, annual volume of runoff water, and total land area, compared to pastureland and forestland
    ii. P concentrations in stream water were lower than estimated values in runoff water
    iii. High pH values observed in stream water
b. Estimates of average runoff volume (area-weighted)
    i. Rates ("in good agreement with observed average runoff [from USGS stations in other Hardy County watersheds]")
        1. Cropland: 4835 m^3/ha per year (53.8% of annual rainfall)
        2. Pastureland: 4580 m^3/ha per year (51.0% of annual rainfall)
        3. Forestland: 4296 m^3/ha per year (47.8% of annual rainfall)
    ii. Total annual runoff volume in watershed from estimated rates
        1. Forestland: 52.9 million m^3/year (from 12,304 ha)
        2. Pastureland: 17.5 million m^3/year (from 3,820 ha)
        3. Cropland: 1.3 million m^3/year (from 266 ha)
c. Runoff Phosphorus
    i. Estimates of average P concentration in runoff water (with ranges of varying soil types)
        1. Cropland: mean 1146 μg/l (range: 604-1650 μg/l)
        2. Pastureland: mean 432 μg/l (range: 113-790 μg/l)
        3. Forestland: mean 133 μg/l (range: 101-185 μg/l)
    ii. Estimates of average runoff P
        1. Cropland: 5.51 kg/ha per year
        2. Pastureland: 1.98 kg/ha per year
        3. Forestland: 0.57 kg/ha per year
    iii. Average P concentrations in stream water
        1. CR watershed: 5.99 μg/l (range: 1.3-12.6 μg/l)
        2. CR/UCR watershed: 3.47 μg/l (range: 0.0-13.3 μg/l)
d. pH: "High pH values… were measured in stream water that ranged from 7.6 to 8.4…"
    i. Values from a previous long-term study (Cacapon Institute 2002)
        1. 1 site in CR watershed: median 7.4
        2. 2 sites in UCR watershed: medians of 8.1 and 8.4

4. **Discussion**
    a. The modest volume of runoff generated by cropland reflects the small percentage of watershed occupied by this land type
    b. "Land cover (i.e., forest, pasture, and crop) could affect the amount of P released from surface soil by rainfall in two different ways:"
        i. "it affects the volume of surface water runoff generated by rainfall"
        ii. "it minimizes the area of surface soil exposed to direct rainfall energy"
        iii. Related to canopy coverage of plant community

c. Contributing factors to high P runoff from cropland
    i. Runoff water volume
    ii. Soil exposed to direct kinetic energy of rainfall (impermanent canopy coverage)
    iii. "The high P loss by runoff from cropland was probably associated with application of P fertilizer or poultry manure to these soils."
d. While forestland and pasture generally experience lower P input, manure may still be added to pastureland through soil amendment or litter disposal
e. Observed low P concentrations in stream water compared to runoff P concentrations
    i. May have been due to elevated stream water pH, which alters the dominant orthophosphate, monocalcium phosphate [$Ca(H_2PO_4)_2$], to phosphate complexes with lower solubility (Lindsay 1972)
    ii. "Furthermore, the presence of large populations of algae, weeds, and aquatic plants in the stream water could assimilate P and decrease the concentration in the water."

Title: Periphyton control on stream invertebrate diversity: is periphyton architecture more important than biomass?

Authors: Tonkin, J. D., R. G. Death, and J. Barquín

Journal: Marine and Freshwater Research 65:818–829, 2014

Study Objective:

"We set out to (1) test the response of stream invertebrate-diversity metrics, often used in biomonitoring, to periphyton biomass (assessed as chlorophyll *a*) and(2), because biomass measurements do not account for variation in the growth form of primary producers, to examine whether this link can be better explained by underlying responses to different growth forms of periphyton categorized coarsely into two major groups (i.e. all non-filamentous films and mats and streaming filamentous green algae)."

"We also use a common stream-specific metric, %EPT (Ephemeroptera, Plecoptera and Trichoptera), to assess whether this metric is more sensitive to environmental gradients in streams than are simple invertebrate diversity measurements."

Abstract: (Tonkin et al. 2014)

There is little consensus on the form of the periphyton biomass–macroinvertebrate diversity relationship in streams. One factor that these relationships do not account for is the growth form of primary producers. We (1) examined the periphyton biomass–macroinvertebrate diversity relationship in 24 streams of Cantabria, Spain, in July 2007, and (2) determined whether this relationship was underpinned, and better explained, by specific responses to the growth form of the periphyton community. We hypothesised that macroinvertebrate diversity would be a log-linear function of periphyton biomass and would respond differently to two coarse divisions of the periphytic community; i.e. positively to %cover of non-filamentous algae and negatively to %cover of streaming filamentous algae. There was no relationship between benthic periphyton biomass and macroinvertebrate diversity in these streams but, as predicted, this relationship was underpinned by responses to the growth form of periphyton community. Generally, macroinvertebrate diversity responded positively to %cover of non-filaments and negatively to %cover of streaming filaments, although results were variable. These findings suggest that periphyton biomass–macroinvertebrate diversity relationships in streams can be underpinned by interactions with specific growth forms of periphyton. We suggest that further research is required to develop robust thresholds of % cover of filamentous algae cover that would benefit managers wishing to minimize negative effects of eutrophication on stream communities.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**

- **a.** Compared with lentic, few have investigated if high productivity increases biodiversity in lotic systems
- **b.** Different growth forms of algae fulfill different functional roles in the community
  - **i.** When epilithic films are replaced by macroalgae… "interactions between grazers and periphyton can shift from simple plant-herbivore interactions to more complex relationships."
- **c.** Hypotheses:
  - **i.** "We hypothesise that invertebrate diversity, including richness and rarefied richness, will increase logarithmically with increasing periphyton biomass, but we predict that this relationship will be under-pinned by particular responses to different growth forms of periphyton."
    - **1.** "… invertebrate diversity will respond positively to %cover of non-filamentous and negatively to %cover of streaming filamentous algae."

2. Methods

- **a.** Sites
  - **i.** 24 streams in the Cantabria region of Northern Spain
    - **1.** Sites selected *a priori* in pairs (high productivity and low productivity), with two from each of six river basins sampled in the region
- **b.** Water samples and analysis
  - **i.** Depth and velocity: Marsh-McBirney flowmate current meter
  - **ii.** Conductivity, temperature, DO, pH: YSI 556 MPS meter
  - **iii.** Nitrates, phosphate, ammonia:
    - **1.** Unfiltered samples kept in dark on ice during transport
    - **2.** Measured with Beckman Coulter spectrophotometer
- **c.** See paper for substrate-size composition methods
- **d.** Algal biomass and coverage
  - **i.** Periphyton biomass: chl *a* extracted from five stones collected randomly from riffles within a ~50m reach at each site
  - **ii.** Kept in cool, dark environment during transport, then frozen until analysis
  - **iii.** Chl *a* and phaeophytin extracted with 90% acetone at 5 °C for 24 hrs in the dark
    - **1.** Measured with Beckman Coulter spectrophotometer
  - **iv.** %Cover non-filamentous algae
  - **v.** %Cover streaming filamentous algae
    - **1.** "Visually assessed along three randomly located transects across the entire width of the stream bed within riffles along the sample reach, using modified rapid assessment protocols from the New Zealand Stream Periphyton Monitoring Manual (Biggs and Kilroy 2000)
      - a. Every stone across each transect
      - b. Grouped all algae into the two forms

c. Used same observer at all 24 sites to reduce bias

e. Macroinvertebrates

- **i.** Sampling
  - **1.** Five 0.1 m^2, 500 µm mesh Surber samples at each site from random locations
  - **2.** Preserved in 10% formalin in the field
  - **3.** Washed through 500 µm and 1 mm sieves before identification
- **ii.** Rarefied Richness Calculation
  - **1.** Calculated for lowest average number of animals at a site
  - **2.** "Rarefaction accounts for the passive increase in the number of taxa collected with increasing number of individuals collected… This, in effect, standardizes sites by predicting richness per a set number of animals rather than a set area."

f. Statistical Analysis (all done with R)

- **i.** Pearson Correlation Coefficient (w/rcorr() function in R): Correlation of inverts, periphyton, and physiochemical variables
- **ii.** Linear Regression: "… relationships among periphyton biomass, %cover of non-filaments, %cover of streaming filaments and invertebrate metrics…" (using lm() function in R)
  - **1.** Log-transformed data to remove heteroscedasticity
- **iii.** Thresholds: where apparent in response of inverts to streaming filaments, "we tested these using the cpt.mean() procedure in the R package 'changepoint'… We used Bayesian information criteria (BIC) and 'at most one change' (AMOC) to select the location of single change-points if present."
- **iv.** Also constructed non-metric multidimensional scaling (NMDS) ordination to visually examine non-linear effects of periphyton

3. Results (averages shown with SE unless otherwise noted)

a. Physiochemical and Periphyton

- **i.** Periphyton biomass (chl *a*): 5.8 ± 0.7 µg cm^{-2}
  - **1.** Non-filaments %cover: 48.3 ± 5.6 %
  - **2.** Streaming filaments %cover: 24.0 ± 6.1
- **ii.** Chlorophyll *a* positively correlated with %cover of filamentous algae
- **iii.** Conductivity positively correlated with chl *a* and %cover of streaming algae

b. Invertebrate Community Composition

- **i.** Average taxonomic richness: 15.6 ± 0.6 (range 9.6 – 21.0) taxa / 0.1 m^2
- **ii.** Density of individuals: 928.2 ± 406.7 individuals/ 0.1 m^2
- **iii.** %EPT: 56.8 ± 3.5 %
- **iv.** Most abundant families:
  - a. Ephemeroptera: 47.9 3.2 %
  - b. Diptera: 25.9 ± 4.1 %
  - c. Coleoptera: 5.1 ± 0.7 %

d. Trichoptera: 4.6 ± 0.8 %
e. Plecoptera: 3.6 ± 0.8 %

2. "These patterns largely due to the abundance on three individual taxa…" (*Baetis* spp., *Prosimulium* spp., *Echinogammarus* spp.)

c. Density and Diversity Patterns (graphs from article referenced for visual aid)

i. Chl *a*: only response to this metric of biomass seen in number of individuals *(graph d)*

1. Although significant, r^2 was low

ii. "Density and diversity measures exhibited opposing responses to the two growth forms of periphyton measured."

1. Non-filamentous algae

a. Taxonomic and rarefied richness: increased log-linearly with increasing non-filamentous cover *(graphs b, h)*
b. Density of individuals not related *(graph e)*
c. %EPT: "… peaked strongly at intermediate levels and declined at higher levels of %cover of non-filamentous algae…" *(graph k)*

i. Largely influenced by one site with 95% non-filamentous algae cover

2. Filamentous streaming algae

a. Density of individuals exhibited quadratic increase with increasing %cover *(graph f)*
b. Taxonomic richness not related *(graph c)*
c. Rarefied taxonomic richness and %EPT exhibited a curvilinear decline with increasing %cover *(graphs i, l)*

i. "However, removing [a] site with 92% cover of streaming filamentous algae removed any relationship with %EPT…"

iii. Changepoint at 40% cover of streaming filamentous algae (no other significant changepoints identified)

1. Taxonomic richness dropped from 16.27 taxa to 13.60 taxa

2. Rarefied richness dropped from 15.32 taxa to 11.73 taxa

4. Discussion

a. "There was no relationship between periphyton biomass and invertebrate diversity in the present study."

b. "Although invertebrate communities did not respond clearly to changes in periphyton biomass, the growth form of the periphyton community was important in determining diversity patterns."

i. Shows that a focus on biomass can mask important underlying relationships

ii. This bottom-up control of algal communities on invertebrates has not been well studied in streams, because most has focused on top-down control via grazing invertebrates

c. Response to periphyton growth forms were mainly due to changes in three dominant taxa (*Baetis* spp., *Prosimulium* spp., *Echinogammarus* spp.)
    i. Appeared to be species-specific and influenced by feeding habits (i.e., drift feeders, grazers, etc..)

d. "Percentage of non-filaments was the best predictor of diversity, with both taxonomic and rarefied richness increasing log-linearly as cover increased."
    i. "Diatoms are likely the most important food source for a high proportion of benthic invertebrates, because grazers tend to be able to assimilate diatoms better than other algal taxonomic classes (Lamberti et al. 1989)."

e. %EPT responded to growth form of periphyton, but not biomass
    i. Declined at highest levels of non-filaments possibly due to palatable diatoms being replaced by less palatable taxa
    ii. Saw %EPT remain high up to about 75% streaming filament cover (had expected decline)
        1. Could be due to filamentous algae hosting palatable diatoms
            a. These epiphytic diatoms can also reduce shear stress on streaming filaments (Hansen et al. 2014), which "could in fact alter the hydrodynamic environment for grazers."
            b. May also retain more detritus, providing additional food for other functional groups

f. Rarefied richness declined strongly at intermediate levels of streaming filaments
    i. Because of marked increase in *Prosimulium* spp.
    ii. More complex relationships involving habitat provisioning and feeding interactions develop with higher density of filamentous algae
    iii. Dense filamentous algae can cause large DO fluxes, leading to replacement of more sensitive inverts by other taxa

g. Dense levels of streaming filaments in this study possibly a result of selective grazing where filamentous algae is rejected in favor of more palatable taxa with lower cellulose content and less thick cell walls
    i. Same force can lead to increases in abundance of cyanobacteria (Hart 1985)
    ii. This selectivity can be so strong that some grazing insects may even remove unpalatable forms to clear substrate for more palatable forms to grow (Hart 1985)

h. Management Implications
    i. "Given we only spot-measured nutrients in the present study, we cannot directly attribute nutrients as the main underlying stressor here."
    ii. "We suggest that the cover of streaming filamentous algae could be a useful threshold indicator in streams."
    iii. "Ideally, this should occur throughout the year and at least during summer low-flow periods, as was the case in the present study, but not following a high flow events."
        1. Filamentous green algae vulnerable to high-flow events

Title: Effects of macroalgae on a stream invertebrate community

Authors: Dudley, T. L., S. D. Cooper and N. Hemphill

Journal: Journal of the North American Benthological Society 5(2):93-106, 1986

Study Objective:

"… examined the relationships between disturbance, macroalgae and the winter and summer faunas of a southern California stream."

Abstract: (Dudley et al. 1986)

The effects of macroalgae on stream invertebrates were studied in riffle zones in a coastal southern California stream. Dense growths of macroalgae (Cladophora glomerata, Nostoc sp.) were experimentally removed at different times of the year and in different years, and the insect communities which developed were compared with those in unmanipulated controls. Marl precipitated by algae was also removed in one experiment. The presence of macroalgae was associated with greater total densities and taxon richness of invertebrates, and nearly all taxa responded significantly to algal removal on at least some dates. Insects formed most of the community and were classified according to three categories of macroalgal effects on benthic densities: 1. Negatively affected by macroalgae (and marl) due to competition for space--e.g., Blephariceera (strong response to both algal taxa); large Simulium (strong with Cladophora, weak with Nostoc). 2. Positively affected due to structural habitats created by algae--e.g., Micrasema, Rhyacophila and Hydropsyche (all strong); Tinodes (weak-Nostoc); Rheotanytarsus (strong-Nostoc). 3. Positively affected by both macroalgal structure and associated food resources (macroalgae or epiphyton)--e.g., Baetis and Chironomidae (strong-Cladophora, weak-Nostoc); Hydroptila, Ochrotrichia, and Euparyphus (strong-Cladophora); and endosymbiotic Cricotopus (strong-Nostoc). Natural disturbances will indirectly affect invertebrate distributions and abundances by affecting the distributions and abundances of macroalgae.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
   a. "Macroalgae may: 1. Provide food, either directly or indirectly, to herbivores; 2. Alter physical conditions; and 3. Compete with invertebrate taxa for attachment space."
   b. Macroalgae alter habitat in several ways
      i. Plants themselves provide substrate for attachment
      ii. Modify habitat:
         1. Water flow
         2. Light penetration
         3. O2 fluxes

iii. Trap detritus

c. "In our study, we examined the relationships between disturbance, macroalgae and the winter and summer faunas of a southern California stream… we used controlled field experiments to clarify these relationships."

2. **Methods**
   a. Study Site (Rattlesnake Creek)
      i. Second order coastal stream
      ii. Well-developed but open riparian zone and dense chaparral upslope
   b. Single winter experiment
      i. Done on rock and concrete bridge face with 20% slope
      ii. 10 x 10 cm quadrats initially cleared and succession followed
      iii. Treatment quadrats paired with adjacent control quadrats in a randomized block design
      iv. Algal percent cover estimated with a Plexiglas viewer, marked with 33 randomly placed circles (presence of absence in each circle recorded
         1. "… this technique underestimated the small insects… and those within the algal matrix.."
   c. Two summer experiments
      i. Done on natural boulders as well as bridge face
      ii. Sampling and monitoring
         1. 10 x 10 cm wire grid separated into 64 squares; presence of algae and number of macro-invertebrates recorded
         2. 29 of these quadrats randomly assigned to four treatments:
            a. *Nostoc* removal
            b. *Cladophora* removal
            c. Both algae removed
            d. Undisturbed controls
         3. Treatments maintained by removing algae with forceps weekly
         4. Quadrats destructively sampled (scraped clean) at conclusion
   d. Statistical Analysis
      i. Two-way ANOVA and regression analyses used to examine relationships between algae and invertebrate responses
         1. Log transformed invertebrate density values where appropriate for analysis
      ii. Invertebrates present in study:
         1. *Simulium*: genus black flies of order Diptera - true flies
         2. *Euparyphus*: genus of soldier flies of order Diptera - true flies
         3. Chironomids: family of non-biting midges of order Diptera - true flies
         4. Dixidae: family of order Diptera, "meniscus midges" - true flies
         5. *Blepharicera*: genus of "net-winged midges" of Order Diptera - true flies
         6. *Baetis*: genus of order Ephemeroptera - Mayflies

7. *Rhyacophila*: genus of order Trichoptera- Caddisflies
8. *Micrasema*: genus of order Trichoptera- Caddisflies
9. Psychomyiidae: family of trumpet-net-spinning Trichopterans- Caddisflies
10. *Ochrotrichia*: "microcaddisfly" genus of order Trichoptera- Caddisflies
11. Hydracarina: unclassed group of water mites of class Arachnida

3. **Results**
   a. Summer Experiment
      i. Sites prior to experiment
         1. Density of *Nostoc* and *Cladophora*, and *Simulium* larvae, did not differ between sites
         2. large simuliids negatively correlated to spaces occupied by macroalgae
      ii. Total invert density increased with *Cladophora* biomass
      iii. Both algae had positive effects on number of taxa
      iv. Effects of *Cladophora* on inverts
         1. Several taxa declined with Cladophora removal
            a. *Baetis*, chironomids, *Ochrotrichia* (microcaddisflies), *Rhyacophila* (caddisflies), *Micrasema* (caddisflies), *Euparyphus* (soldier flies), Hydracarina (water mites)
            b. Dixa (meniscus midges) near significant decline (p = ~0.06)
      v. Effects of *Nostoc* removal on inverts
         1. Reduced number of mites and chironomids
      vi. Effects of algae on simuliids were size-dependent
         1. "These results suggest that small simuliids responded positively to some algal attributes associated with algal biomass, whereas large blackflies were inhibited by the presence of filamentous algae. This is consistent with field observations: small larvae perched on *Cladophora* filaments, while large simuliids were usually attached to primary substrates."
         2. No size-based relationships were found between simuliids and *Nostoc*
      vii. Mites only found in control quadrats (suggests complex interaction between both algae and mites)
   b. Winter Experiment 1984
      i. Effects of Cladophora on inverts
         1. Removal increased abundance of two Dipterans: *Blepharicera* and *Simulium*
         2. Removal decreased abundance of *Baetis*, *Hydroptila*, *Micrasema*, and *Hydropsyche*
   c. Winter Experiment 1984-1985

i. Authors were not able to keep treatment replicates statistically identical (in algal density and composition) through the entire experiment. See article for details.
ii. Some taxa decreased with *Nostoc* removal, "but the relationships were only significant on some dates."
iii. Most insect taxa were positively associated with *Nostoc* (these insects decreased in abundance when alga was removed)
    1. *Hydrpsyche*, *Micrasema* and *Tinodes*, and also *Rheotanytarsus*
    2. "All these taxa are firmly attached or slow-moving (*Micrasema*) and appeared to be taking advantage of crevices provided by the structure of the *Nostoc* colonies."

4. Discussion
    a. "Our results show that macroalgae have highly significant effects on the local distributions of many stream insects in Rattlesnake Creek… Three general patterns of invertebrate association with macroalgae are apparent."
        i. Invertebrates that are inhibited by macroalgae due to pre-empted or modified habitat
            1. *Blepharicera* (net-winged midges): use a ventral row of hydraulic suckers to hold position on substrate, and are thus inhibited by macroalgae
            2. *Simulium* (black flies): response is size dependent
                a. Larger individuals are inhibited by macroalgae
                    i. Need a firm substrate to attach and hold position.
                    ii. They are subject to mechanical disturbance by filaments, and flow and seston disruption.
                b. Conversely, smaller individuals can use macroalgae as secondary attachment substrate, thus exhibiting a positive association.
                c. Larvae of all sizes may also be subject to less predation in clear areas
        ii. Invertebrates that are positively associated with macroalgae due to habitat provision
            1. *Micrasema* (caddisfly with cylindrical hard case): decreased currents may provide refuge for bulky case, or *Nostoc* mats/marl may provide surer foothold
            2. Some caddisflies may build nets more easily with attachments to *Nostoc*/marl
                a. *Hydropsyche* (net-spinning caddisflies that use openings of cases):
                b. Psychomyiidae (trumpet-net-spinning caddisflies)
        iii. Invertebrates that are positively associated with macroalgae due to food provision, either directly or indirectly (periphyton or detritus)

1. "Insects depending on macroalgae for food responded to algal removal as expected."
2. Algae piercing caddisfly larvae: *Hydroptila*, *Ochrotrichia*,
3. *Euparyphus* (soldier flies)
4. *Baetis*: in addition to providing food as periphyton, larger individuals likely benefited from decreased currents and rougher surface provided by macroalgae
5. Chironomids: "In the summer, Chironomidae probably responded in the same way as *Baetis*…

b. "Though grazers can inhibit the establishment of algae, once macroalgae are established, they usurp space from taxa which use rock substrates directly for food acquisition and/or attachment… Consequently, macroalgae will depress natural populations of species like *Blephariceria*, which are unable to survive in any alternative habitat within the stream."

c. "The extent to which macroalgae provide secondary substrate is a matter of scale."

- **i.** In this relationship, inverts may even promote algal growth by removing periphyton from surface of macroalgae

d. "... some invertebrate populations may be dependent on a combination of disturbance and herbivory to maintain populations on hard substrates."

e. "The effects of macroalgae on local distributions of invertebrates appear to be predictable. Because the distribution of algae may, in turn, be dependent upon unpredictable processes such as localized disturbance, the local distributions of stream invertebrates are determined by a combination of stochastic and deterministic processes."

Title: Relation of algal biomass to characteristics of selected streams in the lower Susquehanna River basin

Authors: Brightbill, R. A., and M. D. Bilger

Journal: USGS Water-Resources Investigations Report 98-4144, 1998

Study Questions:

"Nutrients affect algal growth, and algae are present in both tidal and non-tidal waters. Are nutrients the controlling factor, or are there other environmental factors that control algal growth? Can algal biomass be used as an indicator of whether of whether nutrient reduction strategies are working?"

Abstract: (Brightbill and Bilger 1998)

Seven small tributary streams with drainage areas ranging from 12.6 to 71.9 square miles, representative of both limestone and freestone settings, in the Lower Susquehanna River Basin were sampled for algae, nutrients, water quality, habitat, land use, hydrology, fish, and invertebrates. Nutrients, site characteristics, and selected characteristics of the invertebrate and fish communities known to influence algal growth were compared to chlorophyll a concentrations. Nitrogen was not found limiting in these streams; however, phosphorus may have been limiting in five of the seven streams. Concentrations of chlorophyll a in riffles increased with the degree of open canopy and as bottom substrate reached the gravel/cobble size fraction. These increased chlorophyll a concentrations and the substrate size in turn raised the levels of dissolved oxygen in the streams. Freestone streams had increased chlorophyll a concentrations associated with increases in percentage of omnivorous fish and in pH and decreases in percentage of collector/gatherer invertebrates. Concentrations of chlorophyll a in limestone riffles decreased as the percentage of omnivorous fish increased. Depositional chlorophyll a concentrations increased as the Bank Stability Index decreased and as the riffle velocity increased. Depositional chlorophyll a concentrations increased in limestone streams as collector/gatherer invertebrates increased and as phosphorus concentrations decreased. No relations were seen between chlorophyll a concentrations and land-use characteristics of the basin. In this study, there were too few sampling sites to establish statistically based relations between algal biomass and nutrient concentrations. Further study is needed to generate data suitable for statistical interpretation.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. "The [USGS NAWQA] Program is a long-term effort to describe the status of, and trends in, the quality of the Nation's surface and ground water resources and to provide an understanding of the natural and human factors that affect the quality of these resources…"

- **i.** Reports from this program assess algae, invertebrates, and fish, in community-based assessments of water quality.
  - **1.** "It represents a compromise between the greater sensitivity of species indicators or physiological responses to individual stresses and the lower variability, but broader response, of ecosystem responses (Gurtz 1994)."
- **b.** "This report evaluates the influence of nutrient concentrations and other selected environmental characteristics on algal concentrations in stream representing seven environmental settings in the Lower Susquehanna River Basin."

2. Methods

- **a.** Study area and site selection
  - **i.** Lower Susquehanna River Basin
    - **1.** Land Use: 47% Agriculture, 47% Forested, ~4% Urban, ~2% waterbodies or barren lands
  - **ii.** Site Selection
    - **1.** Basin area subdivided into 12 relatively homogenous units using GIS (based on physiography, lithology, land use, and land cover)
    - **2.** Basins in 7 of the 12 subunits were chosen for monitoring based on an approach focused on agricultural land underlain by carbonate bedrock
      - a. Secondary consideration was land that had been converted from agricultural to commercial, urban, industrial, or residential
    - **3.** Within each of these 7 basins, "a site was selected so that the most apparent factors influencing the water quality were bedrock type and land use."
    - **4.** Four sites are freestone and three sites are limestone
- **b.** Algal biomass determination
  - **i.** Periphyton collected from riffles according to NAQA protocols (Porter et al. 1993)
    - **1.** Circular wire brush scrapings analyzed for chl *a*, chl *b*, ash-free dry mass, total dry mass
- **c.** Water quality characteristics
  - **i.** Measured water temperature, DO, pH, and specific conductance, suspended sediments, TP, nitrates
- **d.** Environmental characteristics/habitat parameters
  - **i.** Canopy cover, vegetative bank stability, stream-channel embeddedness, width-to-depth ratio, Wolman pebble ranked size
- **e.** Hydrologic variables
  - **i.** Streamflow records collected for 2-3 years prior sampling, which is "too short to develop any meaningful streamflow statistics for a comparative analysis to previous years."

  - ii. Because of this insufficient data, "surrogate streamflow-measurement stations with longer periods of record were chosen to represent each of the sites where algae data were collected." (1940-1994)
  - iii. Data analyzed included range and mean of daily mean stream flows for each of the 5 years preceding the study
- f. Collection of fish and invertebrates
  - i. Annually collected in June, 1993-1995
  - ii. Fish collected using electrofishing
  - iii. Invertebrates collected following NAWQA protocols
    1. Data used in analysis is only from 1995 collection; "These 1995 data were the only data available at the time of analysis."
- g. Statistical Analysis
  - i. Linear regressions compared environmental, water quality, and nutrient characteristics to algal biomass
  - ii. Scatterplots used to visualize possible relationships between free-stone and limestone stream characteristics (too few data for meaningful statistical analysis)

3. **Results**
- a. Relationships between environmental characteristics and algal biomass were different between freestone and limestone sites
- b. Nutrients and water quality characteristics
  - i. No significant relationship observed between chl *a* and nitrogen
  - ii. Depositional chl *a* negatively related to phosphorus in limestone streams
  - iii. Riffle chl *a* positively related to pH and specific conductance in freestone streams
  - iv. Chl *a* negatively related to specific conductance in limestone streams
  - v. Riffle chl *a* positively related to DO in both stream types
- c. Fish and Invertebrates
  - i. Fish
    1. Riffle chl *a* positively related to percentage of omnivorous fish in freestone streams
    2. Riffle chl *a* negatively related to percentage of omnivorous fish in limestone streams
  - ii. Invertebrates
    1. Collector/gatherer invertebrates negatively related to chl *a* in freestone streams
    2. Collector/gatherer invertebrates positively related to depositional chl *a* in limestone streams
    3. Scraper invertebrates negatively related to depositional chl *a* in limestone streams
- d. Habitat and land use
  - i. Riffle chl *a* concentrations positively related to degree of open canopy
  - ii. Land use "showed no relation to chlorophyll *a* concentrations."

   e. Hydrology
      i. Depositional chl *a* positively related to riffle velocity
      ii. In limestone streams, chl *a* negatively related to width-to-depth ratio

4. **Discussion**
   a. Factors influencing algal biomass
      i. Freestone stream on average had lower chl *a* concentrations than limestone streams (limestone streams more productive)
         1. Limestone more enriching due to dissolution, relative to freestone
      ii. Nitrogen did not appear limiting in this study, likely due to high degree of agricultural land use in the basin
         1. N exceeds critical low for algal growth (0.35 mg/l TN)
      iii. Chl *a* unaffected by pH in limestone riffles
         1. Water buffered by carbonate lithology and pH is relatively constant
   b. Use of algal biomass in describing nutrient cycling
      i. "This study shows that bedrock plays an important role in algal production."
      ii. "This study had a small sample size and this, the conclusions cannot be rigorously supported statistically. For a better understanding of this complex system at least 40 representative streams from each bedrock type would need to be studied…"

Title: Linking nutrient enrichment and streamflow to macrophytes in agricultural streams
Authors: Mebane, A. M., N. S. Simon, and T. R. Maret
Journal: Hydrobiologia 722:143–158, 2014
Study Objective:

"The objectives of our study were to determine whether nutrients, streamflow, or other environmental variable best explained macrophyte abundance in streams in an agriculturally dominated landscape."

Abstract: (Mebane et al. 2014)

Efforts to limit plant growth in streams by reducing nutrients would benefit from an understanding of the relative influences of nutrients, streamflow, light, and other potentially important factors. We measured macrophytes, benthic algae, nutrients in water and sediment, discharge, and shading from 30 spring-fed or runoff-influenced streams in the upper Snake River basin, ID, USA. We hypothesized that in hydrologically stable, spring-fed streams with clear water, macrophyte and benthic algae biomass would be a function of bioavailable nutrients in water or sediments, whereas in hydrologically dynamic, run- off-influenced streams, macrophyte and benthic algae biomass would further be constrained by flow disturbance and light. These hypotheses were only partly supported. Nitrogen, both in sediment and water, was positively correlated with macrophyte biomass, as was loosely sorbed phosphorus (P) in sediment. However, P in water was not. Factors other than nutrient enrichment had the strongest influences on macrophyte species composition. Benthic algal biomass was positively correlated with loosely sorbed sediment P, lack of shade, antecedent water temperatures, and bicarbonate. These findings support the measurement of bioavailable P fractions in sediment and flow histories in streams, but caution against relying on macrophyte species composition or P in water in nutrient management strategies for macrophytes in streams.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. Macrophyte beds can act as a nutrient buffer and increase nutrient processing, P residence time, denitrification capacity and overall N removal
    b. Macrophytes can also reach nuisance levels, and determining what factors influence macrophyte abundance has been a focus of many previous studies
        1. Current, nutrients, light and substrate are important factors
        2. "However, reports have been contradictory on the relative importance of these factors, or even whether a factor had a positive or negative influence on macrophyte abundances."

c. Studies analyzing nutrient enrichment and its impact on algae may be incomplete if analysis does not include sediment nutrients and macrophytes
d. "We selected a suite of field and laboratory measurements that we expected would capture the expected interplay of current, light, nutrients, and substrate, and we selected study sites that we anticipated would encompass a broad range of conditions."
e. "More specifically, **we hypothesized** that
    i. in hydrologically stable, spring-fed streams with clear water, macrophyte and benthic algae biomass would be a function of bioavailable nutrients in water or sediments; and
    ii. in hydrologically dynamic, runoff-influenced stream, macrophyte and benthic algae biomass would further (also) be constrained by flow disturbance, light, and substrate."

2. **Methods**
    a. Study Sites
        i. 30 site in Snake River basin (Idaho/Nevada)
            1. Agriculturally dominated
            2. Selected with goal of obtaining range of nutrient conditions and two distinct stream types: runoff-fed and spring-fed
                a. Runoff-fed streams represent range of influences from irrigation wastewater conveyances to those located in watershed reserves
            3. Calculated index with four variables: % agricultural land use, % riparian within 100m of the channel, and estimates of TP and TN
                a. Index has 0-100 point scale; sites ranged from 0-95
    b. Physical, chemical, and biological measurements
        i. Water/stream characteristics: nutrients in water and sediment, substrate composition, light availability, and discharge
            1. Stream flow determined from gauges
        ii. Biological measurements:
            1. Benthic algae from 25 rock scrapes per site
            2. Macrophyte % cover visually estimated at 55 points per site; 11 transects with 5 points each
            3. Macrophytes for species counts and biomass: clipped "all plant material above substrate from five quadrats along each of three transects within the reach"
    c. Statistical analysis
        i. Focused interpretations on light, current, substrate type, nutrients in sediment and water, inorganic carbon availability, and algal density
        ii. Raw data graphing, multivariate ordinations of species composition, and multiple regression models
            1. "The ordinations were constructed to explore associations between environmental variables and species occurrence."

2. "Multiple regression models were constructed to relate the abundances of macrophytes to potentially predictive variables."

3. **Results**
   a. Macrophyte species composition and environmental correlates
      i. 13 taxa identified, with the most common being Sago pondweed (*Stuckenia pectinata*)
      ii. Ordinations identified best explanatory variable between macrophyte taxa matrix and environmental matrix data (listed in order):
         1. Elevation
         2. Agricultural intensity index
         3. Max velocity in year prior to sampling
         4. Al in sediment
         5. % fine-grained sediments
   b. Macrophyte biomass and environmental co-variates
      i. Nutrients
         1. Nitrogen
            a. TN in water and sediments strongly correlated with macrophyte biomass
            b. Total N mostly dissolved in spring-fed sites
               i. Median DIN was 87% of TN in spring-fed sites, and just 23% of TN in runoff sites
         2. Phosphorus
            a. Loosely sorbed P (extracted using $MgCl_2$) in sediment correlated with macrophyte biomass
            b. Less distinction between site type seen in dissolved P
               i. Median dissolved OP was 77% TP in spring-fed sites, and 63% of TP in runoff sites
      ii. "Shading limited macrophyte abundance at some sites. Sites with less than about 70% unshaded channels consistently had low or no macrophytes present, and all sites that had abundant macrophytes had open channels."
      iii. Turbidity negatively related to macrophyte abundance
      iv. "Highest macrophyte biomasses occurred at sites with nearly stable flows, and macrophyte biomasses declined at sites with increasing flow disturbance…"
      v. Periphyton and macrophyte biomass not strongly correlated
         1. Periphyton biomass positively correlated with loosely sorbed P, antecedent stream temperature, bicarbonate, and percent of channel without shade
   c. Multiple regression models
      i. Model for macrophyte and periphyton biomass

1. **1.** "We constructed a multiple linear regression model that reflected [light availability (as % unshaded channel), current, nutrients in sediment (as Al/P ratio), and nutrients in water (as TN)]…"
2. **2.** "… was able to account for 75% of the observed macrophyte biomass values."

ii. Model for benthic algae (periphyton chl *a* biomass)

- **1.** Most important environmental variables (in order of importance)
  - **a.** Loosely sorbed P in sediment
  - **b.** Stream temperature in 30 days prior to sampling
  - **c.** % unshaded channel
  - **d.** DIC as bicarbonate
- **2.** Accounted for 58% of observed benthic algae biomass

4. Discussion

- **a.** Hypotheses only supported in part
  - **i.** Macrophyte biomass correlated with sediment and water nitrogen, loosely sorbed P in sediment. However TP in water was not correlated.
  - **ii.** Substrate size was not correlated with macrophyte biomass
  - **iii.** "Factors other than nutrient enrichment had the strongest influences on macrophyte species composition."
- **b.** TN had strongest correlation with macrophyte biomass (of any variables measured), and TP not correlated at all
  - **i.** Could be related to macrophytes obtaining P through roots, or it could reflect N being the limiting nutrient rather than P
- **c.** Al and Fe partly control release of riparian P to groundwater, and consequently to stream water
  - **i.** In this study, normalizing P to Al or Fe improved correlation strength of this variable with macrophyte biomass
  - **ii.** Fe:P ratio has been used to estimate mobility of P in sediments to overlying water and bioavailability of P
    - **1.** In this study, streams with high macrophyte biomass correspond with low Fe:P ratios, and vice versa
    - **2.** Sites with ratios greater than 6 had low macrophyte biomass. This is supported by a previous study observing that sediments with a ratio of 5.5 are unable to retain P from releasing to overlying water (Jensen et al. 1992)
- **d.** In multiple regression model for algal biomass, "the stronger correlation between algal biomass and loosely sorbed P in sediment than TP in water suggests that loosely sorbed P may be a more meaningful measure of the P available to benthic algae than was P in water during the growing season."
  - **i.** Sediments likely acting as a diffusing substrate, and "loosely sorbed P in sediment reflects the ongoing availability of P to the stream."

   ii. P in water during growing season would only reflect that which was excess to algal growth requirements. Therefore correlation with algal biomass would not be expected

e. Loosely sorbed P obtained using $MgCl_2$ extraction also strengthened correlations and was a good approach to measuring bioavailable P in sediments

Title: Connecting phosphorus loss from agricultural landscapes to surface water quality

Authors: McDowell, R. W., B. J. F. Biggs, A. N. Sharpley, and L. Nguyen

Journal: Chemistry and Ecology 20:1–40, 2004

Study Objective:

"… our objectives in this review are to outline the major interactions and processes involved in connecting P loss from the landscape to the site of impact, whether that is a stream, river or lake. This review is split into three sections. The first deals with P loss from the landscape, whilst the second deals with processes that alter the behavior and impact of P once in the aquatic system. Finally, a third section outlines a secondary objective, which is to examine some management practices for P in relation to the connectivity between mitigating losses from the landscape and associated impacts on freshwater ecosystems."

Abstract: (McDowell et al. 2004)

The loss of phosphorous (P) from the landscape is commonly viewed as deleterious for surface water quality. However, the quantities lost and the impact this can have on surface waters depends on numerous mechanisms that occur whilst en route. The aim of this review is to give an outline of these mechanisms and thus how sources of P in the agricultural landscape are connected to the impairment of surface water quality. Processes are dealt with by examining the potential for P loss from the landscape and its availability to aquatic plants during flow overland and subsurface flow and once in streamflow or a lake or reservoir. By examining the connectivity between P loss and the impact on surface water quality, potential mitigation and management of P losses are discussed for various aquatic systems.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. Primary producers can be "extremely sensitive to even minor increases in P (can sequester P at the scale of picomoles)
    b. Economic impact of eutrophication on fishing and water treatment industries in last decade has amounted to more than $1 billion
    c. "… intensification and specialization of farming systems has led to regional surpluses of P imported in fertilizer and animal feed compared with P exported in farm produce. Now many farms have soil P concentrations well in excess of plant needs with increased potential for P loss."
    d. "Numerous reviews have dealt with P loss from land to water or the impact once in the stream. Little attempt has been made to link the two systems together."
2. **The potential for P loss from the landscape**

a. P in soil
    i. In acidic soils, P generally exists as Al- and Fe- phosphates
    ii. In neutral/alkaline soils, P generally exists as Ca- and Mg- phosphates
    iii. P most mobile at pH 6-7
b. P loss from soil
    i. Erosion
    ii. "During most high-flow events, the major release of P occurs at the start of the flow event when the labile pool of P is lost along with any mobile PP [particulate P]."
    iii. "Eroded particulate material is enriched with P compared to surface soil, due to the preferential transport of light and highly P-sorptive fines compared to coarse-sized particles."
    iv. Previous studies have shown that "P lost from low intensity rainfall events [can be] equally important as from high intensity rainfall events."
        1. However, more overall TP is generally lost in overland flow (carrying P from a thin layer of P-rich topsoil) typical during high intensity, low-frequency rainfall events
c. P in surface waters
    i. "… P retention by streams is dominated by physical processes such as flow velocity, discharge, and water depth."
    ii. Algal uptake and growth is an important determinant of DRP concentrations/gradients, and P-spiraling
        1. P-spiraling refers to the "distance travelled down-stream by one P molecule as it completes one cycle of uptake and transformations from dissolved to organic forms and back into flow."
        2. During high-intensity

3. **Impact of P transport to streams and rivers**
    a. In streams with prolonged periods of stable flow, periphyton is less limited by boundary layer diffusion of P because this layer is continuously eroded by moving water
        i. Therefore the rate limiting step in P assimilation becomes P uptake kinetics of algae
    b. Algal mats and P diffusion
        i. Algal cells at surface of mat will not likely experience P depletion
        ii. Cells at the bottom of the mat will eventually become P depleted, causing cell death and sloughing of the mat
        iii. Higher P concentrations in the water will increase diffusion to the lower layers and subsequent biomass accrual prior to sloughing
    c. N:P ratios
        i. Shifts seen in periphyton community composition as N:P ratio is altered (Biggs and Smith 2002)
            1. N:P less than 10 – high proportion of N-fixing cyanobacteria
            2. N-fixing cyanobacteria rare in streams with high N:P ratio

            3. Algal diversity greater at low N:P

4. **Measuring P in surface water**
    - **a.** TP has been used traditionally
    - **b.** "The use of mat P concentrations (i.e. TP of the periphyton mat normalized to mar biomass…) is appealing because of the potentially [closer] relationship between concentrations and algal growth rates…"
        - **i.** Can be biased due to non-phototrophic sediments, and analyses are expensive
5. **Managing P loss from the landscape**
    - **a.** Streams and rivers that experience frequent floods can assimilate more P before nuisance algae levels are reached. This is due to washouts of periphyton mats.
        - **i.** "… it is possible to have high P concentrations without eutrophic conditions if flood events are frequent, resulting in short accrual periods."
    - **b.** "Due to variability in soil types and management, generalizations across scale are rarely applicable."
    - **c.** "Due to the non-specific nature of farm boundaries in relation to catchment boundaries, management is best achieved at the scale where most benefit is evident, the catchment scale. This requires knowing where within the catchment most P loss is occurring…"

Title: Effects of chromic hypoxia and reduced temperature on survival and growth of burrowing mayflies, *Hexagenia limbata* (Ephemeroptera: Ephemeridae)

Authors: Winter, A., J. J. H. Ciborowski, and T.B. Reynoldson

Journal: Canadian Journal of Fisheries and Aquatic Sciences 53:1565–1571, 1996

Study Objective:

"Here, we report results of a laboratory study evaluating the survival and growth of *Hexagenia* nymphs over a range of dissolved oxygen concentrations and temperature levels comparable with conditions that can be found in western Lake Erie."

Abstract: (Winter et al. 1996)

Hexagenia nymphs are sensitive to hypoxia, and their abundance has been proposed as an ecosystem indicator for assessing the recovery from eutrophication of shallow, esotrophic lakes. Acute oxygen tolerance limits are known for *Hexagenia* spp., but effects of prolonged exposure to sublethal levels of hypoxia are not. A series of 21-day laboratory experiments was conducted to determine the influence of hypoxic stress (range 2-12.6 mg/L dissolved oxygen) and temperature (range 4-20°C) on survival and growth of *Hexagenia limbata* nymphs. Oxygen and temperature together explained 89% of the variability in survival among *H. limbata*, and 71% of the variability in average growth. Survival increased with increasing oxygen concentration, reaching an asymptote at 7-8 mg/L. Survival increased with temperature to an asymptote at approximately 9.5°C. Higher temperatures magnified the effects of hypoxia on survival. Growth increased with both oxygen and temperature, and did not reach an asymptote at levels up to 12.6 mg/L oxygen and 20°C. Persistent, sublethal oxygen stress (concentrations <7 mg/L) and reduced temperature (<20∞C) can influence both survival and size of *H. limbata*.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. *Hexagenia* mayflies formerly abundant in extensive regions of the Great Lakes
    b. Populations declined drastically in the past due to organic pollution, however recent recovery of the *Hexagenia* populations has been recommended as an "indicator of the ecosystem's rehabilitation from eutrophication."
        i. *Hexagenia* nymphs sensitive to hypoxia (< ~1 mg/l)
    c. The use of *Hexagenia* populations as an indicator of ecosystem health "requires an understanding of the mayflies' physiological responses to the effects of chronic, low dissolved oxygen concentrations and temperature…"
    d. "Our study provides information needed to model the recovery of mayfly communities in systems recovering from cultural eutrophication."
2. **Methods**
    a. Experimental Organisms

i. Nymphs obtained by rearing eggs collected from gravid females
    1. Stored at 8°C until needed, at which point they were returned to room temperature and would hatch after ~7 days. They were then reared for at least 3 months, and a broad size range (~4-16 mm) were selected for experimentation.

b. Experimental Design: Chambers and Conditions
    i. Chambers
        1. 125x40x40 cm Plexiglas waterbaths, covered and sealed to encapsulate atmosphere inside
            a. Inside the waterbaths were 1L tubs containing Lake Erie sediment and dechlorinated tap water
        2. 24 different combinations of oxygen and temperature levels in 3 waterbaths
            a. Water DO controlled indirectly through controlling atmospheric oxygen levels (with nitrogen gas)
            b. Location of nitrogen gas and air inflows resulted in varied levels of DO in the tubs
    ii. 21 day trials, after which growth and survival were determined
        1. Used %-change in head width as a measure of growth

c. Statistical Analysis
    i. Survival and growth analyzed by multiple regression analysis
        1. Independent variables analyzed were $[O_2]$, temp, $[O_2]^2$, $temp^2$, and O_2 X temp
        2. Quadratic terms included to detect non-linearity of relationships
    ii. "Because field surveys and records of physical data are often incomplete, oxygen and temperature measurements may not both be available for a given location or period of interests. Therefore, we also calculated regressions using oxygen and temperature variables separately."

3. **Results**

a. Survival
    i. Increased with DO to an asymptote at 7-8 mg/L ($r^2 = 0.84$)
        1. DO explained 84% of variability among treatments
    ii. Increased with temperature to an asymptote at 9.5°C ($r^2 = 0.38$)
        1. Temperature explained 38% of variability among treatments
    iii. High temperature produced a steeper response to DO
    iv. Least favorable hypoxic conditions (0.82-0.84 mg/L) killed fewer than 80% of nymphs over 21 days
    v. Larger sized nymphs were more likely than smaller nymphs to survive oxygen stress

b. Growth
    i. Increased linearly with DO ($r^2 = 0.25$)
    ii. Increased with temperature following polynomial model ($r^2 = 0.57$)

- **iii.** DO x temperature accounted for 44% of variation in growth among treatments
- **iv.** Temperature^2 accounted for 27% of variation in growth among treatments

4. Discussion

- **a.** Survival
  - **i.** Results suggest that even moderate DO stress can negatively affect survival
  - **ii.** "… response relationship to moderate oxygen stress may be as important as [a] tolerance threshold."
  - **iii.** Previous population fluxes in response to anoxia, and subsequent rebounds, suggest that individual and extreme climactic events will not necessarily eradicate a population
    - **1.** Eggs potentially have a greater tolerance for anoxia and allow population rebounds via emergence under more favorable conditions
    - **2.** Therefore population collapses are more likely due to prolonged periods of moderate oxygen stress
  - **iv.** Dead nymphs were observed to die after varying amounts of time under stress across the 21 day treatments
    - **1.** DO stress likely affects population mortality through an increased chance of mortality rather than outright mass kills
  - **v.** The asymptote in survivorship reached at 9.5°C could be due to developmental stasis at lower temperatures, and an indirect increase in mortality stemming from an inability to repair damaged tissue
  - **vi.** High temperatures led to a steeper mortality response to DO
    - **1.** Important when considering annual summer stratification and subsequent anoxia
      - a. Temperature differences of just several degrees during these stratification periods could determine whether nymph populations experience mass kills or are able to sustain a surviving population
  - **vii.** "Our results suggest that a summer regime of ≥8 mg/L oxygen and ≥10°C is adequate to sustain *Hexagenia* nymphs without limiting their survival."
- **b.** Growth
  - **i.** "… as temperature rose, metabolic rates increased, resulting in faster growth, presumably creating higher oxygen demand for *Hexagenia* nymphs."
  - **ii.** Increased in growth at high DO levels could be due to a decreased need to actively ventilate and/or increased feeding efficiency
    - **1.** This relationship between feeding efficiency and DO levels has been shown in various fishes and may be valid in mayflies

Title: Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: Implication to nutrient criteria development

Authors: Wang, L., and D. M. Robertson

Journal: Environmental Management 39:194–212, 2007

Study Objective:

"The objectives of this study were to (1) determine how macroinvertebrate and fish assemblage measures correlate with different forms of nutrients (phosphorus and nitrogen); (2) examine the relationships between key biological measures and nutrient forms to quantify potential threshold concentrations for nutrients beyond which macroinvertebrate and fish assemblages are likely to be substantially degraded; and (3) evaluate the importance of nutrient concentrations in influencing macroinvertebrate and fish assemblages relative to other physiochemical variables at different spatial scales."

Abstract: (Wang et al. 2007)

We sampled 240 wadeable streams across Wisconsin for different forms of phosphorus and nitrogen, and assemblages of macroinvertebrates and fish to (1) examine how macroinvertebrate and fish measures correlated with the nutrients; (2) quantify relationships between key biological measures and nutrient forms to identify potential threshold levels of nutrients to support nutrient criteria development; and (3) evaluate the importance of nutrients in influencing biological assemblages relative to other physicochemical factors at different spatial scales. Twenty-three of the 35 fish and 18 of the 26 macroinvertebrate measures significantly correlated ($P < 0.05$) with at least one nutrient measure. Percentages of carnivorous, intolerant, and omnivorous fishes, index of biotic integrity, and salmonid abundance were fish measures correlated with the most nutrient measures and had the highest correlation coefficients. Percentages of Ephemeroptera–Plecoptera–Trichoptera individuals and taxa, Hilsenhoff biotic index, and mean tolerance value were macroinvertebrate measures that most strongly correlated with the most nutrient measures. Selected biological measures showed clear trends toward degradation as concentrations of phosphorus and nitrogen increased, and some measures showed clear thresholds where biological measures changed drastically with small changes in nutrient concentrations. Our selected environmental factors explained 54% of the variation in the fish assemblages. Of this explained variance, 46% was attributed to catchment and instream habitat, 15% to nutrients, 3% to other water quality measures, and 36% to the interactions among all the environmental variables. Selected environmental factors explained 53% of the variation in macroinvertebrate assemblages. Of this explained variance, 42% was attributed to catchment and instream habitat, 22% to nutrients, 5% to other water quality measures, and 32% to the interactions among all the environmental variables.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. EPA's 1996 water quality report to congress identified excessive nutrients to be the second leading cause of impairment in streams
        i. Leading to impairment of 40% of surveyed waters
    b. Nuisance levels of algae and macrophytes produce potentially harmful detritus and lead to large diurnal swings in DO and pH (Welch et al. 1992)
        i. Subsequently: increased toxic substances, alterations of aquatic habitat and macroinvertebrate community composition, decrease in overall ecosystem functioning (Miltner and Rankin 1998), (Dodds and Welch 2000)
    c. Difficulties in setting nutrient criteria for management purposes
        i. Identifying critical nutrient species and their concentrations at impairment
        ii. "A generally accepted system for defining [an impaired] trophic status of streams or rivers is lacking."
        iii. "General approaches recommended by the EPA's Nutrient Criteria Technical Guidance Manual … are identification of reference reaches for each stream class based on professional judgement or percentile selections from data plotted as frequency distributions; use of predictive relationships of trophic state classifications, models, and bacteria; and application/modification of established nutrient-algal thresholds from the literature."
            1. Challenges with these approaches:
                a. No information on nutrient concentrations related to stream health/utility
                b. A predictive relationship between algal biomass and nutrient concentrations has not been found in all streams, and models from one water body won't necessarily be applicable to others
                c. The relationship/thresholds established between nutrients and algae may not be the same as those between macroinvertebrates and nutrients, or fish and nutrients
    d. Study objectives:
        i. "… determine how macroinvertebrate and fish assemblage measures correlate with different forms of nutrients (phosphorus and nitrogen)…"
        ii. "… examine the relationships between key biological measures and nutrient forms to quantify potential threshold concentrations for nutrients beyond which macroinvertebrate and fish assemblages are likely to be substantially degraded..."
        iii. "… evaluate the importance of nutrient concentrations in influencing macroinvertebrate and fish assemblages relative to other physiochemical variables at different spatial scales."

2. **Methods**
   a. Sites
      i. 240 sites, 1^{st}-4^{th} order streams across Wisconsin
         1. Selected for accessibility and to represent a range of anthropogenic influence and natural variation in catchment characteristics
   b. Catchment landscape data
      i. Boundaries of each stream catchment digitized from ISGS topo map
      ii. Land cover quantified with ARC/INFO software
      iii. Soil data quantified from USSOILS database and a previous geological study
      iv. Runoff calculated from "a digital linear coverage of average annual runoff for the continuous United States."
      v. Temp and precipitation data gathered from National Climate Data Center
      vi. Slope of each runoff determined using digital elevation model
   c. Water quality sampling: middle of each month from May-September from 2001-2003
      i. Stream discharge with current meter or continuous gauge
      ii. Temp, DO, specific conductance, pH measured in field with sonde
      iii. Water clarity with Secchi tube
      iv. Nutrients
         1. "… equal-width-increment method using a hand-held DH-59 depth-integrating sampler
         2. If low current velocity ($<0.04\ m^3/sec$) or depth (<0.15 m) a grab sample was collected instead at center of flow
         3. Analyzed TP, DP, TKN, NO_x, NH_4, and TN (calculated as TKN + NO_x)
   d. Instream habitat and fish sampling (collected once for each site between 1997 and 2002)
      i. Sampling reach was 35 times stream width, or a minimum of 100 m
      ii. 30 habitat variables measured or visually estimated along 12 transects
         1. Channel morphology, bottom substrates, cover, bank conditions, riparian vegetation and land cover, etc…
      iii. Fish collected by electrofishing entire length of site
   e. Macroinvertebrate sampling (October, 1999 and 2002)
      i. D-net in riffles and snags (overhanging grasses, logs, woody debris, and leaf packs)
         1. Average of riffle and snag collections used in all analyses
   f. Data summary measures
      i. 35 fish assemblage measures computed
      ii. 24 macroinvertebrate measures computed, and 2 Wisconsin-specific measures (HBI, MIBI)
   g. Statistical Analysis

i. Thresholds calculated using regression tree analysis and Kolmogorov-Smirnov techniques
ii. "Redundancy analysis (RDA) partition procedures… were conducted to separate the effects of nutrients on biological assemblages from the effects of other physiochemical variables at different spatial scales using CANOCO software…"

3. **Results**
   a. Ranges in landscape/stream habitat coverage
      i. Agriculture: 0-94%
      ii. Urban: 0-14%
      iii. Macrophyte coverage: 0-55%
      iv. Cobble and gravel substrate: 0-86%
      v. Sand and silt substrate: 1-100%
   b. Ranges in nutrient and biological conditions
      i. Median TP: 0.012-1.641
      ii. Median TN: 0.13-21.26
      iii. Intolerant fish: 0-98%
      iv. Fish IBI: 0-100%
      v. EPT: 0-99%
      vi. MIBI (inverts): 0-10 (max 10 on scale)
      vii. HBI (inverts): 1-9 (max 10 on scale)
   c. Correlations between biological assemblages and nutrients
      i. 23 of 35 fish assemblage measures significantly correlated with at least one nutrient measure
         1. Not correlated with any nutrient measures: absolute and relative abundances; % darter species, lithophilous, and suckers; total number of species; insectivore absolute abundance
         2. Most correlated with nutrient measures: Measures of carnivorous, intolerant, omnivorous, salmonid fish, and fish IBI (highest r^2 values and correlations with the highest number of nutrient measures)
      ii. 18 of 26 macroinvertebrate assemblage measures correlated with at least one nutrient measure
         1. Not correlated with any nutrient measures: % individuals and taxa of Diptera and herbivores; % individuals of filterers, gatherers, and midges; 5 scraper taxa
         2. Most correlated with nutrient measures: % EPT individuals and taxa; HBI; mean tolerant value (highest r^2 values and correlations with the highest number of nutrient measures)
         3. "Medians of TP, NH_4, TN, and TKN correlated with the most macroinvertebrate measures."
   d. Relationships between selected biological measures and nutrients

- **i.** Fish measures most strongly correlated with nutrient concentrations (% carnivorous and intolerant abundance, , salmonid abundance, and IBI) decreased as concentrations of P and N increased
    - **1.** At low nutrient concentrations these measures were highly variable, but were "generally low (wedge-shaped response)" at high concentrations
    - **2.** Not linear: Fish measures decreased dramatically with small nutrient increases
    - **3.** TN had weakest relationship with these measures
- **ii.** Macroinvertebrate measures most strongly correlated with nutrient concentrations (HBI, % EPT individuals and taxa, and total number of taxa) decreased as concentrations of P and N increased
    - **1.** These measures were most sensitive to TP, and NH4, and moderately sensitive to TKN and TN
    - **2.** Wedge-shaped response seen in fish measures also seen here, with high variability at low nutrient concentrations and generally low macroinvertebrate measures at high nutrient concentrations
        - **a.** However, HBI scores increased with TP, TN, TKN, and NH4

e. Thresholds and reference nutrient concentrations (regression-tree values are reported here, unless denoted by '*' which identifies Kolmogorov-Smirnov values)

- **i.** Fish IBI, % intolerant and carnivorous abundance, and salmonid abundance all had similar nutrient thresholds
    - **1.** TP: 0.06-0.07 mg/L
    - **2.** NH_4: 0.02-0.03 mg/L
    - **3.** TN: 0.54-0.61 mg/L
    - **4.** DP: 0.06-0.09 mg/L*
    - **5.** Other nutrient measures "quite variable"
- **ii.** Macroinvertebrate % EPT individuals, EPT taxa, and HBI all had similar nutrient thresholds as well
    - **1.** TP: 0.09 mg/L
    - **2.** NH_4: 0.03 mg/L
    - **3.** TKN: 0.54-0.61 mg/L
    - **4.** DP: 0.06-0.08 mg/L
    - **5.** Number of total taxa had lower threshold values for TP and DP
- **iii.** Reference nutrient concentrations
    - **1.** Those determined with 25th percentile of all data within a specific region after (EPA 2000))
        - **a.** Similar for NH_4 at 0.02-0.03 mg/L, but other nutrient concentrations varied among ecoregions analyzed in this study

2. Those determined by regression modeling after (Dodds and Oakes 2004)
    a. Very similar among ecoregions
    b. TP: 0.03-0.04 mg/L
    c. DP: 0.02-0.03 mg/L
    d. NH_4: 0.02-0.04 mg/L
    e. Other nitrogen species "varied considerably" among ecoregions

f. Relative importance of nutrients in influencing the health of stream biota
  i. Fish assemblage
    1. RDA procedures used to correlate fish and environmental characteristics "retained seven instream habitat variables, 13 catchment variables, eight nutrient variables, and three other water quality variables."
      a. These variables combined explained 54% of variation in fish assemblage
        i. 46% from instream habitat /catchment characteristics:
          1. Canopy shading, fish cover, gravel substrate, silt substrate, macrophyte cover, thalweg depth, width of buffer
          2. % agriculture land use, % forest cover, % forest wetland cover, % non-forest wetland cover, % open water land cover, % urban land use, loam surficial deposits, non-glaciated surficial deposits, soil-clay % to 5 ft depth, soil-organic material % to 5 ft depth, soil permeability, catchment area, catchment annual surface water runoff
        ii. 15% from nutrients:
          1. DP in July, TKN in July, TKN seasonal median, NH_4 seasonal median, NO_x seasonal median, TP in July, TP seasonal median, TP from point source
        iii. 3% from other water quality parameters:
          1. conductivity, flow, secchi tube clarity
        iv. 36% from "interactions among all the variables."
  ii. Macroinvertebrate assemblage
    1. RDA procedures found 7 instream habitat variables, 10 catchment variables, 9 nutrient variables, and three other water quality variables
      a. These variables combined explained 53% of variation in macroinvertebrate assemblages

i. 42% from instream habitat/catchment characteristics:
   1. Agriculture/urban % land use in riparian area, % riffle habitat, % run habitat, rubble and cobble substrate, sand substrate, macrophyte cover, width of buffer
   2. % agriculture land use, % forest cover, % forest wetland cover, % non-forest wetland cover, % open water land cover, % urban land use, peat surficial deposits, soil-organic material % to 5 ft depth, soil permeability, catchment area

ii. 22% to nutrient variables:
   1. DP in July, DP seasonal median, TKN in July, TKN seasonal median, NH_4 seasonal median, NO_x seasonal median, TP in July, TN seasonal median, TP from point source

iii. 5% from other water quality parameters:
   1. conductivity, flow, secchi tube clarity

iv. 32% from "interactions among all the variables."

4. Discussion

a. Correlation between macroinvertebrate/fish measures and nutrient concentrations suggests that nutrients have direct or indirect effects on these species assemblages
   i. Past research on these links is limited but supports the trends seen in this study with observed declines in macroinvertebrate and fish indices with increasing nitrogen and phosphorus concentrations (Miltner and Rankin 1998, Heiskary and Markus 2003)

b. Wedge-shaped response of assemblage measures to nutrient concentrations suggests that factors other than nutrients are primary contributors dictating macroinvertebrate and fish community composition at low nutrient levels. However at high nutrient concentrations these assemblages are likely strongly effected by nitrogen and phosphorous levels (Cade et al. 1999)

c. Nutrient criteria established by 25^{th} percentile and regression analysis were different than thresholds established by analysis of macroinvertebrate/fish assemblages
   i. Both methods suggested for establishing nutrient criteria (EPA 2000, Dodds and Oakes 2004)
      1. 25^{th} percentile approach highly effected by anthropogenic impact, however regression modeling "generally provide[s] better estimates of reference conditions" in areas with high anthropogenic activity

2. "The criteria identified by threshold concentrations generally do not change among target [ecoregions] within zoogeographic region."

ii. Thresholds identified by macroinvertebrate assemblages were generally higher than those from fish assemblages (TP and TN)

d. "Our results, in conjunction with previous findings, imply that relationships between nutrients and fish or macroinvertebrates are as sensitive as benthic periphyton in detecting TN and TP degradation."

i. A previous study of Wisconsin streams identified nutrient thresholds through analysis of diatoms and benthic chl*a* (Robertson et al. 2006)

1. Criteria established by Robertson et al. (2006) were similar to those established in this study by macroinvertebrates, but lower than criteria established from fish assemblages

ii. In a study analyzing data from 620 North American and New Zealand streams, Dodds et al. (2002) analyzed nutrients and benthic chl*a* and found thresholds of 0.03 mg/L for TP and 0.54 mg/L for TN. These values are lower than those of the present study

e. "Results of the RDA analyses indicated that nutrients by themselves had relatively small direct influence on biological assemblages in wadeable streams."

f. Use of macroinvertebrate or fish assemblages in establishing nutrient criteria may provide more accurate values due to the indirect effects of anthropogenic nutrient degradation and effects of associated industrial/agricultural activities

i. In addition to inorganic nutrients, these activities can generate "oxygen-demanding substances, organic solids, and other pollutants that impact the health of aquatic ecosystems."

ii. "… using fish and macroinvertebrates as indicators provides additional insight on how nutrients impair stream ecosystems rather than algae alone."

g. "Established relationships between nutrient concentrations and macroinvertebrate or fish assemblages can be used to validate and refine criteria based on nutrient-chlorophyll relations and to identify exceptions to which the generalized nutrient criteria may not be applied."

Title: Environmental nutrient additions accelerate terrestrial carbon loss from stream ecosystems

Authors: Rosemond, A.D., J. P. Benstead, P. M. Bumpers, V. Gulis, J. S. Kominoski, D. W. P. Manning, K. Suberkropp, and J. B. Wallace

Journal: Science 347:1142–1145, 2015

Study Objective:

"To determine how moderate nutrient pollution affects terrestrially derived POC [particulate organic carbon] at stream-reach scales, we tested how long-term (2- to 5-year), continuous, flow-proportional nitrogen (N) and phosphorus (P) additions affected its loss rates and fates in headwater forest streams."

Abstract: (Rosemond et al. 2015)

Nutrient pollution of freshwater ecosystems results in predictable increases in carbon (C) sequestration by algae. Tests of nutrient enrichment on the fates of terrestrial organic C, which supports riverine food webs and is a source of CO2, are lacking. Using whole-stream nitrogen (N) and phosphorus (P) additions spanning the equivalent of 27 years, we found that average terrestrial organic C residence time was reduced by ~50% as compared to reference conditions as a result of nutrient pollution. Annual inputs of terrestrial organic C were rapidly depleted via release of detrital food webs from N and P co-limitation. This magnitude of terrestrial C loss can potentially exceed predicted algal C gains with nutrient enrichment across large parts of river networks, diminishing associated ecosystem services.

Information most pertinent to Mid-Atlantic management issues

**** denotes information from supplementary materials***

1. **Introduction**
    - **a.** Nutrient pollution strongly effects C cycling
        - **i.** Promotes growth of algal biomass (C addition) but can also promote C mineralization (C loss by releasing CO_2)
        - **ii.** Production in freshwater streams is based largely on terrestrial C inputs
            1. Inputs of leaves and wood are main sources of POC
                - a. Nutrients stimulate breakdown of this POC by microorganisms
                - b. Also broken down by detritivore feeding
2. **Methods**
    - **a.** "We conducted two manipulative experiments at large spatial and temporal scales and focused our measurements on forest-derived leaf litter, because it is the most biologically active pool of terrestrial C in forest streams and is renewed annually."

- **b.** Measured terrestrial C loss rates in 70-150m 1st order stream reaches
  - **i.** *Coweeta Hydrologic Laboratory Long-term Ecological Research Site, NC
  - **ii.** *Sites heavily shaded, resulting in primarily heterotrophic streams relying on terrestrial organic C
- **c.** Experiment 1 (N + P)
  - **i.** Paired watershed design, with one experimentally enriched (N and P) stream and one unaltered reference stream
    1. Ran for 5 years after a pretreatment year
    2. *Continuously enriched with N (NH_4NO_3) and P (K_2HPO_4 and KH_2PO_4) at molar ratio of 16:1. Enrichment proportional to discharge.
- **d.** Experiment 2 (N x P)
  - **i.** Expanded N and P enrichment gradients in 5 other streams for 2 years after a pretreatment year
    1. *Designed to create inverse scales of N and P to create treatments with differing nutrient limitations
  - **ii.** *Continuously enriched for two years with N (NH_4NO_3) and P (H_3PO_4) at target molar ratios of 2:1, 8:1, 16:1, 32:1, and 128:1. Enrichment proportional to discharge.
- **e.** Litterbag Experiments (*conducted in both N + P and N x P experiments)
  - **i.** "We further tested the importance of biological versus physical control of C loss rates by comparing the degree to which small-scale (i.e., litterbag) measurements aligned with reach-scale rates, using measurements made at the same time; relatively good alignment between reach-scale and litterbag-scale rates indicates that biological, rather than physical, processes drove reach-scale rates."
  - **ii.** *Leaf litter standing stocks determined by collecting all leaf material down to 1 cm in width/length by hand at 10 randomly selected transects along reach. Then ash-free dry mass was determined.
  - **iii.** *Litter breakdown determined with bags of red maple, rhododendron, tulip polar, and chestnut oak
    1. 12-35 bags per species, per stream, each year
- **f.** *Quantification of C pools and Outputs (only in N + P experiment)
  - **i.** POC and DOC export determined from collecting traps and Coshocton proportional subsamplers at weirs (POC), and biweekly water samples at seeps and weirs (DOC)
  - **ii.** Pools determined from benthic core samples in dominant substrate

3. **Results**
- **a.** Experiments 1 and 2
  - **i.** Terrestrial C loss rates increased with all concentrations of N and P enrichment
    1. Median C loss rates increased 1.65 times w/enrichment

a. A range of 1.02-4.49 times reflects variation in N and P concentrations, discharge, and temperature

2. Out of 27 annual measured variables, the following explained 83% of variation in C loss rates:

a. Discharge, N and P concentrations, temperature, and "associated random effects (stream and year)…"

3. N and P enrichment levels explained ~75% of variation seen in litter loss rates "as annual cumulative discharge"

a. N and P concentrations ($r^2 = 0.79$), and discharge and temperature ($r^2 = -0.76$), were correlated and therefore individual effects could not be determined

4. Similar effects of N and P concentrations on litter loss rates suggest co-limitation of these nutrients and a synergistic effect resulting from their enrichment

ii. Average terrestrial POC residence time halved with N and P enrichment

1. 167d in reference stream, 75 days in enriched streams

iii. Authors estimated litter quantity using average annual litter input and median loss rates. They predicted litter quantity to be higher in reference stream relative to enriched stream. With time this difference increases.

1. Reference litter was 2.8x higher after 6 months and 7.7x higher after 12 months

iv. Authors quantified role of microbial decomposers (leading to C lost via respiration) and detritivores (C lost to POC export) using data from enrichment experiments

1. C pools declined with N and P enrichment

a. POC export increased

b. C export via respiration increased in first 2 years

2. In later years (3-5), elevated C output was due more to POC export by detritivores than by microbial respiration

b. Litterbag Experiment

i. "Our results generally support the use of litterbags to measure larger-scale C dynamics, but with consideration of differences among litter species and potential divergence in rates due to the degree of biological processing."

ii. Authors cite the following issues:

1. Litterbags with more labile litter (relative to actual reach-wide litter composition) would over-estimate reach-wide C loss and vice versa with more refractory litter

2. Litterbags would be expected to experience higher C loss than full reaches because reaches receive additional C input over time

iii. "When litterbag-scale rates exceeded reach-scale rates, this suggested strong and increased biological processing of C under nutrient-enriched conditions."

4. Discussion

a. "Our results suggest N and P co-limit terrestrial C loss rates in streams…"
b. Increased fine POC export with enrichment was observed
    i. *Supported by previously published results from this study
    ii. Due to stimulation of microbial respiration and detritivore feeding, which decreases C storage in the stream
    iii. *Detritivore-driven losses of terrestrial C increased 2.3x with nutrient enrichment
    iv. Fine POC also experiences heightened transport distance within stream reaches
    v. The relative strengths of microbial and detritivore influences will determine whether exported C is primarily lost as CO_2 or transported downstream
c. *Nutrient effects on macroinvertebrate assemblages (limited to observations in N + P experiment, previously published results)
    i. Nutrient enrichment led to increased litter nutrient content due to increased bacterial and fungal colonization
    ii. Initially, macroinvertebrate production increased with nutrient addition
        1. Nutrient addition over time increased flow of C to detritivores and decreased prey consumption by predators
        2. Predator-prey relationships were further decoupled over time and energy flow to predators was truncated
    iii. "These findings showed that long-term enrichment may have unforeseen and unpredictable effects on ecosystem efficiency and productivity."
d. "In agricultural streams with diminished abundance of detritivores, nutrients stimulate largely microbial breakdown of crop residues that enter streams."
e. "Similarly, greater CO_2 flux than export due to increased microbial, but not detritivore, processing of terrestrial C is predicted with elevated temperature."
f. "When animals are reduced in streams due to biogeographic or land-use factors, nutrients or temperature accelerate losses of C, but primarily via CO2, not export of particles.
    i. Thus, populations of detritivores in streams play complex and important roles: They may limit terrestrial C loss as CO2 and maintain downstream C export, but contribute to depletion of local C resources."
g. Terrestrial C input dominates smaller streams, however in large order streams there is greater autotrophic C influence
    i. The authors conducted simple analyses of their study area that shows, with enrichment, the potential for terrestrial C losses greater than autotrophic C gains in 1^{st}-4^{th} order streams
h. "The mineralization of terrestrial C, similar to the production of algal C, is stimulated at nutrient concentrations that are now common across human-disturbed landscapes. Thus, reduced retention and increased export and respiratory losses of terrestrial C are probably occurring in many aquatic systems because of increased nutrient availability, with consequences for ecosystem

services. The loss of terrestrial C is not as visually obvious as increased algal biomass."

Title: Long-term nutrient enrichment decouples predator and prey production
Authors: Davis, J. M., A. D. Rosemond, S. L. Eggert, W. F. Cross, and J. B. Wallace.
Journal: Proceedings of the National Academy of Sciences 107:121–126, 2010
Study Objective:

"[If] nutrient enrichment disproportionately stimulates predator-resistant prey, it may reduce positive nutrient effects on predators and inhibit predator production… Here we report the results from an ecosystem-level [nutrient enrichment] of a detritus-based headwater stream that is dominated by approximately 20 taxa of macroinvertebrate and salamander predators."

Abstract: (Davis et al. 2010)

Increased nutrient mobilization by human activities represents one of the greatest threats to global ecosystems, but its effects on ecosystem productivity can differ depending on food web structure. When this structure facilitates efficient energy transfers to higher trophic levels, evidence from previous large-scale enrichments suggests that nutrients can stimulate the production of multiple trophic levels. Here we report results from a 5-year continuous nutrient enrichment of a forested stream that increased primary consumer production, but not predator production. Because of strong positive correlations between predator and prey production (evidence of highly efficient trophic transfers) under reference conditions, we originally predicted that nutrient enrichment would stimulate energy flow to higher trophic levels. However, enrichment decoupled this strong positive correlation and produced a nonlinear relationship between predator and prey production. By increasing the dominance of large-bodied predator-resistant prey, nutrient enrichment truncated energy flow to predators and reduced food web efficiency. This unexpected decline in food web efficiency indicates that nutrient enrichment, a ubiquitous threat to aquatic ecosystems, may have unforeseen and unpredictable effects on ecosystem structure and productivity.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    - **a.** Nutrient enrichment shifts trophic pathways and dominance and "represents one of the greatest threats to global ecosystems."
    - **b.** Anthropogenic enrichment can often have unpredicted consequences by diverting energy to alternate pathways (e.g., microbial growth and respiration fed by algal detritus and leading to anoxic "dead zones")
        - **i.** Enrichment can increase dominance of predator-resistant primary consumers and make energy inaccessible to higher trophic levels
        - **ii.** "[If] nutrient enrichment disproportionately stimulates predator-resistant prey, it may reduce positive nutrient effects on predators and inhibit predator production."

   c. **Hypothesis:** "[On] the basis of our earlier results from the first 2 years of enrichment and from a similar long-term enrichment that showed positive effects of nutrient enrichment on predators and primary consumers, we hypothesized a priori that in subsequent years of nutrient enrichment (years 4 and 5), primary consumer and macroinvertebrate predator production would continue to be positively correlated."

2. **Methods**
   a. Study conducted simultaneously with Rosemond et al. (2015)
      i. See above summary for description of study site, paired-stream experimental design, and nutrient enrichment method
   b. Both streams used were fishless
      i. Nutrient enrichment was similar to that of those experiencing agricultural and urban input (Alexander and Smith 2006)
   c. Macroinvertebrate sampling and analysis of primary consumer populations
      i. Monthly mixed-cobble substrate samples collected with a stovepipe corer
      ii. Identified most taxa to genus besides Chironomidae
      iii. "We evaluated trophic level responses to enrichment with community biomass and secondary production. However, because it integrates multiple metrics in assessing taxonomic response to enrichment (i.e., abundance, biomass, growth rate, survivorship, and development time), secondary production was the best metric to quantify the overall response."
         1. Secondary production: identified size-frequency cohort intervals
         2. Community biomass: measured length, but used previously published length-mass relationships to quantify ash-free dry mass
      iv. Also analyzed size-specific production of primary consumers to "help distinguish between the response of preferred prey (small-bodied individuals, ≤10 mm) and predator-resistant primary consumers (large-bodied individuals, >10 mm).
   d. Divided study into three time periods (pretreatment, short-term: 1-2 years, and long-term: 4-5 years) to determine short-term vs long=term macroinvertebrate response
      i. Third year discarded due to "inadequate [macroinvertebrate] preservation"
   e. Statistical analysis: Randomized intervention analysis (RIA)

3. **Results**
   a. "Unexpectedly, during the fourth and fifth years of the experiment, nutrient enrichment produced a trophic decoupling whereby enrichment continued to stimulate primary consumer production with no concomitant increase in macroinvertebrate predators."
      i. Short term, nutrient enrichment promoted production and biomass of primary consumers and predators, indicating efficient transfer between trophic levels

ii. "However, this short-term response contrasted sharply with our longer-term results showing that predator biomass and production did not respond positively to nutrient enrichment, despite continued stimulation of primary consumer biomass."
    1. Predator biomass and production declined to pre-treatment levels in years four and five
iii. "This trophic decoupling reduced overall food web efficiency during the long-term enrichment and contrasted with previous studies showing evidence of highly efficient energy transfer from primary consumers to predators in similar stream food webs."

b. "The alteration of the predator-prey relationship during the long-term enrichment was largely driven by changes in the relative dominance of large- vs. small-bodies primary consumers."
   i. Enrichment… only increased the production of large-bodied primary consumers in the treatment stream during years 4 and 5."
   ii. "Because small-bodied prey declined to pretreatment levels by the fourth year of enrichment, long-term enrichment primarily stimulated those consumers that were relatively resistant to predation."

4. **Discussion**
   a. "Our results provide strong evidence that nutrient enrichment reduced energy flow to predators and decreased the trophic transfer efficiency between primary consumers and predators."
   b. "Our results further demonstrate our limited ability to predict how higher trophic levels in aquatic ecosystems will respond to nutrient enrichment and highlight the difficulties in predicting long-term food web responses from few large-scale experimental manipulations."
   c. "… increased nutrient supplies do not always propagate up food webs to increase the productivity or biomass of higher trophic levels."
      i. Agrees with previous analysis that food chain length is primarily dictated by ecosystem size rather than productivity
   d. Ecosystems dominated by predators that are gape-limited (i.e., can only consume prey smaller than their mouth) may be more susceptible to this decoupling, because primary consumers will benefit from a size-based predator refuge
   e. "The mechanism by which large-bodied taxa became dominant in this ecosystem is likely a function of several factors."
      i. Differential predation pressure
         1. Size refuge
      ii. Declines in leaf litter standing crop and habitat complexity
         1. If large primary consumers reach a size refuge, smaller primary consumers would be more dependent on habitat refuge
         2. The large-bodied caddisfly, *Pycnopsyche* spp., increased in relative dominance throughout this study and likely decreased leaf litter habitat availability

   a. This caddisfly is a competitive dominant that eats leaf litter

f. "Because prolonged enrichment would likely strengthen, not weaken, these benefits, the observed trophic decoupling is unlikely to be easily reversed with continued enrichment. However, this decoupling would have likely reversed when enrichment ceased because of the strong donor-controlled aspects of these ecosystems: both basal resources via seasonal litter-fall and larval aquatic insect populations are renewed annually to a large extent."

g. This decoupling is extendable to headwater streams worldwide: "… our results indicate an important nutrient enrichment response that is applicable to globally distributed aquatic food webs and helps increase our understanding of how such stream networks may respond to enrichment."

Title: A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration

Authors: Boyero, L., R. G. Pearson, M. O. Gessner, L. A. Barmuta, V. Ferreira, M. A. S. Graça, D. Dudgeon, A. J. Boulton, M. Callisto, E. Chauvet, J. E. Helson, A. Bruder, R. J. Albariño, C. M. Yule, M. Arunachalam, J. N. Davies, R. Figueroa, A. S. Flecker, A. Ramírez, R. G. Death, T. Iwata, J. M. Mathooko, C. Mathuriau, J. F. Gonçalves, M. S. Moretti, T. Jinggut, S. Lamothe, C. M'Erimba, L. Ratnarajah, M. H. Schindler, J. Castela, L. M. Buria, A. Cornejo, V. D. Villanueva, and D. C. West

Journal: Ecology Letters 14:289–294, 2011

Study Objective:

"… we set out to test across a broad latitudinal gradient, whether microbial decomposition rates are positively related to temperature, leading to higher rates in the tropics, and whether the commonly observed dearth of litter consumers in the tropics counters this latitudinal pattern, leading to higher decomposition rates in temperate climates."

Abstract: (Boyero et al. 2011)

The decomposition of plant litter is one of the most important ecosystem processes in the biosphere and is particularly sensitive to climate warming. Aquatic ecosystems are well suited to studying warming effects on decomposition because the otherwise confounding influence of moisture is constant. By using a latitudinal temperature gradient in an unprecedented global experiment in streams, we found that climate warming will likely hasten microbial litter decomposition and produce an equivalent decline in detritivore-mediated decomposition rates. As a result, overall decomposition rates should remain unchanged. Nevertheless, the process would be profoundly altered, because the shift in importance from detritivores to microbes in warm climates would likely increase CO_2 production and decrease the generation and sequestration of recalcitrant organic particles. In view of recent estimates showing that inland waters are a significant component of the global carbon cycle, this implies consequences for global biogeochemistry and a possible positive climate feedback.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    - **a.** "Plants produce c. 120 billion tons of organic carbon each year, only a small fraction of which is annually sequestered or removed by herbivores. This suggests that the reverse process of plant production – the decomposition of plant litter – is one of the most important ecosystem processes in the biosphere."
    - **b.** "… recent estimates have shown that decomposition processes in inland waters, including streams and rivers, contribute significantly to the global carbon cycle as

a result of high metabolic rates, facilitated by constant water availability and nutrient supply, especially in flowing waters."

- **c.** "The non-limiting role of water is a key feature that makes aquatic systems a suitable model in which to assess the significance of factors controlling decomposition other than water availability. This is particularly true for temperature, which in terrestrial systems is inexplicably linked to moisture as a mutually confounding factor. Aquatic systems are thus particularly valuable when assessing effects of climate warming on decomposition."
- **d.** "A simple and yet powerful approach to study litter decomposition in the field, widely used in both terrestrial and aquatic systems, is to measure the mass loss of leaves enclosed in mesh bags, which allow water flow and microbial colonization while excluding detritivores or allowing their access, depending on the mesh size of the bags."
    - **i.** "Distinction between these two agents of decomposition – microbes and detritivores – is important because their activities are partly governed by different factors and result in varying proportions of decomposition products such as CO_2 and dissolved and fine particulate organic matter."
- **e.** "Thus, we set out to test across a broad latitudinal gradient, whether microbial decomposition rates are positively related to temperature, leading to higher rates in the tropics, and whether the commonly observed dearth of litter consumers in the tropics counters this latitudinal pattern, leading to higher decomposition rates in temperate climates."

2. Materials and Methods

- **a.** 22 study streams in all inhabited continents (not Antarctica) in both hemispheres
    - **i.** Mean water temp 1.9-26° C and strongly related to latitude
    - **ii.** pH in all streams circumneutral: 7.06 ± 0.18 SE
    - **iii.** Conductivity was low: 871 $\mu S/cm^2$ ± 23 SE
- **b.** Experiments conducted at season of max leaf litter accumulation (dry season at tropical sites, Autumn at temperate sites)
- **c.** At each site chose single stream with little human influence
- **d.** Litter Bags
    - **i.** Used black alder. Determined initial dry weight.
        - **1.** Chose this species because they are a widely distributed and important riparian species. Also black alder has been shown to be highly palatable to both temperate and tropical stream detritivores.
    - **ii.** Three coarse and three fine mesh bags collected at days 0 (to determine weight-loss from handling), 14 28, and 56. Leaves dried and weighed.
- **e.** Data analysis
    - **i.** "We used linear mixed models to test the null hypothesis that decomposition rates do not vary with latitude."
        - **1.** Analyzed mass remaining relative to elapsed time, latitude, elevation, water temp, mesh size, and interactions between factors

ii. "To further predict the consequences of climate warming on decomposition rates across latitudes, in a second model we regressed the natural logarithm of the proportion of litter mass remaining on temperature-normalized elapsed time expressed in degree days, latitude, mesh size and their interactions."
iii. Models fitted with generalized least squares (GLS) using restricted maximum likelihood (REML) procedures implemented in R
iv. Relationships between temperature and decay rate explored using metabolic theory of ecology (MTE), providing a framework to quantitatively assess the relationship between temp and biological activity

3. Results and Discussion

a. "Patterns across the latitudinal temperature gradient were strikingly different between microbial decomposition in fine-mesh litter bags and combined microbial activity and detritivore feeding in coarse-meshed bags."
b. Microbial decomposition rate increased with latitudinal temp increase
    i. However decomposition contribution by detritivores was opposite that of microbes
    ii. "The most parsimonious explanation for this pattern is that litter decomposition mediated by detritivores increases with latitude – whether caused by higher detritivore densities, higher individual feeding capacities related to body size, or a combination of both. In line with this explanation, detritivore densities found in natural leaf packs at half our study sites increased with latitude."
c. "Our results provide a basis for predicting the effects of climate change on litter decomposition in streams."
    i. "First, they suggest that microbial decomposition rates in a warmer world can be forecast largely on the basis of projected water temperature."
        1. Temperature rise would increase microbial decomposition rates, which could be predicted using these models
    ii. "Second, it appears that total decomposition rates involving invertebrates are determined by the interplay between water temperature and the occurrence of litter-consuming detritivores, with the balance of these two driving forces resulting in unchanged total decomposition rates in a warmer world."
        1. This conclusion only valid if climate change results in rapid range shifts according to new thermal regimes
            a. Insect detritivores are unlikely to respond by rapid adaptation or acclimatization, "retraction towards higher latitudes and elevations is much more likely."
d. Alternative hypotheses possibly contributing to observations
    i. Physical breakdown could have contributed to litter mass loss at some sites
        1. Still wouldn't account for latitudinal observations

- **ii.** DIN differences across sites
    - **1.** Unimportant because black alder is a nitrogen fixing plant that provides large amount of N to decomposers
- **iii.** Stream [P] could have played a role despite all streams having similar characteristics and little human influence
- **iv.** These all may have contributed to residual variability in models

e. "If, as indicated by our results, climate change produces an increase in microbial decomposition and a concomitant decrease in rates of detritivore-mediated decomposition, then total decomposition rates at a given latitude would remain unchanged in response to climate warming."

- **i.** Climate change could still have large effects on litter decomposition
    - **1.** Microbe decomposition produces more CO_2
    - **2.** Detritivores produce more fine particulate matter
        - **a.** More easily exported downstream

f. "Conventional models of the global carbon cycle describe inland waters as passive pipes of organic carbon from terrestrial ecosystems to the oceans. Recent estimates have shown, however, that streams and rivers receive and transform a sizeable fraction of terrestrial net ecosystem production and thus contribute to significantly to the global carbon cycle."

g. "Thus, beyond the proof of principle that our study provides, our findings and their implications appear to be directly relevant to global biogeochemistry, with possible positive feedbacks on the climate system."

Title: Trends in the nutrient enrichment of U. S. rivers during the late 20th century and their relation to changes in probable stream trophic conditions

Authors: Alexander, R. B., and R. A. Smith

Journal: Limnology and Oceanography 51:639–654, 2006

Study Objective:

"We used a trend assessment method to estimate changes in the probable trophic state (i.e., "oligotrophic," "mesotrophic," and "eutrophic") at 250 monitored locations on major U.S. rivers, based on measured changes in nutrient enrichment (i.e., total nitrogen and total phosphorous concentrations) at these sites over approximately the last quarter of the 20th century."

Abstract: (Alexander and Smith 2006)

We estimated trends in concentrations of total phosphorus (TP) and total nitrogen (TN) and the related change in the probabilities of trophic conditions from 1975 to 1994 at 250 nationally representative riverine monitoring locations in the U.S. with drainage areas larger than about 1,000 km2. Statistically significant (p , 0.05) declines were detected in TP and TN concentrations at 44% and 37% of the monitoring sites, and significant increases were detected at 3% and 9% of the sites, respectively. We used a statistical model to assess changes in the probable trophic-state classification of the sites after adjusting for climate-related variability in nutrient concentrations. The probabilistic assessment accounts for current knowledge of the trophic response of streams to nutrient enrichment, based on a recently proposed definition of ''eutrophic,'' ''mesotrophic,'' and ''oligotrophic'' conditions in relation to total nutrient concentrations. Based on these trophic definitions, we found that the trophic state improved at 25% of the monitoring sites and worsened at fewer than 5% of the sites; about 70% of the sites were unchanged. Improvements in trophic-state related to declines in TP were more common in predominantly forested and shrub–grassland watersheds, whereas the trophic state of predominantly agricultural sites was unchanged. Despite the declines in TP concentrations at many sites, about 50% of all monitoring sites, and more than 60% of the sites in predominantly agricultural and urban watersheds, were classified as eutrophic in 1994 based on TP concentrations. Contemporaneous reductions in major nutrient sources to streams, related to wastewater treatment upgrades, phosphate detergent bans, and declines in some agricultural sources, may have contributed to the declines in riverine nutrient concentrations and associated improvements in trophic conditions.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. History of U.S. waters and nutrient excess
        i. Diffuse nutrient sources at peak in 70's and early 80's

ii. "Soil conservation and government-sponsored retirements of farmlands have received increasing attention since the 1980's, although knowledge of their effects on stream nutrients are limited."
iii. Despite this variation in management and nutrient sources little is known about how streams have responded
iv. Challenges in establishing a trophic-state classification system:
    1. Long-term monitoring records of algal biomass is sparse across the U.S.
    2. State bio assessments have only recently been implemented in most states
    3. Streams respond variably to nutrient enrichment
    4. "Moreover, published trend information has limited utility for assessing the ecological relevance of historical changes in stream nutrients; frequently, trends are reported as an absolute or percentage change in mean concentrations without any information about how the trends relate to trophic conditions."
v. Recent efforts have been made to establish a trophic state classification scheme using nutrient and chlorophyll concentrations (EPA 2000), (Dodds et al. 1997b)
vi. "Here, we use this classification system as a framework for evaluating the potential trophic importance of changes in the nutrient enrichment of U.S. rivers over approximately the last quarter of the 20^{th} century."
    1. "Using long-term monitoring data collected at 250 locations on major rivers throughout the U.S. from 1975 to the 1990's, we statistically model changes in ambient nutrient concentrations and concentrations standardized for seasonal and stream flow variability."
    2. "This assessment method addresses the difficulty of using conventional measures of trend (e.g., absolute or percentage change) and their statistical uncertainties to evaluate the relation of changes in stream nutrient concentrations to concentrations that have relevance to trophic conditions."

2. **Methods**
   a. Water-quality data
      i. Collected from USGS long-term monitoring stations
      ii. 250 stations with data from 1973-1994
          1. "The set of 150 stations provides a geographically representative description of the water quality of major rivers of the U.S."
      iii. Measurements included TN, TP, DIN, TKN, DP, flow, turbidity, suspended sediment, fecal coliform bacteria
   b. Inferences about the causes of nutrient trends
      i. Nutrient trends analyzed for relation to other water-quality constituents, including turbidity, suspended sediment, and fecal bacteria

- ii. Monitoring sites classified by watershed land-use (urban, agricultural
  - 1. 94 of 250 stations fell into one of the categories, all others classified as mixed
- iii. Considered "major national changes in municipal wastewater and diffuse nutrient sources and agricultural management during the study period…" when interpreting data and sources of observed trends

3. Results

- **a.** Trends in nutrient concentrations and water-quality measures
  - **i.** Downtrends in TN and TP concentrations were more numerous among sites than uptrends from 1975-1994
    - **1.** Downtrends in TP occurred at 6x more sites than uptrends in TP
  - **ii.** Uptrends in DIN occurred at 2x sites with downtrends
  - **iii.** More downtrends than uptrends in suspended sediment (3/1) and turbidity (10/1) nationally
    - **1.** These were coincident in about half of all sites with downtrends in TN (50%) and TP (40%) concentrations
  - **iv.** 24% of sites nationally exhibited downtrends in fecal bacteria, 6% exhibited uptrends
- **b.** Trends in trophic-state classifications (based on P or N concentrations)
  - **i.** Initial State (as of 1975)
    - **1.** Agricultural and Urban frequently eutrophic (>50%)
    - **2.** Few forested sites classified as eutrophic, >70% were meso- or oligotrophic
    - **3.** Many shrub-grasslands classified as eutrophic based on P concentrations
      - **a.** Fewer classified this way based on TN
  - **ii.** Improvements as of 1994
    - **1.** Shrub-grasslands: 52% improved based on TN
    - **2.** Forested: 42% based on TP
      - **a.** Some of these sites were located in the Susquehanna River watershed
      - **b.** Agricultural Sites: ~1/3 of sites (n=8) improved based on TN
        - i. Most in Missouri/Mississippi regions
  - **iii.** Worsening in trophic state as of 1994
    - **1.** Fewer than 10% of sites within each of the land-use categories worsened based on TP
    - **2.** At worsening or consistently eutrophic agricultural sites
      - **a.** At these sites "manure nitrogen declined from 1982 to 1992 by an average of 15%, whereas nitrogen fertilizer increased from 1975 to 1994 by an average of about 60%"
      - **b.** Choptank River in Maryland declined from mesotrophic to eutrophic

            c. Significant uptrends observed in total nitrogen at four agricultural sites, three of which were eutrophic from 1974-1995
    iv. Recent trends in nutrient concentrations and trophic conditions, 1994-2001
        1. Examined data from 27 sites
        2. In 1994, 65% eutrophic based on TP, 40% eutrophic based on TN
            a. 1 site improved based on TP, 3 sites improved based on TN
            b. Worsened at 5 sites for TP and two sites for TN

4. **Discussion**
    a. "Our conclusions about changes in the probable trophic conditions at the monitoring sites are based exclusively on estimated long-term changes in total nutrient concentrations (both observed and those adjusted for seasonal and flow effects) in relation to the nutrient concentration boundaries of the trophic-state classes; information on changes in chlorophyll concentrations or other measures of algal biomass was unavailable for these sites. The estimates of changes in the probable trophic conditions assume that algal production at the sites was primarily controlled by nutrient availability; these estimates do not account for cases where algal growth was primarily limited by physical properties (e.g., light, temperature, water velocity and depth, and substrate conditions)."
    b. These criteria developed (Dodds et al. 1997b) with the understanding that trophic-state-reassessments would take place as new literature became available
        i. The trophic-state classifications proposed here may change with changing nutrient concentration boundaries

Title: Toxicological evaluation of microcystins in aquatic fish species: Current knowledge and future directions

Authors: Pavagadhi, S., and R. Balasubramanian

Journal: Aquatic Toxicology 142–143:1–16, 2013

Study Objective:

"The main objective of this review is to consolidate and integrate the major findings reported on the toxicological impacts of MCs [(microcystins)] on fish under various environmental conditions in a systematic manner… The last section of this review article points out future research directions of this relatively new research area. In addition, it presents suggestions to evaluate the toxicity of MCs with the use of high throughput platforms such as the use of "omics"-based techniques among others."

Abstract: (Pavagadhi and Balasubramanian 2013)

Microcystins (MCs) are algal toxins produced intracellularly within the algal cells, and are subsequently released into the aquatic systems. An increase in the frequency and intensity of occurrence of harmful algal blooms has directed the global attention towards the presence of MCs in aquatic systems. The effects of MCs on fish have been verified in a number of studies including histological, biochemical and behavioral effects. The toxicological effects of MCs on different organs of fish are related to the exposure route (intraperitoneal injection, feeding or immersion), the mode of uptake (passive or active transport) as well as biotransformation and bioaccumulation capabilities by different organs. This paper reviews the rapidly expanding literature on the toxicological evaluation of MCs in fish from both field studies and controlled laboratory experimental investigations, integrates the current knowledge available about the mechanisms involved in MC-induced effects on fish, and points out future research directions from a cross-disciplinary perspective. In addition, the need to carry out systematic fish toxicity studies to account for possible interactions between MCs and other environmental pollutants in aquatic systems is discussed.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    - **a.** Factors influencing cyanobacterial growth and bloom events (all as a function of depth)
        - **i.** Light intensity (500-600nm)
        - **ii.** N/P ratio (10:1-16:1)
        - **iii.** Temperature regimes (20-35° C)
    - **b.** "Recent research evidences suggest that the interactive effects of future eutrophication and climate change would increase the likelihood of HABs [(harmful algal blooms)] occurrence and severity."

c. Toxins produced by cyanobacteria
    i. Cyanobacteria produce a variety of toxins. The most commonly found in aquatic systems are microcystins (MCs), a class of cyclic peptide hepatotoxins (affecting the liver).
        1. MCs produced by *Nostoc*, *Microcystis*, *Oscillatoria*, and *Anabaena*, among other genera
        2. MCs most widely studied are "potent acute liver toxins"
    ii. Liver damage by MCs
        1. Early signs include increased liver weight and serum of liver enzymes (a sign of liver cell death)
        2. Also promote tumor growth
    iii. Phosphatase Inhibition
        1. Phosphatases 1 and 2A are key enzymes in cell regulation,
            a. Responsible for removing phosphates from proteins, "a common step in many biochemical pathways."
        2. Inhibition of these enzymes "is believed to be a major mechanism by which MCs destroy livers
        3. "Hepatocytes from animals treated with MCs appear to die by a process of programmed cell death…"
d. MC-induced poisonings
    i. Usually occur among domestic animals that drink from waters containing HABs, or those that bathe in them
    ii. "Thousands of livestock fatalities and numerous poisonings in dogs have been linked to the ingestion of cyanobacteria."
e. MC effects on aquatic life
    i. First contact point for MCs because they are released from cyanobacterial cells actively or upon lysis
    ii. Bioaccumulation of MCs in aquatic food webs is a possibility
        1. Certain groups, such as rotifers and protozoans, are highly sensitive to these toxins "which implies that processes such as microbial loop are disturbed with negative consequences for other organisms in the food web."
        2. "Fish typically either ingest cyanobacteria, or their released MCs, or prey upon organisms that have fed on cyanobacteria."

2. Toxicological impacts
a. "Inhibition of protein phosphatases, leads to phosphorylation of cytoskeletal proteins and cytoskeletal-associated proteins and consequent redistribution of these proteins."
b. Oxidative stress can result from MC exposure, and potentially result in molecular damage
c. "The uptake of MCs by aquatic organisms has been associated with the production of reactive oxygen species (ROCs).." leading to:
    i. Increased lipid peroxidization

ii. DNA damage
iii. DNA-protein crosslink
iv. Mitochondrial damage
v. Alteration of antioxidant defense system

d. Uptake

i. "… fish species tend to bathe in water containing MCs following bloom lyses, when MCs persist in aquatic systems for long periods of time due to their chemical stability and resistance to breakdown."

1. Referred to as "balneation" or immersion

2. Would mainly cause uptake dermally or actively through the gills during respiration

a. "Normally, the first-pass effect (also known as first-pass metabolism or presystemic metabolism) is a phenomenon whereby the concentration of a chemical species is greatly reduced before it reaches the systemic circulation… The liver may metabolize many toxins to such an extent that only a small amount of active chemical component emerges from the liver to the rest of the circulatory system. The *first pass* through the liver thus greatly reduces the bioavailability of the chemical species."

b. The *first pass* effect is only present when cyanobacteria or MCs are ingested, and therefore balneation is a more powerful method considering MC delivery to systemic tissue other than the liver

e. Resultant disease

i. "In adult fish, field studies demonstrated that after ingestion, MCs were first detected in gut contents, which then accumulated in liver and also in muscle and viscera."

ii. Fish fed with cyanobacterial cells have exhibited reduced growth, and exhibit stress response when exposed to these cells or MCs

iii. Immunological indices are negatively affected

1. Red blood cells

2. Plasma enzymes

iv. "Chronic exposure of fish to the cell contents of cyanobacteria has been shown to promote osmoregulatory imbalance, increase volume of fluid in the gut, and inability to remove excess water. Studies strongly suggest that the massive fish kill from microcystis blooms may result from the loss of ion homeostatic processes produced by the inhibitory action of MCs on the ion pumps."

Title: Ecological assessments with algae: A review and synthesis

Authors: Stevenson, J.

Journal: Journal of Phycology 50:437–461, 2014

Study Objective:

"The goals of this review were to describe the relationships among environmental management and the many types of information generated in algal sciences, thereby increasing the potential for algal research to be integrated and applied in environmental policy."

Abstract: (Stevenson 2014)

Algae have been used for a century in environmental assessments of water bodies and are now used in countries around the world. This review synthesizes recent advances in the field around a framework for environmental assessment and management that can guide design of assessments, applications of phycology in assessments, and refinements of those applications to better support management decisions. Algae are critical parts of aquatic ecosystems that power food webs and biogeochemical cycling. Algae are also major sources of problems that threaten many ecosystems goods and services when abundances of nuisance and toxic taxa are high. Thus, algae can be used to indicate ecosystem goods and services, which complements how algal indicators are also used to assess levels of contaminants and habitat alterations (stressors). Understanding environmental managers' use of algal ecology, taxonomy, and physiology can guide our research and improve its application. Environmental assessments involve characterizing ecological condition and diagnosing causes and threats to ecosystems goods and services. Recent advances in characterizing condition include site-specific models that account for natural variability among habitats to better estimate effects of humans. Relationships between algal assemblages and stressors caused by humans help diagnose stressors and establish targets for protection and restoration. Many algal responses to stressors have thresholds that are particularly important for developing stakeholder consensus for stressor management targets. Future research on the regional-scale resilience of algal assemblages, the ecosystem goods and services they provide, and methods for monitoring and forecasting change will improve water resource management.

Information most pertinent to Mid-Atlantic management issues

1. **Introduction**
    a. Scientific use of algal indicators began over a century ago, and even this was predated by use by coastal Native Americans
    b. "Contemporary ecological assessments with algae can be defined broadly as the application of algal biology to understand relationships among human and natural determinants of algae and ecosystem services. Thus, assessments determine the

causes and consequences of growth, accumulation, and death of nuisance and toxic species of marine and freshwater algae for safe use and productivity of drinking water and fisheries."

- **c.** "Assessments use the species composition of algae in all aquatic habitats to characterize deviations from minimally disturbed condition, which has regulatory significance in many countries for protecting water quality."
- **d.** "Solving environmental problems requires understanding complex systems, draws from many disciplines of phycology and other sciences, and has many intermediate objectives; but the ultimate goals are relatively focused on the protection and restoration of the final ecosystem goods and services (EGS) that support human well-being."

2. Ecological Assessment and Management

- **a.** "EAM can be summarized in four, multistep phases: designing the ecological assessment, characterizing condition, diagnosing causes and threats, and selecting management options."

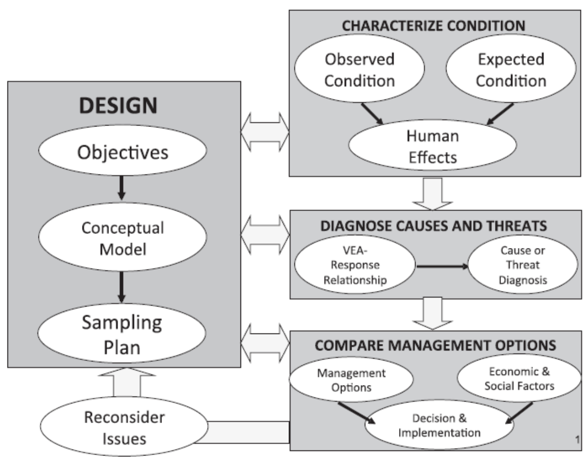

FIG. 1. A framework for assessing and managing ecological systems (Stevenson 2004a, b).

3. Designing Assessments: Goals and Conceptual Models

- **a.** "Conceptual models provide an overview of how healthy and safe communities are related to specific attributes of ecosystems. They provide a list of variables that could be measured as well as the relationships among variables that will be important for diagnosing causes of problems and developing management plans. Conceptual models should include the ecological system as well as elements of the fully coupled human and natural systems (CHANS)…"
  - **i.** Thus, an overarching conceptual model for integrated assessments of CHANS should emphasize at least four CHANS elements..." (A possible fifth element is added here)
    1. Physical, chemical, and biological characteristics
    2. Elements of human well-being
    3. Economic activities
    4. Stressors resulting from human activities
    5. Environmental policy

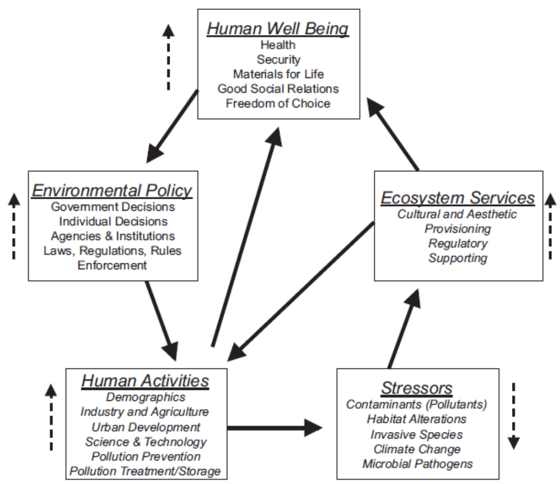

Fig. 2. A conceptual model coupling human and natural systems (CHANS) by linking ecosystem goods and services (EGS) to human well-being and economic activities to stressors. EGS are linked to human well-being directly and indirectly via economic activities within the human activities element. The model includes negative feedbacks on ecosystem services from human activities in the form of stressors. The model also includes feedbacks on the economic activities that produce stressors by individual and institutional decisions about products to buy and regulations to support. The vertical dashed arrows indicate that management goals are high or low levels of conditions within a CHANS element, such that if stressors are managed at low levels by good environmental policies, we can have high levels human activities in watersheds, EGS, and human well-being. From Stevenson (2011).

   - **b.** "Algae complexly support a set of final EGS that support human well-being directly with aesthetics and moral services and indirectly by provisioning the economy with natural resources that provide water, food, and chemicals for many economic products. The complexity in these relationships is caused by the possibility that modest increases in algal production have positive effects on some EGS and negative effects on others."
   - **c.** "The ecological determinants of algal metabolism and thereby species composition, biomass, and diversity of assemblages are remarkably similar among habitat types, which should enable transferring problem solving approaches from one ecosystem to another."

4. **Designing Assessments: Sampling Plan**
   - **a.** "The sampling plan for ecological assessments varies with goals and phase of the assessment."
   - **b.** "Repeated sampling of a site, or a set of sites, is important for evaluating trends in ecological conditions resulting from human disturbance or restoration, or to confirm current status."
      - **i.** On shorter timescales, used to determine success of management/restoration, or whether site meets management criteria
   - **c.** "Experimental and modeling approaches are also study designs used in algal assessments, primarily to diagnose stressors and model success of management options."
      - **i.** Confirm cause-effect relationships and parameterization of relationships in models
      - **ii.** "Modeling has also been used for assessment when probabilistic sampling was not practical, such as extrapolations of results from small-scale assessments to all sites in a region, nation, or the world…"

5. **Designing Assessments: Selecting Indicators**
   - **a.** "Good indicators change substantially across a gradient of human disturbance and have little spatial or temporal variability (i.e., high signal:noise ratios)… [and] are ecologically and socially relevant, broadly applicable, cost-effective, previously measured, technically feasible, diagnostic, and complementary."

- **i.** Biomass
  - **1.** Can be indicator of harm to drinking water quality, aesthetics, recreation, fisheries productivity, and ecological stressors
  - **2.** "Algal biomass is considered a good indicator of human disturbance because it monotonically responds to resource and toxic stressors…"
    - **a.** Challenges:
      - **i.** Varies greatly with temporal weather change, nutrient loading in coastal zones, scouring in streams and rivers
      - **ii.** Varies spatially within habitat with light, current, and substrate composition
    - **b.** Remote sensing for biomass rapidly developing
      - **i.** Historical images can even be utilized
- **ii.** Nutrient chemistry and toxins
  - **1.** "Determining the relative importance of N and P regulation of algal accumulation in habitats is challenging because nutrients are sequestered in cells and can be removed from the water column by settling planktonic algae and benthic algae."
  - **2.** "Presence of toxin-producing algae is an indicator of a threat, but toxin-producing algae often do not produce toxins. Toxin analysis is challenged by the lack of analytical standards and toxicity equivalence factors."
- **iii.** Diversity
  - **1.** "Algal diversity is a good indicator of human disturbance because diversity if highly relevant for management; but challenges exist because some algal diversity indices are not accurate measures of species richness, and they vary non-monotonically along gradients of human disturbance."
  - **2.** "… Not consistently related to human disturbance for three basic reasons…"
    - **a.** "First, species richness is poorly estimated because only minute proportions of algae in habitats are examined and many rare taxa are not observed."
    - **b.** "Second, we might expect the number of species observed in counts to be proportional to the number of species in samples, however evenness of species abundances strongly affects the number of species observed in counts."
    - **c.** "Third, species richness could vary non-monotonically along stressor gradients, for example, positive responses at low levels of resource stressors and negative effects at high levels."
- **iv.** Taxonomic Metrics and taxa traits

1. "Species environmental optima and ranks are also usually related to specific environmental gradients, such as pH, nutrient concentrations, conductivity, salinity, and organic pollution."
2. "Another way to determine species ecological preferences is regression and indicator species analysis, which have been used to characterize species habitat preferences, such as taxa characteristically found in minimally disturbed habitats, highly disturbed habitats, and low or high nutrient conditions."
3. "Morphological traits (filamentous, heterocystous, motile, stalked, monoraphid, biraphid), growth forms (colonial, unicellular, planktonic, benthic), taxonomy, and potential toxicity of taxa are traits related to species function in habitats."

v. Taxonomic Metrics of Stressors

1. Algal biomass in streams across US ecoregions better related to "diatom-inferred TP" than average TP concentrations
   - **a.** Use diatom assemblage to predict algal biomass via diatom-TP relationship

vi. Taxonomic Metrics of Biological Condition

1. "… metrics of biological condition should unambiguously and independently characterize changes in sensitive and tolerant taxa. Therefore, separate metrics should be used for sensitive and tolerant taxa."

vii. Multimetric indices (MMI)

1. "MMIs are calculated by averaging the values of more than one metric after ranges of metrics are recalculated to be equal."
2. "They are hypothesized to respond more consistently to a wide range of human alterations of ecosystems than individual metrics."

6. Characterizing Condition: Expected Condition

a. "Ecological condition is characterized by answering two questions."

- **i.** "What is the natural or expected condition?"
- **ii.** "Have human activities affected the natural or expected condition?"

b. "Expected condition can be defined according to uses with legislated criteria, reference condition, or desired condition."

c. "Reference condition is a common goal for water resource management because low levels of primary production and high levels of biological condition (i.e., biological integrity) support many goals of management. Reference condition can be determined by expert opinion, historical data, paleolimnological reconstruction, ecological surveys, and modeling."

- **i.** "Reference condition is also characterize by assuming that current conditions in minimally disturbed watersheds represent historical natural conditions."

- **ii.** "Routinely, reference condition is characterized by selecting a subset of sites that meet criteria for minimally disturbed, best available, or best attainable sites."
- **iii.** Can also use all sampling sites (not just best available) to determine reference conditions by statistical modeling of habitat criteria as related to human impact level (i.e., 25^{th} percentile values of statistical models). This is referred to as a "dirty model."
    - **1.** "…may be necessary in ecoregions with few reference sites because watersheds are extensively altered by human activity… can also be valuable for providing a standardized scale for quantifying reference condition when minimally disturbed and best available conditions are known to vary among regions with varying extents of alteration by human activity."

7. Characterizing Condition: Stressor-Response relationship (SRR) and Criteria

- **a.** "Quantitative relationships among elements in CHANS [coupled human and natural systems] are important for understanding feedbacks, tradeoffs, and thresholds in these complex systems."
- **b.** "SRRs describe the loss of valued ecological attributes (i.e., EGS) with increasing pollution or habitat alterations. They can be used to diagnose the stressors causing or threatening to cause problems, and they can quantify the costs and benefits of better stressor management."
    - **i.** Complement reference approaches for establishing criteria
- **c.** Criteria development involves three steps:
    - **i.** "…find an ecosystem service that responds nonlinearly to a stressor;"
    - **ii.** "… establish the stressor criterion at a level that protects the ecosystem service;"
    - **iii.** "… establish a complementary biological criterion using a biological indicator or MMI [multi-metric index] that responds linearly to the stressor and at an indicator or MMI level predicted to occur at the stressor criterion."

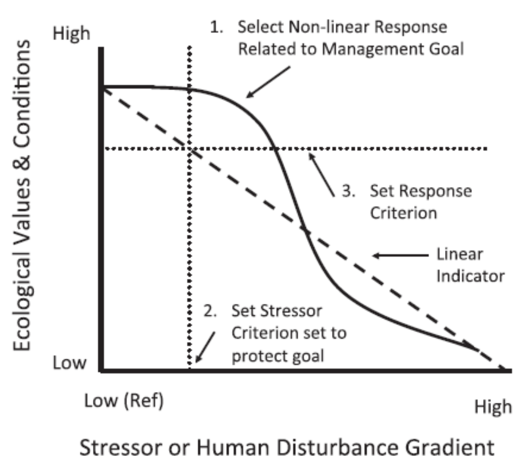

FIG. 3. Steps for using threshold responses to set both stressor and biological criteria (modified from Stevenson et al. 2004b).

d. Thresholds: SRRs that have some resilience at low disturbance levels are ideal because they can be used to identify a threshold, "where risk of losing a desirable attribute or gaining an undesirable attribute suddenly increases with steady incremental increases in stressor level."
   i. Particularly useful and identifiable in response of algae to nutrient concentrations
   ii. Other thresholds are useful as well. Ex: stream diatom species composition exhibits greater changes "in the circumneutral range of pH than high or low pH ranges."
   iii. "Thresholds can be found in many relationships in CHANS and are particularly important for managing ecological systems. Threshold responses can propagate through CHANS to stimulate management decisions.'
      1. *Cladophora* sp. response to TP provides an excellent example of this
         a. "Filamentous green algal cover of stream bottoms increases from less than 5% to greater than 20% cover at thresholds of 23 and 27 μg TP/L in Kentucky and Oklahoma (Stevenson et al. 2012)"

e. "Resolving nonlinear responses requires sufficiently high sample size and often repeated sampling of habitats to precisely characterize conditions at a site."

f. "Propagating thresholds" can occur where a threshold response in one stressor-EGS relationships impacts another, creating a cascade of effects.

g. Classifying systems by "tiered uses" allows for different criteria and management strategies based on intended use and desired condition

h. "Refinement of SRRs becomes even more important as environmental management policies start to incorporate ecosystem services as management endpoints and to ensure more likely achievement of management goals without overprotection."
   i. "Tradeoffs exist in uses of waters for different ecosystem services"

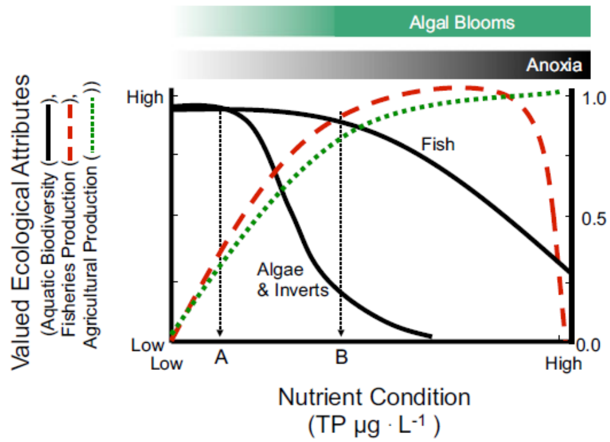

FIG. 4. Tradeoffs among uses of rivers indicated by hypothetical relationships between a resource stressor (e.g., nutrient concentrations) and a suite of ecosystem services of catchments: drinking water impairment by algal blooms; algal, invertebrate, and fish biodiversity; fisheries production; and agricultural production. The vertical lines (A and B) indicate nutrient criteria that could be used to protect different uses in different watersheds, e.g., all aquatic biodiversity at A and fish biodiversity, fish productivity, and agriculture at B (modified from Stevenson and Sabater 2010).

8. **Diagnosing Stressors**
   a. "Diagnosing stressors in ecological assessments calls for listing possible human alterations of ecosystems that could be causing problems, characterizing likely stressors, and evaluating a wide range of evidence."
   b. Nine causal criteria "should be considered when designing assessments by including variables in the conceptual model that are plausible stressors with conceptually sensible reasons for causing the problem."
      i. **Coherence/Plausibility**: "cause-effect relationship is consistent with known information."
      ii. **Analogy**: "refers to similar stressors causing similar problems."
      iii. **Temporality**: "refers to timing with cause occurring before effect."
      iv. **Strength/consistency/specificity** of the association: "call for evidence that the problem has a high probability of occurring if exposed to the stressor, that stressor and problem co-occur in other ecosystems, and that no other stressors cause this problem."
      v. **Experiments**: "important for establishing that a stressor or interaction among multiple stressors can cause a specified effect."
      vi. **SRRs**: "show that incremental increases in stressors correspond to magnitude of the effect, and they provide the basis for developing stressor criteria."
   c. "If we assume that we have gathered information on all plausible stressors, including the correct stressor, then stressor diagnosis should be a matter of comparing observed condition to predicted responses of the ecosystem based on SRRs to determine which stressor is sufficiently great to cause the problem."

- **d.** "Erroneous conclusions can result from this approach if multiple stressors covary with the causal stressor and we have incomplete information on SRRs of individual stressors. Multiple interacting stressors can confound SRRs."

9. Management

- **a.** "Managing algal conditions provides a good example of the challenges for resource managers because tradeoffs exist for managing aquatic EGS along nutrient gradients. Nutrients positively affect food webs and some provisioning ecosystem services and negatively affect water clarity, dissolved oxygen, water chemistry, and related drinking water, aesthetic, and cultural EGS (including biodiversity). In addition, EGS derived from economic activities that produce nutrient pollution in urban and agricultural ecosystems are important in the management model."
  - **i.** Relative, to ecological researchers, "resource managers face challenges for managing waters for the diversity of their "uses" without major compromises to economic activities."
- **b.** "To optimize natural resource and economic performance across a region, as well as sustainable resource use, resource managers need more quantitative understanding of SRRs between EGS, metrics commonly used to assess ecosystems, and stressors… Policies that allow tiered uses of waters and managing different waters for different uses provide the regulatory mechanism for integrating ecosystem services and ecological condition into a water policy framework."

10. Conclusions and Future Directions

- **a.** Large-scale assessment programs are providing data with sufficient detail, sample size, and scale to fuel an explosion in ecological knowledge."
- **b.** "We know that taxonomy based on morphology alone is not telling us everything we need to know about algal biodiversity. In addition, our sample analysis protocols grossly underestimate species richness in assemblages."
- **c.** "Evolution of algae may not be sufficiently rapid to adapt to global change and support important ecosystem functions. If we are to protect resilience of algal function in aquatic ecosystems, can this be accomplished by protecting high levels of biodiversity in a subset of ecosystems? If so, which ecosystems and how many ecosystems must be protected to maintain resilience of algal function in aquatic ecosystems?"
- **d.** "Algal biologists need to work more closely with economists and social scientists, as well as biogeochemists, hydrologists, engineers, and policy makers, to better understand how their research can be related to valuation of ecosystem services, developing management strategies, and informing environmental policy."

Title: Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters

Authors: Porter, S. D., D. K. Mueller, N. E. Spahr, M. D. Munn, N. M. Dubrovsky

Journal: Freshwater Biology 53:1036-1054, 2008

Study Objective:

"This study utilized a large national data set to address two study objectives: (i) to assess relations between published algal-autecological metrics and nutrient and suspended-sediment concentrations at large (national) and intermediate (regional) spatial scales and (ii) to determine whether differences in algal-metric values occur among undeveloped and developed land-use categories. We then compare the efficacy of algal and water chemistry approaches for assessing eutrophication and organic enrichment, and discuss implications for establishing nutrient and biological criteria for U. S. A. streams and rivers."

Abstract: (Porter et al. 2008)

1. Algal-community metrics were calculated for periphyton samples collected from 976 streams and rivers by the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program during 1993-2001 to evaluate national and regional relations with water chemistry and to compare whether algal-metric values differ significantly among undeveloped and developed land-use classifications.
2. Algal metrics with significant positive correlations with nutrient concentrations included indicators of trophic condition, organic enrichment, salinity, motility and taxa richness. The relative abundance of nitrogen-fixing algae was negatively correlated with nitrogen concentrations, and the abundance of diatom species associated with high dissolved oxygen concentrations was negatively correlated with both nitrogen and phosphorus concentrations. Median algal-metric values and nutrient concentrations were significantly lower at undeveloped sites than those draining agricultural or urban catchments.
3. Total algal biovolume did not differ significantly among major river catchments or land-use classifications, and was only weakly correlated with nitrate (positive) and suspended-sediment (negative) concentrations. Estimates of periphyton chlorophyll a indicated an oligotrophic-mesotrophic boundary of about 21 mg m^{-2} and a mesotrophic-eutrophic boundary of about 55 mg m^{-2} based on upper and lower quartiles of the biovolume data distribution.
4. Although algal species tolerance to nutrient and organic enrichment is well documented, additional taxonomic and autecological research on sensitive, endemic algal species would further enhance water-quality assessments.

Information most pertinent to Mid-Atlantic management issues

1. Introduction

   a. "Autecological attributes, the physiological requirements or tolerances of algal species, can be aggregated into metrics or autecological classes that indicate nutrient conditions, trophic status and indices of biotic integrity."
   b. Much more work on diatom indices in Europe, including application
   c. Efficacy of these algal metrics as indicators of nutrient/water quality, and land use, has not been thoroughly tested in U.S. streams and rivers
2. **Methods**
   a. Study area and scope
      i. Periphyton sampled throughout US under USGS National Water Quality Assessment Program (NAWQA) 1993-2001
      ii. 7000 algal samples from >1500 streams and rivers
      iii. 976 sites in continental U.S.
   b. Algal Indicators
      i. Periphyton collected from substrate during low-stream flow
      ii. Biovolume/density
         1. 600 algal cells enumerated from each sample.
         2. "Biovolume was estimated by measuring the dimensions of 15 or more representative cells and calculating cell volume in accordance with the nearest geometric shape."
         3. "… total cell density and biovolume were calculated by summing results for all taxa in each sample."
      iii. Autecological characterization based on previous literature describing taxa
   c. Water chemistry indicators
      i. WQ samples collected with depth-integrating sampler, multiple locations along stream cross section
         1. Dissolved ammonia, nitrite/nitrate, orthophosphate
         2. Total Kjeldahl Nitrogen (TKN), total phosphorous (TP),
         3. Suspended Sediment
   d. Land-cover classification
      i. 6 categories determined from National Land Cover Data digital maps
         1. Agricultural, Urban, Mixed,
            a. Each of the three categories above further separated as "Partially Developed" and "Undeveloped"
   e. Analysis of Data
      i. "Spearman's rank correlation analysis was used to evaluate algal-metric relations with nutrient and suspended-sediment concentrations. Redundancy analysis (RDA) was used to investigate relations between the 35 algal metrics and water chemistry."
3. **Results**
   a. National algal-metric relations with water chemistry
      i. 30 of 35 metrics correlated with one or more nutrient forms
         1. **Correlation stronger in total rather than dissolved nutrients**

   - **ii.** Taxon richness and relative abundance of tychoplanktonic algae (organisms carried into water column, i.e., algae floating after benthic disturbance) increased with suspended sediment and all nutrients except nitrate
   - **iii.** Algal indicators of tolerance positively related to most nutrient forms and suspended sediment, "demonstrating the efficacy of the metrics for indicating nutrient and organic enrichment."
      - **1.** "Based on the magnitude of correlation coefficients" the best indicators were (in order):
         - **a.** Nitrogen heterotrophic diatoms
         - **b.** Diatoms tolerant of low dissolved-oxygen concentrations
         - **c.** Pollution-class most-tolerant diatoms
         - **d.** Brackish water diatoms
   - **iv.** Nitrogen fixer abundance negatively correlated with nitrate and total nitrogen (TN)
   - **v.** Sensitive diatoms and "less-tolerant" diatoms negatively correlated with phosphorous concentrations
   - **vi.** "Based on the strength of algal-metric correlations, the best overall indicator of good water-quality conditions (low nutrient and suspended-sediment concentrations) was [diatoms requiring always high DO], whereas [nitrogen fixing algae] was the best indicator of low nitrogen concentrations."
      - **1.** Nitrogen fixing algae not good indicator of suspended sediment or phosphorous concentrations however
- **b.** Regional algal-metric relations with water chemistry
   - **i.** Regional algal-metric associations were mostly similar to those observed nationally
- **c.** Algal biovolume relations with water chemistry
   - **i.** Algal biovolume correlated with nitrate and suspended sediment, however correlations weak
- **d.** Algal metric and nutrient relations with land use classification
   - **i.** "Nutrient relations with land-use classifications generally were consistent with algal-metric results"
   - **ii.** Median values for indicators of nutrient and organic enrichment larger at developed sites
   - **iii.** Median values for indicators of dissolved nutrient lowest at undeveloped sites
   - **iv.** "Comparison of algal-metric and nutrient values at large-river sites with those in other developed land-use classifications suggests that algal indicators of eutrophication may persist with increases in stream size, whereas dissolved-nutrient concentrations decrease longitudinally with increases in stream size…"

e. Comparison of algal-metric values and nutrient concentrations in developed and undeveloped catchments
    i. Efficacy of algal/nutrient indicators to indicate eutrophication varied among major river catchments and with land use
    ii. "Based on the number of significant differences between undeveloped and developed sites, the best overall eutrophication indicators for contrasting developed and undeveloped stream catchments were total nitrogen, total phosphorous, [pollution-tolerant diatoms], and [brackish water diatoms]."

4. **Discussion**
    a. Algal metrics correlated with many water quality conditions they are reported to indicate
        i. Many useful for contrasting undeveloped vs developed drainages
    b. Poor algal-metric relationships in Midwest and Southeast regions likely due to excess nitrogen fertilizer due to high agriculture
        i. "Because algae integrate water-quality conditions over time, periphyton-community structure may have been influenced by ambient nutrient conditions during the period of algal colonization and growth more than by those during the time of algal sampling."
    c. Differences in efficacy between developed and undeveloped sites not well understood
    d. "We suggest that algal metrics might be preferable, or at least complementary, to nutrient concentrations for assessing the trophic condition of streams draining partially developed catchments…"
    e. Algal biovolume weakly correlated with nitrate and suspended sediment concentrations overall
    f. "We hypothesize that nitrogen could be a limiting resource (or a prime determinant) for periphyton growth in relatively undeveloped, western regions of the U.S.A."
    g. "Negative biovolume correlations with TP probably are related to strong association (e. g. adsorption) of phosphorous with suspended sediment."
    h. "Benthic algal-nutrient processes most probably differ depending on whether algal communities are sparse and actively growing (nutrients stimulate algal growth) or whether communities are dense and mature (algal uptake rates approach in-stream rates of nutrient transport). By the first process, biomass would be expected to increase with dissolved nutrient concentrations (positive correlation), whereas by the second process, nutrient concentrations would be expected to decrease with increases in algal biomass (negative correlation)."
    i. "… more emphasis needs to be placed on the taxonomy and autecology of non-diatom algae, particularly the benthic Oscillatoriaceae and cyanobacteria capable of producing toxins."
    j. "Although the tolerance metrics used in this study generally were successful for separating developed from undeveloped sites, improved understanding of the identity and autecology of 'sensitive' algal species, many of which appear to be

undescribed (beyond genus) and probably endemic to North America, should greatly enhance the use of periphyton communities as water-quality indicators."

k. "Algal-tolerance metrics can indicate that eutrophication problems exist or that water-quality conditions are favorable for such problems to develop; however, the relation of those metrics with nuisance growths of filamentous green algae or blooms of eutrophic cyanobacteria (evidence of eutrophication to the public) will require some understanding of recent hydrologic disturbance or days of accrual since the last scouring event."

l. "The efficacy of evolving approaches for algal indices of stream condition may be dependent on innovative approaches for characterizing stream hydrology and grazer effects to place indicators of algal standing crop (e.g. chlorophyll a) in context with indicators of specific water chemistry conditions."

Title: Assessment of filamentous algae in the Greenbrier River and other West Virginia streams

Authors: Summers, J.

Journal: Report by the West Virginia Department of Environmental Protection – Division of Water and Waste Management, December 17, 2008

Abstract: (Summers 2008)

During the summer of 2007, WVDEP received numerous complaints regarding the amount of algae in the Greenbrier River. Most of the complaints centered on the Caldwell to Alderson section of the river; and at least one complaint was received about the level of algae further upstream in the Denmar area. Several employees of WVDEP were familiar with the problem and indicated that the algae bloom had been occurring at various intensities for decades; some asserted that the algae had been getting worse, and perhaps starting earlier, than it had historically. In September 2007, a meeting within the WVDEP Division of Water and Waste Management was held to discuss the problem. Results of water quality samples from the Watershed Assessment Branch sample database (WAB-Base) were summarized at the meeting. The WAB-Base results showed elevated levels of phosphorus in Howard Creek. Howard Creek flows into the Greenbrier River at Caldwell where the algae problem was reported to begin. WAB-Base results also showed that phosphorus levels in Howard Creek were significantly higher below the White Sulphur Springs sewage treatment plant (WSS STP) than above the plant. The WSS STP had a history of solids "washout" which resulted in sludge beds in Howard Creek. Seven golf courses and a fish hatchery are located on Howard Creek, upstream of the WSS STP. Additionally, significant cattle pasturing occurs in the Greenbrier basin upstream of Howard Creek, and the gradient of the river lessens somewhat near Caldwell. It was suggested that the soil particles from the upstream pastures that are high in phosphorus were settling out in this lower gradient section on the river, allowing some of the phosphorus bound to the soil to be released into the water column. Perhaps all these factors were combining to fuel a "perfect storm" of algae. There was no consensus in the September meeting on what the primary source of the algae problem was. A TMDL effort for the Greenbrier river basin was already underway. All the field work for this TMDL had been completed, making available a large amount of recent water quality, biological, and pollutant source tracking data. During the 12 months of monitoring at 130 stations, the only violation of water quality standards was for fecal coliform bacteria. Nutrient samples were taken at several locations; there were no violations listed for nitrate, and currently there is no water quality standard for phosphorus in WV streams. Therefore, TMDLs were not required for nutrients. Pollutant source tracking efforts focused only on the fecal coliform impairments, not on nutrient sources. One outcome of the September meeting was to perform additional source tracking on Howard Creek and the Greenbrier River to better quantify the nutrient sources that could be contributing to the algae problem.

Information most pertinent to Mid-Atlantic management issues

1. **Methods/Data Collection**
    a. "A TMDL effort for the Greenbrier river basin was already underway. All the field work for this TMDL had been completed, making available a large amount of recent water quality, biological, and pollutant source tracking data…. 12 months of monitoring at 130 stations…"
    b. Also utilized data from Watershed Assessment Branch sample database (WAB-Base), "which contains over 30,000 samples from across the state."
2. **Nutrient Source Tracking**
    a. Algae Distribution
        i. Clear starting point for algae in Greenbrier River – just downstream from sewage treatment plant discharge
        ii. "The conclusion drawn from the nutrient source tracking was that the algae in the Greenbrier River results primarily from the dissolved phosphorous in municipal sewage treatment plant effluent combining with nitrogen from a host of sources."
        iii. Algae in Howard Creek (away from Greenbrier main-stem)
            1. Areas where filamentous green algae (FGA) occurred was downstream of "seven golf courses, a trout hatchery, and some minimal pasturing…"
            2. Observed less algae than expected, and this was likely limited by calcium/hardness rather than nutrient input
3. **Keys to Algae Development**
    a. "… if the algae bloom is driven by sewage treatment discharges, why is the algae bloom so severe on some portions of the Greenbrier River and not present on many other rivers in the state."
        i. Analyzed WAB-Base data and found hardness, alkalinity, and pH to be the primary factors resulting in this observation
        ii. "Both alkalinity and hardness are indirect measures of multiple constituents, and are usually expressed as an equivalent concentration of $CaCO_3$. Harness is a property of cations (Ca^{2+} and Mg^{2+}) while alkalinity is a property of anions (HCO_3^{-1}, CO_3^{-2}, PO_4^{-3}, and OH^{-1})."
    b. Threshold Alkalinity
        i. No blooms observed where alkalinity was < 30 mg/L
    c. Hardness Ceiling
        i. Some streams observed where ample P, N, and alkalinity were present that did not cause blooms
        ii. Hardness level on these streams was generally in excess of 100 mg/L
            1. On the Greenbrier, hardness did not exceed 100 mg/L
    d. Nutrients
        i. "In the Greenbrier River, algae blooms were occurring with P concentrations as low as 0.01 – 0.014 mg/L. The amount of algae

formation increased with an increase in P concentrations found below the discharges of the sewage treatment plants."

- **ii.** There is more than enough nitrate loading, even disregarding that from sewage outfall, to stimulate algae growth

- **e.** Other Factors (Turbidity and Temperature)
    - **i.** "Other streams with favorable chemistry which did not have significant levels of filamentous algae had obvious limiting factors for algal growth, most often turbidity (Kanawha River and Brush Fork of Bluestone) or temperature (Piney Creek)."

4. Application in West Virginia

- **a.** "It seems hardness could indeed be used as a basic indicator of a West Virginia stream's propensity to grow filamentous algae. A hardness level less than 100 mg/L appears ideal for algae development. Suppression of algae seems to occur around 120-150 mg/L"

5. Other Factors

- **a.** Turbidity/Substrate
    - **i.** Turbidity can be limiting, and high FGA growth was generally in the least turbid areas
    - **ii.** Sediment-laden stream bottoms can prevent FGA growth, which occurs attachment to hard substrates
- **b.** Temperature
    - **i.** Optimal temperature for *Cladophora* sp. is 15-25°C
    - **ii.** No significant FGA blooms found in cold water streams

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APPENDIX A

TABLE 1. Methods for assessment of nuisance algal blooms and ecological impact.

Article Citation	Targeted Metrics[1]	Methods[2]	Study Organisms	Advantages/disadvantages, relevant results
Anker et al. 2014	a, c	Aerial digital photography - spectral analysis (ADP-SA), ground truthing	*Cladophora glomerata, Nasturtium officinales*	Cost-effective, non-subjective, high resolution (4 cm), particularly suited to small streams
Cattaneo et al. 2013	a, c	Statistical models predicting FGA occurrence using hydrological and climate data (tested against remote sensing with ground truthing)	*Cladophora* sp., *Hydrodictyon* sp., *Spirogyra* sp., *Oedogonium* sp.	Models predicted FGA occurrence with a 74% success rate
Depew et al. 2009	b, c	Single-beam, high-frequency echosounder with ground-truthing	*Cladophora* sp.	Accurate stand height and percent cover estimates on stands >7.5 cm, however this method was unable to accurately estimate biomass.
Dodds and Welch 2004	d	Two-step statistical method: (1) ANCOVA distinguishes regions based on anthropogenic land use; (2) linear regression models describe nutrient concentrations relative to land use. Intercept is reference value (theoretically no anthropogenic impact).	N/A	Able to establish reference values when reference streams are not present or limited. However requires extrapolation outside data range.
Dodds et al. 1997	d	Two methods presented: (1) Mean nutrient levels measured in reference streams, (2) regression and graphical analysis of nutrient and stream quality data from large stream database	N/A	Methods are complementary, with the first approach being site-specific and the second more widely applicable and contextual.
Kilroy et al. 2013	b, c	Visual estimates of periphyton percent cover tested against chl a measurements. Analyzed for differences across operators, rivers, and time.	N/A	Visual estimates distinguished sites and occasions as effectively as chl a. Were able to estimate chl a from visual assessments.
Stevenson et al. 2012	b, c, e, f (nutrient concentrations and macrophyte community responses to poultry house density [PHD])	Biomass: chl a and ash-free dry mass; visual estimates of % cover; land-use GIS; habitat characterization; water quality measures; nutrient analysis; thresholds: classification and regression tree analysis (CART)	*Cladophora* sp., *Rhizoclonium* sp., *Oedogonium* sp., *Spirogyra* sp., *Mougeotia* sp., *Zygnema* sp., *Tetraspora* sp., *Draparnaldia* sp., *Vaucheria* sp., *Batrachospermum* sp.	This comprehensive combination of methods was able to definitively identify a relation between PHD, nutrient pollution, and habitat degradation in the watershed, and also TP thresholds of FGA biomass and % cover. Visual estimates of % cover were more error-proof than benthic chl a as a measure of standing crop.
Dudley et al. 1986	f (macroinvertebrate community)	FGA/BGA removal experiments	*Cladophora* sp., *Nostoc* sp., various invertebrate taxa	Classified taxa of macroinvertebrates based on response to FGA/BGA: (1) Negative - modified habitat, (2) Positive - habitat provision, (3) Positive - food provision
Mebane et al. 2014	b, c, f (macrophyte abundance)	Biomass: chl a, dry/wet weight; Visual estimates of percent cover; habitat characterization; water quality measures; nutrient analysis: loosely sorbed P measured using $MgCl_2$ extraction	*Cladophora* sp., *Potomageton* sp., *Elodea canadensis,* and other SAV	$MgCl_2$ extraction was a good method for measuring bioavailable P. Found TN to be more strongly correlated with biomass than TP.
Wang et al. 2007	d, e, f (macroinvertebrate and fish assemblages)	Reference values: 25th percentile of all data within ecoregions, regression modeling; thresholds: regression tree analysis, Kolmogorov-Smirnov analysis; redundancy analysis (RDA) partition procedures used to test for relationships, and separate effects, between various biotic indices, nutrient concentrations, and habitat characteristics	35 fish and 26 macroinvertebrate species	Regression modeling better than 25th percentile at estimating of reference conditions in areas with high anthropogenic activity; RDA analysis showed that interactions between nutrients and environmental characteristics explained much more variation in macroinvertebrate andcfish assemblages than nutrients alone
Rosemond et al. 2015	f (carbon sequestration)	Long-term nitrogen and phosphorous addition coupled with litterbag experiments	N/A	N and P enrichment led to an increase in detritivore and microbial production which significantly increased terrestrial C loss rates from streams

[1] **a:** species identification, **b:** biomass, **c:** % cover, **d:** reference/target criteria, **e:** chemical/biological threshold, **f:** ecological response (parentheses indicate targeted response)
[2] Listed methods and results are not comprehensive.