United States
Environmental Protection
Agency

Office of Water
EPA-822-R-14-004

www.epa.gov
September 2014

# U.S. EPA Expert Workshop: Nutrient Enrichment Indicators in Streams

## Proceedings

April 16–18, 2013

## Disclaimer

The information in this document was funded wholly (or in part) by the U.S. Environmental Protection Agency under Contract EP-C-08-004, Task Order 71 and Contract EP-C-12-055, Task Order 04, and was subjected to Agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Furthermore, this document is a summary of the views of the individual workshop participants; approval for publication does not signify that the contents reflect the views of the Agency, and no official endorsement should be inferred.

## Acknowledgments

The U.S. Environmental Protection Agency would like to thank the speakers and others who participated in the workshop *Nutrient Enrichment Indicators in Streams*. Their dedication and hard work at the workshop and following the workshop to produce these proceedings are greatly appreciated.

Workshop Speakers: Dr. Michelle Baker (Utah State University), Dr. Candice Bauer (U.S. EPA Region 5), Dr. Max Bothwell (Environment Canada), Dr. Don Charles (Academy of Natural Sciences), Dr. Betty Fetscher (Southern California Coastal Water Research Project), Dr. Stuart Findlay (Cary Institute of Ecosystem Studies), Terry Fleming (U.S. EPA Region 9), Dr. Steve Francoeur (Eastern Michigan University), Dr. Evelyn Gaiser (Florida International University), Dr. Jim Hagy (U.S. EPA Office of Research and Development), Dr. Anne Hershey (University of North Carolina – Greensboro), Lisa Huff (Alabama Department of Environmental Management), Dr. Ryan King (Baylor University), Tina Laidlaw (U.S. EPA Region 8), Dr. Mark Munn (U.S. Geological Survey), Dr. Greg Pond (U.S. EPA Region 3), Dr. Steven Rier (Bloomsburg University), Dr. Bob Sinsabaugh (University of New Mexico), A.J. Smith (New York State Department of Environmental Conservation), Dr. Nathan Smucker (U.S. EPA Office of Research and Development), Dr. Jan Stevenson (Michigan State University), Dr. Mike Suplee (Montana Department of Environmental Quality) Workshop Organizing Committee: Dr. Janice Alers-Garcia (U.S. EPA Office of Science and Technology), Ifeyinwa Davis (U.S. EPA Office of Science and Technology), Dr. Scot Hagerthey (U.S. EPA Office of Research and Development), Susan Jackson (U.S. EPA Office of Science and Technology), Dr. Jacques Oliver (U.S. EPA Office of Science and Technology), Dr. Michael Paul (Tetra Tech, Inc.), Dr. Dana Thomas (U.S. EPA Office of Science and Technology), Brannon Walsh (U.S. EPA Office of Science and Technology), Dr. Lester Yuan (U.S. EPA Office of Science and Technology), Facilitator: Dr. Michael Paul (Tetra Tech, Inc.)

Note Takers: Katelin Kelly (Tetra Tech, Inc.)

## Contents

## Tables

## Acronyms

| | |
|---|---|
| AFDM | Ash-free dry mass |
| BOD | Biological oxygen demand |
| chl-a | Chlorophyll-a |
| DO | Dissolved oxygen |
| EMAP | Environmental Monitoring and Assessment Program |
| EPA | U.S. Environmental Protection Agency |
| GPP | Gross primary productivity |
| IBI | Indices of biotic integrity |
| IPP | Integrated primary production |
| $mg\ L^{-1}$ | Milligrams per liter |
| MMI | Multimetric multiplicative index |
| NDS | Nutrient-diffusing substrate |
| NH3 | Ammonia |
| NH4+ | Ammonium |
| NO2- | Nitrite |
| NO3- | Nitrate |
| PAR | Photosynthetically active radiation |
| POC | Particulate organic carbon |
| SOD | Sediment oxygen demand |
| SOP | Standard operating procedure |
| SRP | Soluble reactive phosphorus |
| TMDL | Total maximum daily load |
| TN | Total nitrogen |
| TP | Total phosphorus |
| UAA | Use attainability analysis |
| $ug\ L^{-1}$ | Micrograms per liter |

## Foreword

The goal of the expert workshop on Nutrient Enrichment Indicators in Streams was to gather scientific insight into how to best measure the effects of nutrient pollution in flowing waters for the purposes of developing numeric nutrient criteria that are protective of designated uses. This workshop represents just one part of a series of EPA led efforts to inform criteria development with the latest scientific thinking, and ultimately, provide revised guidance to State and Tribal partners. The goal of the workshop was not to reach consensus, rather, it was designed to be a critical thinking and information gathering exercise. Therefore, the workshop proceedings below provide a record of the workshop discussions and primary outcomes but do not contain official recommendations.

The workshop proceedings were peer reviewed to ensure that the discussion presented is accurate and clear. The reviewers' comments have greatly improved the document in these respects and also provided useful direction for future nutrient criteria science development efforts. In response to this proceedings and the reviewers' comments, EPA is now working on several fronts: EPA has partnered with USGS to complete a web-based taxonomic key for diatom taxa to facilitate taxonomic training and nationally consistent taxonomic identification by State and Tribal programs; EPA is also working to develop best practices for diatom and soft-bodied algae sampling and identification to help identify and improve inconsistencies in algae identification among laboratories; further, EPA is piloting the use of metagenomic information as a way to assess algal communities in rivers and streams. EPA intends to incorporate the outcomes from these projects into future guidance.

## Executive Summary

For the past 15 years, the U.S. Environmental Protection Agency (EPA) has encouraged states and tribes to adopt numeric criteria into water quality standards to protect waters from the widespread and growing problem of nutrient pollution. Excess nutrients (nitrogen and phosphorus) cause algal growth that degrades aquatic communities and cause fish kills, degrades beaches and shorelines with nuisance algae, and adversely affect human health from algal toxins and trihalomethane formation in drinking water. State progress toward adopting numeric nutrient criteria has been limited in flowing waters in part because of the technical challenge of developing numeric nutrient criteria when multiple factors (e.g., light, flow) can influence responses (e.g., algal biomass) and confound nutrient response models. Such conditions can make it difficult to predict nitrogen and phosphorus concentrations that adversely affect aquatic life. One approach to overcome such challenges and to reduce uncertainty when implementing numeric criteria is to integrate biological response indicators with numeric nutrient criteria in a decisional framework.

EPA's Office of Science and Technology convened a workshop, *Nutrient Enrichment Indicators in Streams*, at the EPA Potomac Yard Office in Arlington, Virginia, on April 16–18, 2013. The workshop was designed to explore science issues involved in developing criteria that integrate biological responses and nutrient concentrations in streams that are protective of aquatic communities, as required under the Clean Water Act. The workshop explored the state of the science and considered innovative, new approaches to numeric nutrient criteria development that could provide early warning of impairment of aquatic systems. Twenty-two invited technical experts in the field of nutrient pollution indicators—representing academic, state, federal, and international institutions—met with Agency staff over the three days. The experts had two tasks. The first task was identifying a suite of indicators most sensitive to changes in nutrient concentrations and predictive of changes to aquatic life or other designated uses. The second task was identifying combined approaches for (1) indicators readily available for most states (total nitrogen [TN], total phosphorus [TP], chlorophyll-a [chl-a], dissolved oxygen [DO], and benthic macroinvertebrates); and (2) any combination of chemical, physical, or biological indicators that would yield a robust assessment of adverse effects on aquatic life from nutrient pollution

This workshop proceedings document captures the insight of the technical experts. This information will be beneficial in efforts to provide technical support for states on the derivation and implementation of numeric nutrient criteria in flowing waters.

The content below describes the primary workshop findings.

**Which indicators are most sensitive to nutrient pollution in streams and most predictive of impacts to higher trophic levels?**

- Nutrients: TN and TP concentrations provide a direct measure of nutrient pollution. Thus, measured concentrations above thresholds known to adversely affect aquatic life should indicate impairment.
- Primary producers: Chl-a, percent visual coverage of algae and in-stream macrophytes, and measures of algal assemblage (e.g., diatoms and soft-bodied algae) are the most sensitive

response indicators of nutrient pollution in streams. Algal assemblage indicators are widely recommended as sensitive nutrient response indicators in all waters.

- Ecosystem function: Continuously measured DO and pH are good indicators that capture heterotrophic and autotrophic responses, are generally sensitive to nutrient stress, and provide a clear linkage to aquatic life. The workshop participants acknowledged the routine monitoring of fish and macroinvertebrates, and the public's recognition of the linkage between adverse effects on fish and invertebrates and impairment of aquatic life. While they concluded that commonly used fish and macroinvertebrate indices may be less sensitive nutrient pollution indicators than other indicators (e.g., algae), refined and/or species-level metrics for macroinvertebrates specifically calibrated to be responsive to nutrient effects continue to show promise as indicators. However, there can be a significant temporal lag between high nutrient concentrations and adverse effects to some higher trophic levels, making it difficult to proactively prevent nutrient impairment.

### How can criteria be structured in a combined approach?

When there is uncertainty around the relationship between nutrient concentrations and the health of the aquatic community, some experts suggested it might be useful to combine numeric nutrient criteria into a decision framework with other indicators, but there was not universal agreement on the defensibility of such an approach. One approach might be to establish an upper nutrient concentration, above which designated uses are impaired, and a lower nutrient concentration, below which designated uses are attained. The concentrations between these upper and lower values make up a "grey zone," within which a numeric nutrient criterion, expressed as a decision framework, could be applied.

- Considering indicators that are commonly available to states, the following combination of indicators are sensitive to nutrient pollution: nutrient concentrations (TN and TP); chl-a; and, to a lesser extent, DO.
- Considering all possible indicators, the following combination of indicators are sensitive to nutrient pollution and may provide early warning of impairment: nutrient concentrations (TN and TP); a measure of algal biomass (chl-a, ash-free dry mass [AFDM], or visual percent cover); a measure of the primary producer assemblage (mostly based on diatoms); and, to a lesser extent, a measure of ecosystem function (e.g., diel DO or pH).
- Adverse responses from any of these possible indicators should be sufficient to indicate nutrient pollution-related impairment.
- Sufficient data and robust stressor-response models are imperative for numeric nutrient criteria development and assessment. Participants were concerned about the lack of sufficient data for nutrient criterion development and assessment.
- Proper classification of data by attributes, such as expected trophic state or physical factors, is fundamental to reducing natural variability in nutrient responses in different types of streams.

### What information gaps exist regarding nutrient criteria development in streams?

The workshop participants identified the following research needs: (1) development of a single standardized primary producer indicator that integrates the productivity of various producers into a single indicator; (2) improved understanding of the linkages between nutrient measures, primary producer measures, and higher trophic levels that often are used to quantify aquatic life impairment; (3) identification of a minimum data set necessary to characterize stressor-response relationships; and (4) development of regional stressor-response relationships linking nutrient concentrations to algal assemblage indicators, algal abundance, and nutrient-sensitive macroinvertebrate indicators.

## Introduction

The U.S. Environmental Protection Agency (EPA) Office of Science and Technology convened a workshop, *Nutrient Enrichment Indicators in Streams*, at the EPA Potomac Yard Office in Arlington, Virginia, on April 16–18, 2013. Twenty-two invited technical experts in the field of nutrient pollution indicators—representing academic, state, federal, and international institutions—met with Agency staff over the three days. A combination of full-group sessions and breakout sessions facilitated the discussion of protective nutrient pollution indicators in streams and innovative criteria development methods.

### Purpose of the Workshop

For the past 15 years, EPA has encouraged states[1] to adopt numeric criteria into water quality standards to protect waters from the widespread and growing problem of nutrient pollution. Excess nutrients (nitrogen and phosphorus) cause algal growth that degrades aquatic communities and cause fish kills, degrades beaches and shorelines with nuisance algae, and adversely affect human health from algal toxins and trihalomethane formation in drinking water. Progress toward adopting numeric nutrient criteria has been limited in flowing waters in part because of the technical challenge of developing numeric nutrient criteria when multiple factors (e.g., light, flow) influence responses (e.g., algal biomass) and can confound nutrient response models. Such conditions can make it difficult to predict nitrogen and phosphorus concentrations that adversely affect aquatic life in streams. States are seeking improved methods to overcome such challenges and to reduce uncertainty when implementing numeric criteria—for example, by integrating response indicators into a numeric nutrient criterion decisional framework. The purpose of the EPA workshop, *Nutrient Enrichment Indicators in Streams,* was to explore the science underlying novel approaches to numeric nutrient criteria development for the protection of aquatic life.

The workshop had two primary goals:

1. Gather independent scientific views on appropriate chemical, physical, and biological indicators to measure the ecological effects of nutrient pollution in streams.
2. Investigate how indicators can be used in conjunction with numeric nitrogen and phosphorus criteria to improve the accuracy and precision of an assessment decision.

---

[1] The word *state* in this document is intended to also include federally recognized tribes.

EPA views this workshop as part of an ongoing commitment to build technical and scientific capacity for criteria development and to assist states in adopting numeric nutrient criteria into their water quality standards (USEPA 1998[2]).

### Workshop Design

The workshop was designed to provide an opportunity to share and listen to ideas, not to reach consensus on any particular topic; therefore, all relevant discussion is included in this document. This discussion reflects expert opinion.

Following this Introduction, the remaining sections of the workshop proceedings document are organized parallel to the workshop agenda (Appendix B).

## Day 1 Summary

### Welcome and Introductions

Betsy Behl, Director of the Health and Ecological Criteria Division in EPA's Office of Water, provided opening remarks describing the purpose and importance of the workshop and welcoming and thanking the participants. The participants then introduced themselves. She explained that EPA's role in the development of numeric nutrient criteria is to provide national guidance to the states and work with the states to ensure that proposed standards are effective for Clean Water Act purposes. She described how state adoption of numeric nutrient criteria—which currently include total nitrogen (TN), total phosphorus (TP), chlorophyll-a (chl-a), and surface water clarity—has been limited because criteria can be difficult to derive and implement. She added that numeric nutrient criteria can be particularly difficult to derive for streams and rivers because of the complexity of confounding factors such as substrate, canopy cover, and temporal changes. She concluded her remarks by highlighting the need to identify and explore innovative, new approaches to criteria development using assessment endpoints that are sensitive to nutrients and can provide early warning of adverse effects on aquatic life in streams.

After the opening remarks, Dr. Mike Paul, Tetra Tech, Inc., introduced himself as the workshop facilitator. He reviewed the agenda and the purpose of the workshop, and he emphasized the workshop goal—finding nutrient indicators that are both sensitive to and predictable of adverse effects from nutrients. He noted that ultimately EPA is seeking a list of the best nutrient indicators to pursue, taking into consideration indicators typically used now (TN, TP, chl-a, and clarity) and new or emerging indicators.

### Indicator Category Presentations Outline

The workshop discussions on day 1 began with presentations describing nutrient pollution indicators in streams. Several weeks before the workshop, the experts had been assigned to

---

[2] USEPA. 1998. *National Strategy for the Development of Regional Nutrient Criteria*. EPA 822-R-98-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

groups and tasked with developing brief presentations on one of six broad indicator categories: (1) Nutrients, (2) Algal Biomass, (3) Algal Assemblage, (4) Microbial Processes, (5) Higher Trophic Levels, and (6) Ecosystem Function. The purpose of the presentations was not to describe at length every indicator available; rather, it was to stimulate thinking and discussion of the potential universe of nutrient pollution indicators in streams. The groups were given a template to help guide the development of their presentations; that template is below.

### Indicator Category Presentation Template

- List the universe of indicators identified within the assigned category
    - If some indicators were deemed not appropriate, briefly explain why
- Describe the indicators identified as appropriate
    - Where does each indicator lie along the causal pathway?
        - E.g., show a conceptual model
- Describe each indicator's relationship to nutrient stress
    - Is there a scientific, peer-reviewed demonstration of a relationship?
    - Is the relationship quantifiable?
    - What are the rate and trajectory of response to nutrient stress?
        - Sensitive to nutrient pollution (strong signal)
        - Low natural fluctuation (low noise)
        - Disappears quickly when nutrient concentrations decline
- Describe each indicator's relationship to aquatic life use
    - Is it predictive of ecological change and higher trophic level impacts?
    - Can thresholds be identified between supporting and not supporting different designated uses (e.g., literature or others)?
- Identify any feasibility considerations
    - Are reliable methods of measurement/evaluation of the indicator available?
        - Currently?
        - Near future (< 10 years)?
        - Is use of the indicator operationally feasible for state monitoring programs?
        - Ease of measurement/evaluation
        - Cost

## Indicator Category Presentations: Summary

### Nutrients

The group described nutrient indicators as direct measures of nutrient inputs. Six potential indicator types were identified in the Nutrients category:

- Soluble inorganic nutrients
- Organic nutrient fractions

- Total nutrients (direct or calculated)
- Carbon-to-nitrogen-to-phosphorus (C:N:P) ratios of algal cells
- Extracellular enzymes
- Nutrient loads.

Of the six identified, four—soluble inorganic nutrients, total nutrients (direct or calculated), C:N:P ratios of algal cells, and extracellular enzymes—were the focus of the group's discussion.

The group explained that the term *soluble inorganic nutrients* includes the following: nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonia ($NH_3$), ammonium ($NH_4^+$), and soluble reactive phosphorus (SRP; mostly $PO_4^{3-}$). It was noted that the relationship between these nutrient forms and algal/plant growth is well established in the scientific literature. The group also suggested that measuring soluble inorganics can be helpful in identifying sources (e.g., high nitrate levels in ground water) and noted that in some cases soluble organics might need to be measured to inform process-based models (e.g., QUAL2K). The group cautioned, however, that measurements of soluble inorganic nutrients can be highly variable because of rapid uptake and remineralization by algae, plants, and bacteria.

The group also discussed total nutrient concentrations, which represent the total amount of nitrogen and phosphorus potentially available for uptake by algae/plants, as useful measurements for ambient stream monitoring and assessment. These measurements were described as conservative because of the continual cycling between inorganic fractions, algae/plants, higher trophic levels, death, and subsequent remineralization. Also, the link between total nutrient concentrations and algal biomass in streams was described as well documented in the scientific literature.

The group described extracellular enzymes (exoenzymes) produced by algae and bacteria and their ratios as potentially useful for elucidating nutrient limitation status in streams. Algae and bacteria expend energy to produce and release these enzymes in order to acquire nutrients that are in short supply. Glycosidase, peptidase, and phosphatase are three examples of exoenzymes. Ratios among enzymes can also be used to indicate which nutrient is limiting in the algal/bacterial community. However, the group cautioned against the use of enzymes in instances where nitrogen or phosphorus saturation or carbon limitation might result in the erroneous interpretation of a measurement.

Nutrient ratios (C:N:P) were also suggested as useful for determining nutrient limitation status in streams. Because the C:N:P ratio of algal cells growing at optimum conditions tends to be near 106:16:1 (Redfield 1934[3]), deviations from this ratio can be used to diagnose nutrient limitation. Algal cell ratios were recommended. Whole-water-sample ratios as surrogates for algal cell ratios were described as less meaningful and uninformative once nutrient concentrations become saturated. As with extracellular enzymes, the group cautioned against applying nutrient ratios when nitrogen or phosphorus saturation or carbon limitation exists because of the possibility of misinterpreting information.

---

[3] Redfield, A.C. 1934. On the proportions of organic derivations in sea water and their relation to the composition of plankton. In *James Johnstone Memorial Volume*, ed. R.J. Daniel, pp. 177–192. University Press of Liverpool.

## Algal Biomass

The group described the relationship between nutrient inputs and algal biomass measures as well established in the scientific literature. They noted, however, that the strength and nature of the relationship can be variable, depending on confounding factors such as light, flow, substrate, grazing, and species interactions. For these reasons, a sampling design that takes spatial and temporal variability into account in order to “calibrate” nutrient-biomass relationships for different geographic regions and ecosystem types was deemed important. In addition, the group suggested that biomass measures temporally lagged behind nutrient inputs because of storage effects.

The group identified four indicator types in the Algal Biomass category:

- Periphyton (streams and large rivers), measured as: Chl-a, ash-free dry mass (AFDM), biovolume from quantitative algal counts, visual assessment
- Phytoplankton (larger rivers, pools), measured as: Chl-a, AFDM/particulate organic carbon (POC), biovolume from quantitative algal counts/particle counts, transparency/Secchi depth
- Aquatic macrophytes, measured as: percent cover, biomass (AFDM)
- Floating algal mats, measured as: percent cover, biomass.

The group discussed the benefits and drawbacks of each indicator. Chl-a was described as easily measurable and sensitive to nutrient inputs but affected by light and other factors. AFDM was also considered responsive to nutrients but more variable than a measurement of chl-a. The group described biovolume calculations from quantitative algal counts as useful for attaining biomass estimates for specific taxonomic groups but noted uncertainty due to assumptions in measurements and calculations. Visual assessment methods were thought to be more time- and resource-efficient measures of nutrient input effects; however, consistency in survey crew protocol and ability, as well as common problems with water clarity, were listed as drawbacks. The group reiterated the importance of classifying streams when determining which algal biomass measures to apply to a system.

## Algal Assemblage

The group presenting the Algal Assemblage category listed four indicator types as potential measures of shifts in primary producer species composition consistent with nutrient pollution:

- Microscopic counts of diatoms and soft algae
- Visual assessment of dominant algal taxa
- Measures of algal pigment and fatty acid ratios
- Emerging techniques using DNA/RNA sequencing.

The indicators above included currently available taxonomic composition analyses and variables as well as emerging techniques. Overall, algal assemblage measures were described as highly sensitive to nutrient pollution, representative of aquatic life uses, and linked to higher trophic levels; however, it was suggested that some measures suffer from moderate to high variability. It was noted that several of the emerging techniques including, DNA/RNA sequencing, currently lack a demonstrated relationship to nutrients.

The group considered microscopic counts of diatoms and soft algae to be the most useful algal assemblage indicators at this time. The group described relative abundances or biovolume metrics of species or genera as sensitive to changes in nutrient concentrations and land use and as representative measures of aquatic life use. It was suggested, for example, that shifts in taxonomic composition indicate a shift to eutrophic species due to competitive differences in nutrient uptake. The group also discussed the ability of these indicators to link nutrient pollution to higher trophic level responses through changes in food quality.

Visual assessment of dominant algal taxa was also discussed as a potential indicator of community composition shifts and the presence of nuisance taxa. The group described these measures as strongly linked to aquatic life and recreational uses. It was noted that visual assessment of nuisance taxa has a demonstrated relationship to nutrient stress, whereas visual assessment of class and genus ratios were considered to be less strongly linked.

Other indicators discussed by the group included measures of algal pigment and fatty acid ratios and emerging techniques using DNA/RNA sequencing. Pigment and fatty acid ratios were described as highly variable, but the group considered them direct measures of aquatic life and linked to higher trophic levels. Techniques using DNA/RNA sequencing were described as promising for assessing taxonomic composition; the science underlying these emerging techniques, however, is still developing.

### Microbial Processes

The group described microbes and the processes they mediate as important links between nutrients and ecosystem attributes such as nutrient uptake and retention. They presented four potential indicator types:

- Molecular measures: Fatty acids, nutrient storage genes, functional gene frequency, fluorometry, nitrate reductase
- Stoichiometric measures: C:N:P, biomass, nitrogen- and phosphorus-acquiring enzymes, polyphosphates
- Process indicators: Nutrient-diffusing substrate (NDS) targeted at heterotrophs, nitrogen and carbon isotopes, nutrient uptake rates, decomposition rates
- Pharmaceuticals/toxins (as covariates).

Generally, indicators were described as sensitive to nutrient stress, but the linkage to aquatic life use was considered lacking. Indirect links to aquatic life were discussed; for example, heterotrophs can affect oxygen availability and are a food source for some higher trophic levels. It was also noted that heterotrophic processes can affect algal communities.

The group focused their discussion on three indicator examples—nitrate reductase, nitrogen- and phosphorus-acquiring enzymes, and decomposition rates. All three indicators were noted to have readily available, cost-effective methods; the indicators were also suggested to be applicable at a national scale.

### Higher Trophic Levels

The group identified numerous potential macroinvertebrate and fish indicators. Many indicators were described as well established, widely available, or both because of their historical use in biological assessment programs. The group also discussed several examples of biological metrics being used by state monitoring programs.

The following higher trophic level indicator types were presented:

- Multimetric indices
- Pollution tolerance metrics
- Abundance, density, and biomass measures
- Observed/expected or similarity metrics
- Functional group metrics
- Nutrient-specific measures.

The group emphasized the strong link between higher trophic level indicators and measures of aquatic life use but added that the observed relationships to nutrient stress can be highly variable. Fish indicators were highlighted as having a particularly weak and variable relationship with nutrients. To improve the nutrient signal and decrease the noise observed with higher trophic level indicators, the group recommended controlling for confounding factors. The methods recommended included classifying sites during survey design, isolating the habitats sampled, and improving taxonomic resolution.

Observed/expected models and nutrient-specific macroinvertebrate metrics were suggested as having the greatest potential for improved relationships with nutrients and the development of aquatic life support thresholds. It was noted that to achieve these improvements, taxonomic expertise could be developed and regional calibrations could be pursued.

The group also mentioned several emerging indicators that could be useful with additional research. For example, chemical analysis of macroinvertebrate body nutrient ratios was described as promising for improving quantitative relationships between nutrients and specific macroinvertebrate taxa. In addition, research into the link between protozoan and bacterial colonization of macroinvertebrate exoskeletons was suggested as a way to improve quantifiable nutrient relationships in streams.

### Ecosystem Function

The group described ecosystem function measures as linking nutrient inputs to higher trophic levels through increased primary productivity, increased organic matter, increased respiration, and subsequent decreases in available oxygen. The group presented three indicator types:

- Gross primary productivity (GPP)
- Primary productivity
- Heterotrophic productivity.

Within each indicator type, several measures were discussed. Overall, the group described most indicators as having a demonstrated, quantifiable relationship with nutrients. However, the indicator's relationship to aquatic life use and feasibility considerations such as measurement cost varied substantially between indicators.

The group described various methods for determining GPP by measuring dissolved oxygen (DO) levels over time. Whole-system measures were suggested to be the most useful of this type because the signal-to-noise ratio is highest. Diel DO metrics were also discussed as having a good signal-to-noise ratio. The group considered both methods relatively difficult and expensive to measure (approximately $5,000) but added that equipment costs are decreasing.

Of the heterotrophic productivity indicators mentioned, some participants thought that bacterial production rates (leucine incorporation), microbial respiration (biological oxygen demand [BOD] or sediment oxygen demand [SOD]), and ecosystem respiration might be the most promising indicators because of their high signal-to-noise ratio. Microbial respiration was also suggested to be strongly linked to higher trophic level impacts. The group described fungal production/biomass and leaf decomposition as more variable measurements.

Finally, the group presented potential indicators of primary production, including NDS, nutrient uptake measurements, stoichiometry, and dose-response curves (e.g., using periphytometers). With the exception of dose-response curves, each indicator was noted as having a quantitative relationship with nutrients. The group described the relationship between each indicator and higher trophic level impacts as largely unknown.

## Indicator Category Presentations: Discussion

After the indicator category presentations, the discussion of indicators was continued amongst the expert workgroup and EPA staff.

Several questions had emerged during the presentations regarding the linkage between indicators and designated uses. Some participants questioned whether indicators should protect not just aquatic life but also recreational uses. EPA staff confirmed that the participants should consider recreation and other factors necessary to support the broader goals of the Clean Water Act when thinking about indicators; it was also noted that criteria must protect the most sensitive use.

Regarding recreational uses, EPA staff noted that it is important to consider spatial variability when selecting indicators. For example, different regions might define recreational uses differently, and therefore different indicators might be appropriate. Sensitivity to nutrient pollution can also vary regionally among recreationally important fishes.

The applicability of pH as an indicator of nutrient pollution was discussed briefly. Some participants considered pH a sensitive indicator of changes in algal biomass—perhaps more sensitive than DO in some large rivers; others considered it useful because of its direct linkage to macroinvertebrate and fish health.

Other points raised during the discussion included genomics and algal species composition as potential indicators that might better reflect the long-term condition of a system than other indicators; the importance of the spatial scale of an indicator; and the issue of interdependency among indicators (for example, contradictory outcomes from algal composition measures and measures of productivity).

The discussion concluded with EPA staff's stating that indicators are needed for both deriving numeric nutrient criteria and identifying impairments in the field. Therefore, participants should think of "ideal" indicators as needing to meet two requirements: (1) they must be sensitive to changes in nutrient concentrations, and (2) they must be predictive of changes to aquatic life or other designated uses. Participants were then divided into breakout groups and asked to select "ideal" indicators from those that had been considered during the day's discussion.

## Breakout Session 1: Ideal Indicator(s) List

For the first breakout session, the experts were asked to rank potential indicators on the basis of each indicator's ability to accurately and precisely predict the effect of nutrient pollution on aquatic life in streams. This session was designed to identify, as broadly as possible, the universe of indicators relevant to development and/or implementation of nutrient criteria based on stressor and response data. Workshop participants were divided into five breakout groups with three to six experts per group; each group was asked to rank potential indicators on the basis of each indicator's ability to accurately and precisely predict the effect of nutrient pollution on aquatic life in streams. In order to develop this ranking, experts were asked to draw from the indicator category presentations, matrices, and discussion, and consider the trade-offs between sensitivity to nutrients and predictability of aquatic life use impairment. Specifically, groups were asked to evaluate each indicator while considering the following questions:

- Is it measureable?
- Is it sensitive to nutrient increases?
- Does it respond to nutrient increases consistently and/or predictably?
- Does it exhibit low natural variability?
- Does it have a known response to natural disturbances and changes over time?
- Is it, itself, predictive (i.e., signifying an impending change to aquatic life)?

### Breakout Session 1: Group Discussions and Resulting Indicator Lists

The breakout groups identified a common set of indicators—TN and TP concentration, algal biomass based on chlorophyll and algal cover (depending on stream type), assemblage measures (with diatom assemblages prioritized), and metabolism indicators (DO and pH). Each group also identified additional indicators for consideration; some are "ready for prime time," but most (such as leaf decomposition rates, nitrogen- and phosphorus-acquiring enzymes, and genomics) require further development and testing. The discussions leading to each group's top-ranked indicators are presented below, followed by summary tables of each group's results.

#### Group 1

Group 1 ranked total nutrients and soluble nutrients, respectively, as being of highest value. The group noted that season affects nutrient levels for both of these indicators. The group also noted that load, as a measure of concurrent flow, is important in many circumstances, such as total maximum daily load (TMDL) development, and therefore could be collected along with nutrient concentration. The group cautioned, however, that load measurements might not be meaningful in all cases. For example, nutrient loads can be very high during spring runoff, but physical limitations on the stream ecosystem (e.g., scouring flows, uptake rate, and light limitation) often abate the effects of high nutrient levels at the local reach level.

The first group's third-highest-ranked nutrient indicator was organic nutrients. They noted that this is a useful indicator of biologically available nutrients in certain situations. For example, organic nutrients would be a valuable indicator in glacially influenced streams where there is high TP, but most of it is not biologically available. Group 1 ranked C:N:P ratios and ratios of exoenzyme activities fourth. The group noted that these indicators are not necessarily predictive of nutrient concentrations, but might be useful if normalized to something else, such as reference stream levels of exoenzymes and their activities. One participant also mentioned that ion exchange resins could be used to quantify nutrients, but the group did not discuss this potential indicator at length.

In addition to nutrient indicators, Group 1 considered algal biomass indicators, taxonomic indicators, and ecosystem functional indicators. The group named visual percent cover as the best indicator of algal biomass. They noted that visual percent cover methods are simple, but would need to be thorough and include documentation of benthic algae cover and mat thickness, filamentous algal cover, macrophytes, and moss. Calibrating these cover values to harm-to-use levels would also be necessary to make them suitable for implementation. Group 1 also discussed benthic and phytoplankton chl-a and AFDM as algal biomass indicators. They discussed both artificial and natural substrates in regard to benthic algae; some group members advocated artificial substrates, others expressed a dislike for them. The group noted that phytoplankton biomass measures are most significant in settings where this flora is important (e.g., large rivers, backwaters/sloughs, river-reservoir interfaces).

The first group's top-ranked taxonomic indicators were diatoms and soft-bodied algae. The group noted that diatom metrics are far more advanced than soft-bodied algae indicators; however, they still thought soft-bodied algae quantification from samples was valuable. Macroinvertebrates were also discussed as a taxonomic indicator, but came in a distant second place to diatoms and soft-bodied algae. Fish were noted as having value as metrics, but more so at the extremes of eutrophication (e.g., only carp and goldfish remaining in a warm-water prairie stream). The group also discussed several future indicators that are not currently ready for use. These future indicators include analysis of diatoms, soft-bodied algae, and macroinvertebrate taxa and abundances via DNA/RNA methods. Flow cytometer methods (for cell counts) could also be used in the future and could have application for phytoplankton counts.

Group 1 named DO (both minima and diel range) as the top indicator of ecosystem function, stating that DO diel range provides a good overall indicator of stream system metabolism. The group also discussed that pH range would be valuable. DO and pH linkages to harm-to-use are generally well understood.

The first group's indicator ranking that resulted from their Breakout Session 1 discussion appears below in Table 1.

**Table 1. Group 1 Indicator Rankings**

| Nutrient Indicators | Algal Biomass Indicators | Taxonomic Indicators | Ecosystem Function Indicators |
|---|---|---|---|
| 1. Total nutrients | 1. Visual percent cover | 1. Algal species composition (diatoms and soft-bodied algae) | 1. DO (diel minima and range) |
| 2. Soluble nutrients | 2. Benthic and phytoplankton chl-a and AFDM | 2. Macroinvertebrates | |
| 3. Organic nutrients | | 3. Fish | |
| 4. C:N:P ratios; ratios of ecoenzyme activities | | | |

### Group 2

The second group's conversation focused on benefits and disadvantages of potential indicator metrics. The group noted that flow rate plays an important role in affecting most biological nutrient pollution-related impairment indicators; therefore, the group structured their rankings by stream size.

Assessing pH and DO as nutrient indicators, Group 2 noted benefits including that DO and pH diel ranges integrate other variables (e.g., indicate changes in respiration and primary productivity), but are also stressors. (High pH and low DO damage fish and other aquatic life.) As a result, measuring these indicators provides more information. However, noted disadvantages included that pH is largely affected by land use (generally elevated in urbanized streams because of concrete and tilling of soil), and is also affected by natural alkalinity; therefore, regional classification is imperative. Further, this group noted that diel monitoring of pH (and also DO) is more useful than synoptic grab sampling.

Assessing percent *Cladophora* cover as a nutrient indicator, the group noted benefits including a strong linkage to the Clean Water Act's fishable/swimmable requirement, as well as a very clear linkage to nutrient pollution-related impairment. However, noted disadvantages included variability among bottom substrates (e.g., it may not grow as well on non-rocky substrates). Another potential disadvantage is the need to consider and understand *Cladophora* autecology; for example, one expert noted that Texas can experience high levels of *Cladophora* under low flow conditions and low nutrients. The alga, however, appears lighter green under these conditions than when it blooms because of the high nutrient conditions, and that would need to be measured to distinguish this bloom from one fueled by high nutrients. Further, oxygen concentrations do not drop as low under low-flow-induced high *Cladophora* biomass as they do when *Cladophora* is growing under high nutrient pollution conditions. Other potential disadvantages include that *Cladophora* is not an early warning indicator, it has high temporal variability, and that a high percent *Cladophora* cover doesn't necessarily indicate non-problematic concentrations of nutrients. The group also noted that aquatic macrophytes might be merged into percent

*Cladophora* cover; however, it is important to be able to discriminate among macroalgae based on their potential to respond to nutrient pollution by growing to nuisance levels.

Group 2 also discussed nutrients (TP, and in some systems, TN) as indicators, noting the disadvantage that SRP has a major diel cycle. This could make monitoring a problem because getting everyone to measure SRP concentrations at the same time of day in all systems is improbable.

C:Chl-a and C:P ratios were discussed as having many benefits, including the ability to predict nutrient concentrations almost as well as taxonomic classification. However, disadvantages include benthic substrate issues (i.e., the indicator is much more useful when looking at benthic assemblages on hard substrates) and reports of confounding variability in periphyton mats.

The group noted that, when using water column (sestonic) chl-a as an indicator, it is important to control for flow, take repeated measurements (e.g., in Kentucky they sample for eight weeks to estimate a temporal average), and conduct habitat specific sampling.

When evaluating nitrogen- and phosphorus-acquiring enzymes as a nutrient indicator, Group 2 specifically discussed NAG (β-1,4-N-acetylglucosaminidase) and phosphatase. These are used exclusively for acquiring the organic forms of nutrients, so high concentrations indicate inorganic nutrient limitation.

The group discussed benefits to using benthic fish as nutrient indicators, including that darters and stonerollers show a decline with increases in nutrient concentrations due to habitat loss (i.e., more areas with lower dissolved oxygen concentrations and benthic areas that they normally inhabit becoming uninhabitable because of algal overgrowth).

Additional metrics listed by Group 2 without further notes on their evaluation include aquatic invertebrates, nitrogen metabolism (particularly $NH_4^+$ oxidation and denitrification capacity), and BOD. Group 2 indicator rankings from Breakout Session 1 appear below in Table 2.

**Table 2. Group 2 Indicator Rankings**

| Shaded Headwater Streams | Open Water | Large Systems |
|---|---|---|
| 1. Algal species composition | 1. Algal species composition | 1. Sestonic chl-a |
| 2. Nitrogen- and phosphorus-acquiring enzymes | 2. Visual percent cover | 2. DO/pH |
| 3. DO/BOD (BOD where there's too much reaeration) | 3. Diel DO/pH | 3. Nitrogen- and phosphorus- acquiring enzymes |
| 4. Macroinvertebrates | 4. Periphyton chemistry | 4. Algal species composition |
| 5. Denitrification capacity and $NH_4^+$ oxidation | 5. Invertebrate communities | 5. Cyanotoxins and geosmin |
| | 6. Nitrogen- and phosphorus-acquiring enzymes | 6. Mussels (have confounding factors but are sensitive responders to nutrient pollution) |
| | 7. Benthic and riffle-dwelling fishes | 7. Benthic macroinvertebrates (have confounding factors but are sensitive responders to nutrient pollution) |
| | | 8. Secchi depth and other turbidity measures |

### Group 3

Group 3 primarily discussed algal biomass and heterotrophic indicators. The group noted that inorganic nutrients can be good in site-specific situations, but that TN and TP are better indicators on a regional scale. Group 3 also discussed that nutrient loading is good for downstream use protection, while nutrient concentration is better for site-specific application.

Overall, the group noted that algal biomass is very sensitive and more specific to changes, but has only moderate predictability. The group also noted that while dominance of a few algal types might be predictive, adding soft algae can complicate the assessment. For evaluation of algal biomass, Group 3 ranked chlorophyll as the top indicator, noting that it is measurable, highly variable, sensitive, and moderately predictive. The group noted variability induced by sedimentation and scouring effects as complications to using periphyton chlorophyll as an indicator. Following chlorophyll, the group named macrophyte coverage as the second-best indicator; however, they noted that it is less sensitive, often light limited, and habitat specific. Macrophytes can be used in a subset of streams with clear water and stable flow, but are modified by other physical attributes and have a slower seasonal response. On the whole, this group decided macrophyte coverage had potential, but requires additional research, since there is currently not a lot of literature on stream macrophytes.

Group 3 discussed phytoplankton as being highly sensitive and predictive, but being confounded by factors such as residence time, color (e.g., colored dissolved organic matter), turbidity, and tychoplankton (i.e., dislodged benthic algae). Group 3 identified diatoms as the best algal assemblage indicator because of their sensitivity and moderate predictability. Other algal species composition was noted as being less variable, but the aggregated information was deemed too general. Group 3 believed that diatoms might be difficult because of limited feasibility for routine monitoring by many programs.

The group discussed heterotrophic indicators as not being sensitive specifically to nutrients; the group stated that heterotrophs are probably more sensitive to organic pollution. Group 3 noted that despite being highly correlated with algal productivity, heterotrophs are process indicators and provide more of an indication of food web and cycling processes. Heterotrophs are sensitive, but not really calibrated or well understood regarding trends. In terms of heterotrophic indicators and sensitivity to nutrients, Group 3 put correlation to DO lower on their list. Leaf decomposition was discussed as sensitive and measurable, but not well-calibrated. The group noted that it would be necessary to classify streams by light limitation and sediment type.

Group 3 described fish and macroinvertebrates as more general indicators, being predictive of aquatic life use impacts but not directly sensitive to nutrients per se. Higher trophic levels, they argue, are influenced by many factors, making it hard to isolate nutrient pollution effects. The group noted that taxonomic indicators work best because they are well documented, but the future will likely include molecular approaches because, although they are expensive and currently have limited documented application in this arena, they are developing quickly. Organismal stoichiometry is sensitive and could be a good indicator, but the literature is relatively limited in streams and just starting to develop. There are a few species and groups that have been shown to reflect the nutrient stoichiometry of their food resources. Once this indicator is calibrated, stoichiometric measures should be sensitive.

The group discussed several indicators of ecosystem function as being sensitive, but possibly needing to be adjusted for discharge. Uptake length was noted as being sensitive, predictive of downstream use impacts, and measurable. Group 3 noted that uptake length provides a measure of how good the stream is at assimilating nutrients over time, and thus, the health of the stream channel. The group also mentioned diel DO as a good indicator because of its sensitivity and predictability. They noted uptake length and diel DO as being feasible to measure, monitor, and apply to site-specific studies in states. Sediment denitrification was discussed as increasing with nitrate loading but not being very predictive of uses per se, but rather is predictive of ecosystem services. NDS was also discussed and noted as working across systems, but being artificially sensitive and not very predictive.

The Group 3 indicator rankings that resulted from their Breakout Session 1 discussion appear below in Table 3.

**Table 3. Group 3 Indicator Rankings**

| Biomass Indicators | Heterotrophic Indicators |
|---|---|
| 1. Chl-a (benthic in wadeable steams and sestonic in larger streams) | 1. Leaf decomposition |
| 2. Macrophyte cover | 2. Diel DO and pH |
| 3. Algal species composition | 3. Sediment potential denitrification |
|  | 4. Nutrient limitation studies |

### Group 4

The fourth group framed their discussion of best indicators by naming all potential indicators of nutrients, algal assemblages, microbial physiology, higher trophic levels, and ecosystem function. The group then discussed the measurability, sensitivity, and predictability of each indicator. The following relays the group's thoughts on each indicator's sensitivity and predictability of nutrient pollution-related impairment:

Nutrient indicators:

- TN/TP: High sensitivity, low predictability
- Soluble inorganic nitrogen: Medium sensitivity, lower predictability
- Algal biomass periphyton
  - Chl-a: Medium sensitivity, medium predictability
  - Visual: Low sensitivity, high predictability
- Algal biomass seston
  - Chl-a: High sensitivity, high predictability
  - Biovolume: High sensitivity, high predictability, more labor-intensive than chl-a

Algal assemblage indicators:

- Algal metrics: High sensitivity, high predictability
- Visual assemblages: Low sensitivity, high predictability, concerns about methodology

Microbial physiology indicators:

- Functional genes: Unknown sensitivity; unknown predictability; future, interpretive potential
- Polyphosphates: Unknown sensitivity; unknown predictability; future, interpretive potential
- Fluorometric measures of autotrophs: unknown sensitivity, unknown predictability

Higher trophic level indicators:

- Multimetric indices, richness/diversity Metrics, and observed/expected richness: Medium sensitivity, high predictability
- Tolerance metrics: Medium sensitivity, high predictability

Ecosystem function indicators:

- Diel DO: Low sensitivity, high predictability
- Leaf Decomposition: Medium sensitivity, medium predictability

After developing the list of potential indicators and discussing their sensitivity and predictability, Group 4 voted for what they believed to be the best indicator in each of the five indicator categories. The group indicator rankings that resulted from their Breakout Session 1 discussion appear below in Table 4.

**Table 4. Group 4 Indicator Rankings**

| Nutrients | Algal Assemblages | Microbial Physiology | Higher Trophic Levels | Ecosystem Function |
|---|---|---|---|---|
| 1. TN/TP | 1. Algal metrics and relative taxa | 1. Functional genes | 1. Multimetric indices, richness/diversity metrics, and observed/expected richness | 1. Diel DO |
| 2. Soluble inorganic nitrogen | 2. Visual assemblages | 2. Polyphosphates | 2. Tolerance | 2. Leaf decomposition |
| 3. Algal biomass periphyton<br>a. Chl-a<br>b. Visual | | 3. Fluorometric measures of autotrophs | | |
| 4. Algal biomass Seston<br>a. Chl-a<br>b. Biovolume | | | | |

### Group 5

Group 5 indicated algal assemblage and species composition as their top nutrient indicators during Breakout Session 1 due to measurability, sensitivity, and consistent, early predictability. At a minimum, the group would measure diatoms, but ideally would also include soft-bodied algae. The group thought that this indicator group could be an end in itself, as well as a conceptual basis for relationships to higher trophic levels (e.g., food source quality, toxin production, tendency to form nuisance blooms that cause physical/chemical habitat changes). Although the group noted that algal assemblage structure does not necessarily have low natural variability, they thought that it was manageable regionally with calibration by stream type. In response to natural disturbances and changes over time, Group 5 noted that the acceptable sampling period can be restricted (e.g., not within 30 days after a scour event). Further, disturbances and changes can be constrained by limiting the sampling period to a specific index period to control for seasonal community shifts. The group also discussed using metrics that are insensitive to taxonomic shifts (e.g., trait-based metrics) rather than individual taxa.

The group’s next-highest-ranked indicator was a nutrient-specific macroinvertebrate trophic index, which they categorized as being measurable, sensitive by design, and having moderate predictability. The group noted that it is harder to quantify confounding natural variable and co-varying stressor effects on macroinvertebrates.

The group’s third-ranked indicator was diel DO (for streams, but not necessarily for large rivers) focusing on diel DO ranges at midstream run/glide locations. Group 5 noted this indicator as being measurable, sensitive, and having comparable predictive consistency to chl-a and AFDM. Due to natural variability, DO measures would have to be calibrated by stream type. Group 5 noted that diel DO is responsive to natural disturbances and changes over time, but that this variability can be reduced by constraining sampling to index periods and specific times since storm events.

Group 5 named enzymes as the last indicator, but did not provide justification for the ranking. The indicator rankings that resulted from the group’s Breakout Session 1 discussion appear below in Table 5.

**Table 5. Group 5 Indicator Rankings**

| Nutrient Indicators |
|---|
| 1. Algal assemblage and species composition |
| 2. Benthic macroinvertebrates |
| 3. Diel DO |
| 4. Nitrogen- and phosphorus-acquiring enzymes |

### Indicator Compilation

Table 6 provides a compilation, by indicator category, of the top indicators named among the breakout groups. Breakout groups that selected the different indicators are noted in parentheses.

**Table 6. Compilation of Top Indicators from all Groups after Breakout session 1***

| **Nutrients** |
|---|
| Total nutrients (1, 2, 3, 4) |
| Soluble nutrients (1, 2, 4) |
| Inorganic nutrients (3 for local scale) |
| Sediment/pore water (3) |
| **Primary Producer Biomass Indicators** |
| Visual percent cover (1, 2 for mid-order streams, 4) |
| Phytoplankton and/or periphyton; chl-a and/or AFDM (1, 2, 3, 4) |
| Sestonic chl-a for large rivers (2, 3) |
| Macrophyte cover (3) |
| Biovolume (4) |
| **Primary Producer Assemblage Indicators** |
| Algal species composition (1, 2, 3, 4, 5) |
| Periphyton chemistry (2 for mid-order streams) |
| Cyanotoxins (2 novel) |
| Geosmin (2 novel) |
| Visual assemblages (4) |
| **Algal/Heterotrophic Physiology Indicators** |
| Nitrogen- and phosphorus-acquiring enzymes (2, 4, 5 for large streams) |
| Genomics (4 novel) |
| Flourometric measures (4 novel) |
| **Higher Trophic Level Indicators** |
| Macroinvertebrates (2, 3, 4, 5) |
| Benthic and riffle-dwelling fishes (2 for mid-order streams) |
| Pearly mussels (2 novel) |
| **Ecosystem Functional Measures** |
| Diel DO and pH (1, 2, 3, 4, 5 for large streams) |
| BOD (2 for headwaters with high reaeration) |
| Denitrification/ammonium oxidation gene frequencies (2 for headwater streams, 3) |
| Leaf decomposition (3, 4) |
| Uptake length (3 novel) |
| Nutrient limitation studies (3) |

* Some groups made a distinction for a particular measure based on stream size or location, which is noted. Others presented indicators that have promise, but still need further development; these indicators are indicated as “novel.”

## Day 2 Summary

The focus of the second day was to discuss whether the participants wanted to add to or remove any of the “top indicators” that had been identified the previous day. There was some disagreement surrounding the use of chl-a and enzymes as nutrient indicators.

## Chl-a as a Nutrient Indicator

Using chl-a as a nutrient pollution indicator has several benefits: It is generally inexpensive to measure, it can be collected relatively easily, and it is simple to understand and communicate. However, several issues need to be carefully addressed for chl-a to be a useful indicator. Most important, chl-a concentration needs to be linked to specific designated uses and contextualized, such that a particular concentration of chl-a is associated with designated use impairment. Chl-a concentration can be associated with aquatic life use (if it is not already considered an aquatic life use) and with recreational use in multiple ways, which were discussed. Some participants suggested that the role of chl-a within existing aquatic life use frameworks could be further clarified, and they suggested two methods by which to do this. First, they suggested that sampling of aquatic life use components like macroinvertebrates be coordinated with chl-a sampling. Currently, large national surveys show poor relationships between chl-a and macroinvertebrates because of issues associated with inconsistent sampling period; addressing this issue would strengthen the relationship of chl-a to macroinvertebrates, which are traditional aquatic life use components. Chl-a concentration can further be linked to aquatic life use through the known relationship between DO and algal biomass. The recreational use link can be developed by using the results of user perception tests, dosing studies, and aesthetics surveys. User perception surveys enable stakeholders to develop a clear relationship between the measured concentration of chl-a and its visual manifestation in the environment, which links the measured chl-a to recreational use impairment. All of these factors can be used to develop context-specific concentrations of chl-a that are indicative of nutrient pollution-related impairment.

Further, the group noted that the benefit of chl-a as an indicator is dependent on how it is used. When using chl-a, several considerations need to be taken into account and clearly described in monitoring and assessment guidelines.

Several participants suggested that chl-a as a nutrient indicator is useful only in certain systems. It was noted by one expert that in Alabama benthic chl-a is a good nutrient indicator in natural rocky systems but is not effective in coastal plain and urban rivers. Further, in coastal plain rivers, the bottom substrate precludes algal attachment; the inconsistent but high flow rates in urban streams lead to desiccation and scour. Uncomplicated low-flow systems might therefore be the best settings in which to use chl-a as a nutrient indicator.

Chl-a is also known to display some seasonal and temporal variability due to factors such as peaks in production or particularly high grazer density. Other physical factors, such as scour, can also cause variability in chl-a measures. There is therefore some irreducible variability, which can be only partially mitigated by taking several replicates at every sampling event, sampling frequently, and measuring other complementary variables. Some participants expressed reluctance to use chl-a as an indicator because it is not as strong an independent indicator as others. One expert noted that the State of Montana measures nutrient concentrations in addition to benthic chl-a, and takes at least 10 chl-a samples at every sampling event to ensure sufficiently high reproducibility; other states also estimate the density of grazers. It was recommended that when filamentous algae are present, even more replicates—20 to 30 samples or more—should be collected to ensure good reproducibility.

The phototrophic community is often more complex than can be estimated using chl-a measures alone. It was discussed that to determine the effect of nutrient pollution on phototrophs, it is frequently necessary to include measures of macrophyte biomass together with measures of chl-a to determine the response of the phototroph community to nutrient pollution. One expert noted that in the rocky-bottomed streams of western Montana, most of the phototroph biomass is benthic microalgae; therefore, provided that sufficient samples are taken, chl-a measures supported by visual assessment are considered sufficient estimates of the phototroph response to nutrient pollution. However, in eastern Montana the complex phototroph assemblage requires further separation and measurement of the individual phototroph groups.

## Enzymes as a Nutrient Indicator

Similar to chl-a, there were questions about whether enzymes are useful as measures of nutrient pollution-related impairment. There was some uncertainty among the group as to whether enzymes would be a good indicator of nutrient pollution-related impairment across a wide nutrient gradient. The presence of nutrient-acquiring enzymes is indicative of nutrient limitation; if the limiting nutrient is already known, it was noted that this information might not be particularly useful.

The production of enzymes such as nitrogenase and alkaline phosphatase is strongly dose-responsive; precipitous declines in the enzymes are seen with increasing nutrient concentrations. However, enzymes might not be particularly useful as predictors of nutrient pollution-related impairment. Although the links to higher trophic levels such as fish and invertebrates and overall ecosystem health are not strong, enzymes provide an easy-to-measure estimate of heterotrophic microbial physiology. Several participants cautioned that when interpreting this type of data, it is important to pay attention to methodological differences such as whether live assays or lysing of cells is used. It was noted that live field assays are generally preferable, especially on rocky substrates. The concentration of individual enzymes is not as useful a measure alone as when it is coupled to biomass measures to generate an estimate of enzyme production per unit mass. It was noted here, too, methodological differences (such as using AFDM or aerial techniques) need to be considered.

## Breakout Session 2: Discussion Outline

The group was charged with developing an annotated outline for the recommended indicators based on the day 1 discussions. The instructions were as follows:

> Describe your decision process and rationale for selecting each indicator (e.g., pros and cons). Expand upon and annotate the body of indicator attribute information. Provide citations for any supporting scientific literature. Use the outline on the following page as a guide and for taking notes as you discuss and develop your outlines.

Each group was asked to produce as detailed an outline as possible for its particular indicator(s). An outline template was provided:

**Indicator Description Template**

- Indicator description
  - Provide a name and a brief (1–2 paragraphs) description of the indicator
- Relevance to nutrients/aquatic life use
  - Summarize why it's an ideal indicator (1–2 paragraphs)
  - Describe the indicator's relationship to nutrient pollution and aquatic life use in streams (3–5 paragraphs)?
    - Is it sensitive to nutrient increases?
    - Does it respond to nutrient increases consistently and/or predictably?
    - Does it exhibit low natural variability?
    - Does it have a known response to natural disturbances and changes over time?
    - Is it, itself, predictive (i.e., signifying an impending change to aquatic life)?
    - Does it predict changes that can be prevented by mitigation and/or does it predictably respond to a decline in nutrients?
    - Is there a scientific, peer-reviewed demonstration of a relationship?
    - Can protective thresholds be identified?
- Assessment methods/considerations (1–3 paragraphs)
  - Are reliable methods available?
  - At what spatial scales is it applicable?
  - How should it be measured (e.g., spatial and temporal scales)?
  - Is it easy/inexpensive to measure?

### Breakout Session 2: Individual Group Reports and Discussions

#### Group 1

Group 1 grouped its ideal indicators on the basis of stream size and reach, grouping them into headwater, mid-order, and large-order streams.

#### Headwater Streams

The indicators chosen were visual cover of benthic phototrophs, nutrient-sensitive diatom assemblages, macroinvertebrate assemblages, and delta DO and pH.

Visual cover was described as needing to encompass an evaluation of algae (including both benthic diatom films and filamentous cover), macrophytes and moss as percent cover, and the thickness of the algal mat. Photographic records should be used in conjunction with the visual assessment. The group chose this indicator because it is sensitive to nutrient increase and generally responds in a consistent or predictable manner. Visual cover displays a medium level of natural variability that can be reduced within geographic zones because its response to natural disturbance and changes over time is well known. Participants noted that phototroph cover is known to be susceptible to scouring and other physical stream effects. It was also described as being predictive of impending changes to aquatic life, provided it is used in a particular geographic context. This indicator was noted to be predictive of other use changes (such as changes to recreational uses) as well. Again provided it is examined within a particular geographic context, it is predictive of changes that can be prevented by mitigation and it responds predictably to declining nutrient concentrations. There is a scientific, peer-reviewed demonstration of a relationship between visual cover and nutrient pollution-related impairment, and protective thresholds for this indicator have been identified. Participants cautioned that these thresholds might require further refinement within a geographic context.

Assessment methods for measuring visual cover are in place, and they are applicable on stream reach scales. Visual cover should be measured on a reach scale (20–40 times wetted width) and, at a minimum, assessed at the beginning, in the middle, and in the late stages of the growing season. Visual cover is easy and inexpensive to measure given a properly trained crew and inter-crew calibration.

Groups 1 described diatom assemblages (and soft algal communities if the resources are available) as showing rapid and diagnostic responses to nutrient pollution-related impairment that can easily be used to develop inference (mechanistic) models that relate to nutrient concentrations. Diatoms are already well-established indicators that are sensitive to nutrient increases and respond to them consistently and predictably. Like visual cover, diatoms exhibit moderate natural variability; however, if calibration, data screening, site selection, and site classification are undertaken properly, this variability can be minimized. Diatom assemblages have well-characterized responses to natural disturbances (such as scouring) and changes over time. Shifts in diatom assemblages are predictive indicators of impending changes to aquatic life, and they predict changes that can be prevented by mitigation. Shifts in diatom assemblages and other soft algae have established links to shifts in higher trophic level assemblages based on functional feeding groups. Diatom assemblages typically show shifts in species composition fairly rapidly after nutrient concentrations decline. Strong relationships between diatom assemblages and nutrient pollution-related impairment in a wide range of areas have been demonstrated in a large body of peer-reviewed scientific literature. Protective thresholds for this indicator have been developed. Thresholds based on reference conditions can be established more easily in small streams than in larger rivers. Change point analyses or changes across nutrient gradients can also be used to establish protective thresholds. As in visual cover, however, it was noted that regional calibration of the diatom metric is essential to be able to discern a strong relationship between the diatom assemblage and nutrient concentrations.

Assessment methods for measuring diatom assemblages are established, and they are applicable on the stream reach and transect scales. Diatom assemblages should be measured every couple weeks over the summer or seasonal growing period. In timing sampling events, the amount of time that has passed since a scouring or desiccation event should be considered. Collecting diatoms is easy (the Environmental Monitoring and Assessment Program [EMAP] method was a suggested protocol), and the cost of sample collection and analysis is moderate ($200–$400 per sample). It was noted that taxonomic expertise is required to assess this indicator well. A suggested mechanism to reduce cost is to use a presence/absence method. Despite the ease of collection, the time lag between sample collection and final results might present a problem.

Group 1 recommended that macroinvertebrate assemblages be specifically designed to encompass species sensitive to nutrients and suggested that indices of biotic integrity (IBI) would not suit this purpose. Macroinvertebrate assemblages were described as ideal indicators because they are sensitive to nutrient increases (by design), have strong links to higher trophic levels, allow for temporal integration because of their relatively long life spans, and are well-established indicators that are already in use in many states.

Although macroinvertebrate assemblages are responsive to nutrient increases, variables that can confound the interpretation of this indicator need to be controlled for. Macroinvertebrates are known to respond to nutrient increases both consistently and predictably, but they are also known to exhibit both seasonal and regional variability. The influence of this variability on the interpretation of results can, however, be mitigated by ensuring proper classification and data categorization. Macroinvertebrate assemblages are responsive to natural disturbance such as scouring and floods; causal analysis may therefore be necessary to account for non-nutrient factors affecting the assemblages. This indicator is predictive and responds to mitigation of nutrient impairment, but lag times need to be expected. Relationships between macroinvertebrate assemblages and nutrient concentrations have been shown in the scientific literature. Dose-response studies or reference condition approaches would be appropriate ways to establish protective thresholds. Indeed, linking aquatic life use to macroinvertebrate assemblages instead of to chl-a might provide stronger, more protective thresholds.

Reliable methods are available to assess macroinvertebrate assemblages, and participants believed that current methods used by states provide good examples. This indicator is most useful at the reach scale; regional calibrations would be necessary to use the indicator over broader spatial extents. Species-level resolution is essential for this indicator to be used effectively. Participants noted that isotope and fatty acid analyses also have great potential in this regard. Although sampling is relatively easy, the entire process is somewhat expensive because of the resources and expertise required. Costs associated with analysis can range anywhere from \$200 to \$500 a sample, but they can be lowered if sampling is undertaken routinely.

The changes in DO (delta DO) and pH were described as overall indicators of system productivity that can be measured reliably. Participants warned, however, that this indicator will not be indicative of nutrient impairment in headwater streams that are at steep gradients and experience high levels of reaeration. With that limited applicability in mind, in other headwater streams delta DO and pH are described as being sensitive to nutrient increases and consistently and predictably responsive. DO and pH exhibit low to moderate natural variability and experience strong seasonal effects. They have known responses to natural disturbances and changes over time, and they are affected by scouring and allochthonous inputs of organic matter. The DO and pH indicator is predictive of impending changes to aquatic life because there are described linkages between DO and fish or macroinvertebrate assemblages. DO and pH can also be used to predict changes that can be prevented by mitigation and are responsive to declining nutrient concentrations. Scientific, peer-reviewed demonstrations have shown a relationship between DO and pH and ecosystem metabolism. Separate protective thresholds for DO and pH, which need to run alongside absolute minimum DO standards, have been developed.

Reliable methods to measure DO and pH are available; sondes are considered the best. DO and pH should be measured on the reach scale by the continuous deployment of sondes. The instruments to measure DO and pH are somewhat expensive and require frequent field maintenance; the upside is that these parameters are easy to measure.

### Mid-Order Streams

The indicators chosen were visual cover of benthic phototrophs, nutrient-sensitive diatom assemblages, and delta DO and pH.

The usefulness, predictability, and assessment methodologies for these indicators in mid-order streams are the same as those in headwater streams. The only difference is that the steep gradient and high reaeration that limit the usefulness of DO and pH as an indicator of nutrient pollution-related impairment in headwater streams are not expected to limit the usefulness of this indicator in mid-order streams.

### Large-Order Streams

The indicators chosen were visual cover of benthic phototrophs, sestonic chl-a and AFDM, nutrient-sensitive diatom assemblages, and delta DO and pH.

Visual cover was described as having the same usefulness, predictability, and assessment methodologies as described for headwater streams, but not all participants were in agreement about its usefulness in large-order streams.

Sestonic chl-a (and AFDM) is an ideal indicator because it provides a measure of spatially integrated water column chl-a, which is known to be very responsive to nutrient changes and is relatively inexpensive to sample. Furthermore, this indicator predictably responds to increases in nutrient concentration. Sestonic chl-a and AFDM exhibit medium to high natural variability due to natural interferences from shading, turbidity, flow, and temperature. These factors can normally be accounted for in sampling and analysis. Sestonic chl-a and AFDM have a known response to natural disturbances and changes over time. Changes in sestonic chl-a and AFDM are predictive of impending changes to aquatic life because this indicator can be linked directly to changes in aquatic life through its role as a food source for zooplankton. Further linkages to aquatic life use can be made through the impact of shading (by suspended phytoplankton) on vision impairment in sight feeders. Participants were not sure whether this indicator is capable of predicting changes that can be prevented by mitigation or whether it predictably responds to a decline in nutrients. Decreasing concentrations of sestonic chl-a and AFDM have, however, been linked to improvements in the drinking water quality use. Scientific, peer-reviewed drinking water quality studies have demonstrated relationships between sestonic chl-a, AFDM, and nutrient pollution-related impairment, and protective thresholds specific to aquatic life and drinking water uses have been identified.

Reliable methods to measure sestonic chl-a and AFDM already exist. Chl-a and AFDM should be measured on kilometer scales using sondes like the algae torch and lab samples. Samples should be depth and width integrated at each site. This indicator is relatively easy and inexpensive to measure, although sondes require maintenance.

The usefulness, predictability, and assessment methodologies for diatom assemblages, delta DO, and pH indicators in large-order streams are the same as those in mid-order and headwater streams. The only difference is that the steep gradient and high reaeration that limit the usefulness of DO and pH as indicators of nutrient pollution-related impairment in headwater streams are not expected to limit the usefulness of these indicators in large-order streams.

**Group 2**

The group noted that visual percent cover, chl-a and AFDM, nitrogen- and phosphorus-acquiring enzymes (perhaps as part of a multimetric indicator), DO and pH, algal assemblage metrics, and macroinvertebrate indices were their choice indicators. Macroinvertebrate indices were not discussed during this session because of time constraints. Thereafter, several indicators received one "vote": periphyton chemistry, denitrification potential and $NH_4^+$ oxidation rates, locally sensitive taxa, and cyanotoxins/geosmin in large rivers.

Visual percent cover was described as measurements of microalgal mat thickness and benthic cover of macroalgae, bryophytes, and macrophytes. The benefits of this indicator are that it is easy and quick to measure and is predictive of several uses—aquatic life, recreation, and aesthetics. It is sensitive to nutrient increases and responds consistently and predictably to increasing nutrient concentrations. Although it exhibits high variability, its response to natural disturbances and changes is known. Visual percent cover is considered potentially predictive of impairment to aquatic life, and it has a high capability to predict changes that can be prevented by mitigation. One expert suggested that peer-reviewed scientific literature in Oklahoma shows a good relationship between visual percent cover and nutrient pollution-related impairment, and protective thresholds have been identified for this indicator.

Group 2 suggested reliable assessment methods are available, but they focus only on erosional habitats. These methods are applicable on reach scales. Visual percent cover should be measured during peak biomass or seasonally replicated, sampling should be coordinated to avoid influence of high flow events, and regional patterns of biota should be considered. Measuring this indicator is easy and inexpensive, the indicator is applicable for all stream types. The participants recommended the use of remote sensing or boat-based sampling for large rivers, noting that snorkeling or boat-based sampling is more useful for mid- to low-gradient streams.

Chl-a and AFDM were described as sensitive to nutrient increases with a predictable, consistent response. Although this indicator exhibits high variability, its responses to natural disturbances and changes are known. Chl-a and AFDM are considered potentially predictive of impairment to aquatic life, but the indicator has a high capability to predict changes that can be prevented by mitigation. Peer-reviewed science has shown relationships between chl-a and AFDM and nutrients. The participants knew that protective thresholds for aesthetic use had been developed for chl-a and AFDM, but they were not certain that they had been developed for nutrient pollution-related impairment.

Methods are already established to measure chl-a and AFDM in erosional habitats, but participants noted that methodological differences among labs can present a problem. They also noted that homogenization of benthic samples is critical. The group suggested that this indicator might be better expressed as a ratio of chl-a to AFDM. Several individual samples should be collected from within a reach for statistical comparison to a criterion. This indicator is relatively time-intensive to process in both the field and the lab, but the chl-a analysis itself is not particularly expensive. Chl-a and AFDM can be used as a nutrient indicator in all types of streams. In headwater and mid-order streams, scraping rocks might be the best collection technique. In mid- to low-order streams, scrapings off other substrates, such as wood and sand, can also be collected, but sestonic chl-a is probably a better measure. In large rivers, seston is the preferable measure of chl-a and AFDM.

Nitrogen- and phosphorus-acquiring enzymes (including nitrogenase for nitrogen fixation) are sensitive to nutrient increase and moderately consistent in their response. These enzymes exhibit moderate variability in the natural environment and have a known response to natural disturbance. Whether enzymes are predictive of impending changes to aquatic life has not been established, but the participants anticipate that the enzymes will have moderate to no predictive capability. Enzymes predict changes that can be prevented by mitigation and predictably respond to declining nutrient concentrations. There are demonstrations of good relationships between nutrient-acquiring enzymes and nutrient concentrations in the peer-reviewed scientific literature, and although it might be possible to establish protective thresholds of enzyme concentrations, none exist at this time.

Methods for assessing enzymes exist, but live assays provide different information from that provided by homogenized freeze-dried assays, and this needs to be taken into consideration. The substrates sampled for enzymes should differ depending on the system. In headwater streams it would be sensible to sample the leaf litter, while in mid-high-gradient streams with erosional habitats, the epilithon should be sampled. In mid- to low-gradient streams, the fine benthic organic matter or seston should be sampled; in large rivers, sampling the seston would give the best estimate of these enzymes. Further, enzymes should be sampled in conjunction with chl-a and AFDM sampling and should be sampled seasonally (because this is a seasonally dependent metric). Enzymes should be sampled at times of peak biomass, times of low flow, and when temperatures are typically high; however, this indicator might be less time-sensitive than others. Although it is easy to measure the enzymes, doing so is moderately expensive; if live assays are used, they can be time-intensive in the field and the lab. However, live assays might be more reflective of the degree of nutrient limitation than preserved specimen assays. To measure the nitrogenase enzyme, which is necessary for nitrogen fixation, live assays are required.

Diel DO and pH are measures of stream metabolism. This indicator is not always sensitive to nutrient increases in that high variation is typically associated with high nutrients but low variation is not necessarily indicative of low nutrients because other factors (such as shading and reaeration) can play a role. Diel DO and pH can exhibit high variability due to photosynthetically active radiation (PAR) and are strongly tied to hydrological variation such as scouring events. Diel DO and pH are also strongly seasonal and temperature-dependent, and they have a known response to natural disturbance. DO and pH track the response of autotrophic and heterotrophic production to nutrient mitigation. There is peer-reviewed scientific literature showing the relationship between DO, pH, and nutrient concentrations, and protective thresholds have already been identified. Several states already have DO minimum standards, and pH maximum values could be adopted in a similar fashion.

Assessment methods have already been developed. They require the use of data sondes with at least a 48-hour deployment. This indicator integrates over the reach scale (on the order of tens to hundreds of meters) and should be sampled, at a minimum, for two days per stream during periods of peak biomass, low flow, and high temperature. Sampling DO and pH is relatively easy although sondes require annual maintenance and replacement of the pH probe every 12–18 months. The initial expense of the sondes is also significant at $1,000–$8,000 per unit. This indicator applies to all types of systems, but a BOD method might be preferable for high-reaeration headwater streams.

Algal assemblage metrics are sensitive indicators of nutrient increase, and they respond to increases consistently and predictably. They exhibit very low variability and are relatively unaffected by natural disturbances. They are potentially predictive of impending changes to aquatic life and indicate changes that affect invertebrates. Algal assemblage metrics are predictive of changes that can be prevented by mitigation, and relationships between these metrics and nutrient concentrations have been established in the peer-reviewed scientific literature. Protective thresholds have been established for algal assemblage metrics.

Reliable methods for assessing algal assemblages have been developed, but to use them, training materials and taxonomic certification of samplers are recommended. Sampling should occur on the reach scale, and separating habitats is not critical. Further, algal assemblages are resistant to storm events, seasons, and even dry streams, so sampling can occur once. Algal assemblages are easy to sample but more expensive to analyze than chl-a or some chemical indicators. Of the different metrics, diatom assays are more reliable but soft algal assays are less expensive.

### Group 3

Group 3 picked chl-a and/or AFDM, plant cover, diatom/soft-bodied algal communities, DO and pH, uptake velocity, microbial heterotrophy (denitrification), and nutrient-sensitive invertebrate and fish assemblages as their top nutrient indicator variables.

The chl-a and/or AFDM indicator is well described and documented, and it controls resource availability to higher trophic levels. It is a useful indicator for all three stream reach types, but the stream reach type influences the relative usefulness of benthic versus sestonic chl-a; chl-a and ash-free dry mass (AFDM) are substrate and habitat dependent. This indicator is sensitive to nutrient increases but is influenced by flow and grazers. The chl-a and AFDM indicator is consistent and predictable, and it exhibits low natural variability. One expert offered that measuring this indicator in per-unit area units as opposed to per-mass units may further decrease this variability. This indicator is highly sensitive to disturbance and has a mixed capability to predict impending changes to aquatic life. Stronger connections (linking chl-a and AFDM to higher trophic levels) need to be developed. The response of chl-a and AFDM to declining nutrient concentrations can be rapid, with sestonic chl-a sometimes responding faster than benthic. There are peer-reviewed, scientifically determined relationships between chl-a and AFDM and nutrients, and protective thresholds for this indicator have already been developed.

Habitat- and stream-type-specific methods for measuring chl-a and AFDM have been developed. Chl-a and AFDM are inexpensive to measure, and samples should be collected seasonally and repeatedly. This indicator is useful in all systems, but whether benthic or seston chl-a should be sampled is system and habitat dependent.

Uptake velocity is a useful indicator in headwater to mid-order streams and is sensitive to nutrient increase. It provides a measure of the demand for nutrients within a stream reach, which is indicative of the nutrient supply to downstream waters. The variability of uptake velocity is stream size specific. It is noted to be influenced by human disturbance such as land use change, but the influence of natural disturbances on nutrient uptake velocity is unknown. Further work and meta-analyses were suggested as still needed to determine whether changes to uptake velocity are predictive of impending changes to aquatic life. Uptake velocity can predict changes that can be prevented by mitigation, but this is stream and habitat specific. Uptake velocity was also shown to predictably decline in accordance with decreasing nutrient concentrations in some restoration work. The response of uptake velocity to changing nutrient concentrations is noted to be different across stream types. Relationships between uptake velocity and nutrient concentrations have been demonstrated in the peer-reviewed scientific literature. Downstream protective thresholds have been developed for uptake velocity, but the participants were not certain whether local thresholds have been developed.

Assessment methods have been developed for measuring uptake velocity, but it was noted that it is important to consider the merits of rapid tests versus detailed reach assessments and whether measurements should be collected on hour or day time scales. The timing of sample collection is reach specific and needs to be done seasonally because this variable exhibits seasonal variation. The cost of collecting and processing uptake velocity data is low to moderate, but it is still more expensive than collecting and processing chl-a.

DO and pH are direct measures of production and thus are directly tied to aquatic life use. These indicators are effective in all stream types except high-gradient headwater streams. Although DO and pH are sensitive indicators of nutrient pollution-related impairment, they are habitat and system dependent. Changes to DO and pH are indirect responses to production and respiration increasing in response to nutrient concentrations. The variability of these indicators is also system dependent, but the variability has been described for many natural systems. Studies on fish have shown that shifts in DO and pH are predictive of changes to aquatic life. The time scale of the response of DO to decreasing nutrient concentrations depends on the type of phototrophic production (benthic versus sestonic, microalgae versus macroalgae). Relationships between DO, pH, and nutrient concentrations have been demonstrated in the peer-reviewed scientific literature. Protective thresholds of DO and pH have been developed, but because of the indirect nature of the response of DO to nutrient pollution-related impairment, additional early warning indicators might be needed.

Methods for measuring DO and pH have been developed, but there are differences between point and continuous measures that need to be considered; continuous measures are useful in measurements of stream metabolism. Many spatial studies of DO and pH already exist, but more studies of temporal changes to DO and pH would be useful to determine at what temporal scales this indicator should be measured. Point measurements can be done cheaply, but continuous measurements require more expensive meters that have a high maintenance requirement.

Invertebrate metrics are only secondarily responsive to nutrient concentrations, but they can demonstrate consistent and predictable responses. For some species, stoichiometric methods can be used to link invertebrates to nutrient pollution-related impairment. The literature on this is still developing, but it appears that some individual taxa might respond strongly to shifts in nutrient inputs and tolerance levels might be identifiable in those taxa. Invertebrate metrics might, however, be harder to link to nutrient Inputs. It appears that both body size and nutrient chemistry need to be considered in interpreting this indicator.

### Group 4

The fourth group picked visual percent cover and gross assemblage, chl-a, diel DO and pH, leaf decomposition, invertebrates, and fish as their top indicators. They also listed microalgal species composition and nitrogen-, phosphorus-, and carbon-acquiring enzymes as indicators but did not further discuss them.

Visual percent cover and algal assemblage were described as an ideal indicator in low- and high-gradient headwater and mid-reach streams (not large rivers) because the indicator is quick and inexpensive to sample, is intuitive to the public, and is a direct measure of nutrient pollution-related impairment. It is sensitive to large-scale changes in nutrient concentration and responds consistently and predictably to nutrient pollution-related impairment. Like chl-a, visual percent cover and algal assemblages exhibit high variability and thus need to be interpreted within a temporal context. This indicator has a known response to natural disturbance; for example, under flood conditions, one would expect to see low levels of visual percent cover. It is predictive of changes to aquatic life, which again should be placed within a temporal context. Visual percent cover and algal assemblage can predict changes that can be prevented by mitigation, and relationships between this indicator and nutrient pollution-related impairment have been described in the scientific literature. Predictive thresholds for this indicator have been identified, but they were acknowledged to be noisy. Reliable methods for assessing visual percent cover and algal assemblage are available.

This indicator is easy and inexpensive to measure. It should be assessed on the reach or transect scale, and the frequency of sampling should be based on weather patterns.

Chl-a is an ideal indicator because it is a direct measure of algal biomass: If high concentrations of chl-a are present, it can safely be deduced that there is a nutrient pollution problem in the water body. Chl-a is a useful indicator in low- and high-gradient headwater and mid-reach streams, but only sestonic chl-a is useful in all gradients of large rivers. This indicator is sensitive to nutrient increases; provided several samples are collected, it has been shown to respond consistently and predictably. As mentioned earlier, chl-a exhibits high natural variability but does have a known response to natural disturbance. One expert suggested that chl-a is also predictive of changes to aquatic life, and relationships between benthic chl-a and nutrient pollution-related impairment have been demonstrated at a regional scale. The participants were not sure whether these relationships have been evaluated for sestonic chl-a. Although the participants were certain that protective thresholds can be identified for benthic chl-a, they were less sure about finding thresholds for sestonic chl-a. Reliable methods are available for assessing chl-a, and multiple samples should be collected. Care should be taken in determining the spatial scales on which to assess chl-a. Although measuring chl-a was noted to be less costly than assessing visual percent cover and algal assemblages, it was also noted that the multiple samples needed to ensure good reproducibility increase the costs of this indicator.

Diel DO and pH make a good indicator because the indicator is already in use and standards for it already exist, although the values of the standards might need some revision. It is relatively inexpensive and straightforward to measure this indicator, and the methods for doing so already exist. Further, this indicator is tightly linked to several trophic levels (algae, invertebrates, amphibians, fish), so when the indicator is not at ideal levels, the impact on ecosystem health is obvious. However, DO and pH are not particularly sensitive to nutrient increases, and therefore they typically respond only when there are big shifts in concentration. DO and pH data can be noisy due to confounding factors and reaeration (especially under lower nutrient conditions). The natural variability of DO and pH depends on the system but can be expected to be similar to that exhibited by algal biomass. The response of DO and pH to natural disturbance is well known and is similar to that of algal biomass and leaf litter. This indicator is predictive of changes to aquatic life use and can predict changes that can be prevented by mitigation. Further, relationships between nutrient concentrations and diel DO and pH have been demonstrated in the scientific literature. Physiology-based protective thresholds have been determined for both DO and pH, but participants noted that these thresholds are context dependent. There are reliable methods for measuring DO and pH, and this indicator is applicable on all spatial scales, although noise and variability are reported to be worst in headwater streams, decreasing as streams get larger. The sampling design should be continuous monitoring that will account for the magnitude and frequency of temporal changes. Once the expensive probes used to continuously monitor DO and pH have been purchased, assessing this indicator is easy and inexpensive; however, sufficient manpower is required to ensure that the probes are maintained in the field.

Participants described leaf decomposition as not ready for adoption and use but still worth discussion as a promising new indicator. This indicator facilitates looking at the effects of nutrient pollution in areas where leaves are an important part of the carbon load, particularly heavily shaded headwaters. It integrates across trophic levels. By altering the mesh size of the sampling apparatus, the effects of various stages of decomposition can be distinguished. This indicator is sensitive to nutrient increases and responds consistently and predictably to nutrient increases provided standardization is done based on leaf species. Standard operating procedures (SOPs) for standardizing leaves based on species need to be developed. Leaf decomposition exhibits medium variability, which can be mitigated by collecting many replicate samples and accounting for current velocity. There is a known response of leaf decomposition to natural disturbance, but, again, SOPs to account for this need to be developed. Leaf decomposition is predictive of ecosystem-level processes and can predict changes that can be prevented by mitigation. Relationships between leaf decomposition and nutrient concentrations have been demonstrated in the literature; see, for example, Suberkropp et al. (2010[4]). No protective thresholds for leaf decomposition are currently available; however, with additional work these could be developed using a reference condition

---

[4] Suberkropp, K., V. Gulis, A.D. Rosemond, and J.P. Benstead. 2010. Ecosystem and physiological scales of microbial responses to nutrients in adetritus-based stream: Results of a 5-year continuous enrichment. *Limnology and Oceanography* 55:149–160.

approach. Methods for assessing leaf decomposition exist, but SOPs have yet to be developed and standardized. At this point, it is known that several replicates would be needed and current velocity would need to be taken into consideration in any method used. Leaf decomposition should be assessed on a localized reach scale, and it should be measured frequently because multiple site visits are required to establish a decay curve. This is an easy and inexpensive indicator to measure because the only values required are values of mass. Students and volunteers could measure AFDM or just dry mass. Leaf decomposition was noted to be a useful indicator only in headwater streams where leaves are known to be an important part of the carbon cycle.

Invertebrates can be ideal indicators in both low- and high-gradient headwater and mid-reach streams because they are both direct and indirect measures of aquatic life. (They are currently less useful in large rivers, but methods are being developed.) In addition, many states already have macroinvertebrate assessment programs in place. Further work is required to determine whether invertebrates are sensitive to nutrient increases, although some states are beginning to develop nutrient-specific tolerance values. The responses of invertebrates to increasing nutrient concentrations are noisy, but they are consistent and predictable on a coarse level; any interpretation should be substantiated by a causal assessment to confirm the role of nutrients. Invertebrates generally exhibit low natural variability; however, if variability is too high, the ideal assemblage composition can be characterized by looking at reference conditions. Invertebrates have a known response to natural disturbance. They are, by definition, predictive of changes to aquatic life, and they have the potential to predict changes that can be prevented by mitigation. It was noted, however, that invertebrate metrics need to incorporate nutrient-specific responses and time lags between changing nutrient concentrations and invertebrate responses. Several states (Wisconsin, New York, and Maine) have developed methods that show relationships between invertebrates and nutrient concentrations, and many of them are developing protective thresholds. Reliable methods are already in place for assessing invertebrates, which should be monitored at least annually on a reach scale. Taking into consideration the field work and analysis associated with assessing invertebrates, this indicator is moderately expensive to measure.

Participants noted that fish as a nutrient indicator are in many ways similar to invertebrates, though less predictable in their responses to changing nutrient concentrations. This indicator would be useful in mid- reach and large rivers.

### Breakout Session 2: Summary

The following common indicators were identified by all four groups:

- Visual cover of phototrophs
- Chl-a and AFDM
- DO and pH
- Algal assemblage metrics
- Macroinvertebrate assemblages

Diatom and soft algal assemblages were identified as an indicator by four groups, macroinvertebrates were identified as an indicator by all four groups, but two of the groups had significant concerns with macroinvertebrates’ ability to link clearly with nutrient impacts.  Fish assemblages and microbial heterotrophy (denitrification) were identified by two groups. Several other indicators were considered promising, including uptake velocity, nitrogen- and phosphorus-acquiring enzymes, periphyton chemistry, $NH4^+$ oxidation rates, leaf decomposition, and cyanotoxins and geosmin.

The common indicators identified by all four groups were found to be sensitive to changes in nutrient concentrations and to be predictive of changes to aquatic life. The responses of these indicators to natural disturbances are well known, and relationships between these indicators and changes to aquatic life are already well described in the literature. Factors that can confound the interpretation of the indicators are generally well understood and can be accounted for or mitigated. In all cases the assessment methodologies are well developed; in many instances they are already in use by states.

## Day 3 Summary

### Breakout Session 3: Discussion Outline

On days 1 and 2 of the expert workshop, the expert workgroup had extensive discussions in Breakout Sessions 1 and 2 regarding sensitive and predictive indicators of nutrient pollution. The output of those discussions was summarized and presented to the expert workgroup in the afternoon on day 2. With this output in mind, EPA charged the expert workgroup to develop two prototype *combined numeric nutrient criteria approaches*—one that uses a prescribed set of indicators and another customized using any set of indicators. Breakout Session 3 consisted of five groups, each of which discussed and prepared a response to the charge. Each group presented its response to the charge to the expert workgroup.

The problem in streams was presented as follows: “Elevated nutrients do not always result in expression and/or observation of adverse biological responses. Single numeric nutrient criteria, therefore, may overestimate nutrient pollution stress to aquatic life.”

The charge to the groups was to identify an approach that combines biological and nutrient information that consistently identifies a condition where nutrient pollution results in stress to aquatic life (i.e., an approach that is sensitive and predictive). The groups were challenged to develop a combined criterion approach for two scenarios, which were described as follows:

- Scenario 1: You can only use the following variables: nutrient concentrations (TN and TP), chlorophyll-a, dissolved oxygen, and invertebrate measures.
- Scenario 2: The world is your oyster.

For each scenario, the goal is to come up with indicators and an approach to combine them (a decision framework, a matrix, a flow diagram, etc.). Think about, but do not limit your thinking to:

- The best indicators to combine
- The best combined approach to use

As output, the groups were to produce “combined indicator option(s), approach(es) for how to combine them in a criterion, and a ready description of these option(s) for group discussion.”

### Breakout Session 3: Scenario 1

#### Group 1

Group 1 identified a factor fundamental to developing criteria in Scenario 1, which was the *a priori* examination of data resulting in accurate and quantifiable dose-response relationships (i.e., empirical models) between nutrient stressors and the prescribed indicators in Scenario 1 (chl-a, DO, and invertebrate measures). Group 1 assumed that the data that would be used in these dose-response relationships were generated from sites that were appropriately classified, the samples were collected and processed under conventional quality control procedures, and the data were handled using clear data quality objectives.

Once dose-response relationships were developed, Group 1 found it useful to quantitatively relate TN and TP with indicators to establish potential indicator thresholds. Group 1 also suggested that the statistical characteristics of these relationships, such as confidence intervals, could be useful in developing the criteria. The group discussed using chl-a, an indicator that contains an upper ceiling threshold such that once that ceiling threshold is exceeded, one would conclude that there is nutrient pollution-related impairment. They suggested looking for dose-response relationships with invertebrates that are responsive to nutrient pollution. Where weak relationships with these invertebrate measures occur, the relationships could be used as supporting evidence for other aspects of the combined criterion approach.

To develop a combined criterion approach using the indicators in Scenario 1, Group 1 suggested establishing a TN and TP value for each indicator (with confidence intervals)—chl-a, DO, and invertebrates. Each relationship could yield a binary decision of “pass” or “fail.” When all four parameters of the combined criterion (nutrients, chl-a, DO, and invertebrates) “pass,” one can conclude the water body is not impaired. Conversely, when all four parameters “fail,” one can conclude the water body is impaired. When there are conflicting outcomes among the four parameters, the water body would require further study to determine the presence of nutrient pollution-related impairment. Group 1 also suggested explicitly embedding in that binary decision the frequency of exceedance, which could help take uncertainty into account. Group 1 observed overall that the creation of a combined criterion approach in Scenario 1 could lead to a complicated decision process.

#### Group 2

In a manner similar to that of Group 1, Group 2 identified a set of assumptions underlying the development of a combined criterion approach in Scenario 1. One assumption was that the stream’s ecological condition was characterized with the understanding that weather-related and small-scale spatial variability might not be accounted for. Another assumption was that the stream sites from which data were derived were classified (i.e., that natural variability in reference condition and response to ecological systems were accounted for). Group 2 suggested that, at a minimum, streams be classified as headwater, low-gradient mid-reaches, high-gradient mid-reaches, or non-wadeable streams/rivers. Finally, Group 2 assumed that stressor-response relationships (relationships between dependent and independent variables in causal pathway) were accurate.

Group 2 constructed a combined criterion approach sequentially by adding one indicator at a time to the stressor parameter. Within each step of the sequence, the group identified nutrient pollution-related impairment decisions based on the quantitative level of the stressor indicator (e.g., TP) and response indicator (e.g., chl-a as periphyton, DO, and index of biological condition). Group 2 suggested independent applicability of each indicator participating in the combined criterion approach in Scenario 1.

#### Group 3

Group 3 suggested an emphasis on chl-a and diel DO in terms of weighting a combined criterion approach in Scenario 1. Nutrient concentrations of TN and TP can be highly variable in time and space; therefore, limited sample sizes for nutrients and DO might not be meaningful in a combined criterion approach in Scenario 1. In addition, the group observed that invertebrate measures might not be good precautionary indicators; nor would DO in a rapidly flushed stream. Instead, peak chl-a during the growing season might be a better indicator.

From a monitoring perspective, Group 3 discussed the representativeness of the data in time and space as an important underlying factor in assessing water quality against the combined criterion approach in Scenario 1. In terms of the assessment outcome, Group 3 favored an independently applicable “pass” or “fail” design for each of the participating parameters in a combined criterion approach in Scenario 1. In cases where only the chl-a is exceeded, the criterion would indicate impairment. In some cases, however, exceedances of TN, TP, or both could still result in attainment, provided the other indicators were not exceeded and there was limited sampling of nutrient parameters.

#### Group 4

Group 4 first emphasized the need to identify the relationships between nutrients and the response variables through a weight-of-evidence approach. The group members observed that from their experience, there is not a clear relationship between nutrients and DO. DO might be informative if there is continuous monitoring data, but a single sample is not informative. If there is no demonstrated relationship with nutrients, an indicator should not be used. When combining the indicators, Group 4 suggested using an upper prediction interval that transcends regions (i.e., a phosphorus level that may not be exceeded regardless of the IBI). Group 4 reasoned that it would be preferable to be protective rather than reactionary in situations where high nutrient concentrations are observed.

Group 4 suggested that once the parameters being used in the criterion are related to each other, the individual variables could be ranked based on sensitivity to the nutrient stressor and the predictive abilities quantified. In Scenario 1 a criterion could be developed through a decision matrix that weights of parameters. Group 4 observed that such weighting might be less necessary with lower-dimension matrices. The group also suggested including a higher trophic level indicator to provide the linkage with what is frequently considered “aquatic life use.” While keeping the more sensitive indicators, Group 4 noted the benefit of adding the less sensitive indicators (e.g., IBI), which maintain a link to aquatic life and are familiar to more people. Shifts in algal species were discussed as transcending water body types, but there is a need to translate such shifts into something that is more meaningful, such as an invertebrate or algal IBI. A macroinvertebrate and/or fish IBI, for example, could be used with the condition that it has a quantified relationship with the nutrient stressor.

Group 4 developed a proof-of-concept criterion in Scenario 1 in which it ranked the parameters in the following order of sensitivity and predictability: nutrients, DO, chl-a, and invertebrates. The group then developed a decision matrix (Table 7) for the criterion that used discrete "decision categories":

- A = Non-impacted, no nutrient stress
- B = Stressed; nutrient impacts possible; bring in additional site-specific information to inform decision
- C = Impaired due to nutrients.

An average category score for each site would be assigned based on predetermined ranges for each parameter in the criterion. These ranges would be developed empirically. For example, a site with A, B, C scores would have an average score of category B. Group 4 suggested that the average should be a "weighted average" that weights the parameters on the basis of sensitivity or through some other empirical method.

**Table 7. Group 4 Example Matrix for Combined Criterion Approach in Scenario 1**

| | A | B | C |
|---|---|---|---|
| **Nutrients (TP) (ug $L^{-1}$)** | 0–29 | 30–60 | > 60 |
| **DO (mg $L^{-1}$)** | > 9.0 | 6.0–9.0 | < 6.0 |
| **Chl-a (ug $L^{-1}$)** | 0–3 | 3–6 | > 6 |
| **IBI** | 0–7.5 | 7.5–5.01 | < 5.0 |
| **Average Assessment** | Non-impacted | Stressed, site-specific | Impaired |

#### Group 5

Group 5 discussed different approaches for developing a combined criterion approach in Scenario 1 but did not agree on any particular approach.

### Breakout Session 3: Scenario 2

#### Group 1

For Scenario 2, Group 1 modeled the development of a combined criterion approach on the same basic framework it had created for Scenario 1 with the exception of the invertebrate measure(s). The group suggested maintaining dose-response relationships between TN, TP, and invertebrates as context for the combined criterion approach, but not including those relationships as explicit components of the combined criterion approach. Instead, Group 1 suggested quantitatively relating invertebrate measure(s) to algal measure(s).

Group 1 suggested replacing the chl-a measure with two other measures of algal productivity—diatom assemblage and macrophyte biomass, with visual surveys (e.g., percent cover) that couple and integrate the latter with periphyton biomass. The group also suggested the integration of a metabolic indicator such as diel pH and/or DO. Group 1 asserted that by substituting these three indicators for the ones prescribed in Scenario 1, a more robust measure of stream primary productivity in response to nutrient pollution could be estimated.

The decision process for Group 1's combined criterion approach in Scenario 2 mirrored the decision process it had envisioned in Scenario 1. The relationships between TN, TP, and the respective indicators (i.e., diatom assemblage, diel pH and/or DO, and stream periphyton and macrophyte biomass) would yield dichotomous, binary decisions of "pass" (when all "pass") or "fail" (when all "fail"). Additional site-specific study over time would ensue when there were conflicting outcomes. In cases where some parameters continued to "fail" and produce a conflicting result, the stream would be considered impaired or site-specific criteria could be pursued. In cases where there were high nutrients above a certain level, the stream condition would indicate potential downstream impacts. As a point of reference, Group 1 discussed and presented an example from Montana DEQ's draft nutrient and biological assessment framework.

### Group 2

For Scenario 2, Group 2 favored slightly different parameters for the combined criterion approach than those they had favored for Scenario 1. The group suggested TN and TP as the stressor parameters of the criterion and diatom metrics (with inferred TP), visual assessment data of benthic algae, and invertebrate measures. Other response parameters such as periphyton-specific nitrogen, phosphorus, and AFDW, and variation in DO and pH could serve as supporting information. Group 2 also observed that the exceedance frequency of these parameters was an important consideration in developing the combined criterion approach in Scenario 2.

In terms of interpreting data used in the combined criterion approach, Group 2 suggested refraining from averaging within each of the parameters (e.g., not averaging TP and inferred TP from diatom assemblage) because of the temporal variation in parameters such as ambient TP concentrations. Group 2 indicated a preference for TP concentrations inferred from diatom assemblage because of the lesser degree of temporal variability as compared to ambient TP concentrations. In this way, in situations where ambient TP concentrations are low and diatom-inferred TP concentrations are high, the latter would lead to the conclusion that the stream is impaired. Group 2 did not suggest a decision framework for interpreting data from each of the other participating parameters.

Group 2 also discussed other important factors that affect the protection of stream designated uses from nutrient pollution. These include the role of nitrogen as a driver (and limitation) of primary production in streams, algal species shifts through physiological adaptation, the role of macrophytes in nutrient pollution dynamics, and inputs of atmospheric nitrogen and how it can affect stream phosphorus inventories. One example the group highlighted was the importance of looking for certain nuisance algal taxa, such as *Didymo*, which can occur and persist across many different stream types under highly variable nutrient conditions. Group 2 suggested that the temporal scales through which nutrient sources manifest their loads as nutrients in streams is slow relative to the manifestation of biological conditions, such as excess algal growth, and impacts on intermediate and final ecosystem goods and services (e.g., designated uses). Based on this observation, Group 2 suggested that integration of biological indictors into a combined criterion approach might not provide “early warning” of nutrient pollution impacts on designated uses; rather, such indicators might be better indicators of “vulnerability” to nutrient pollution.

#### Group 3

For Scenario 2, Group 3 first emphasized the importance of classifying streams based on gradient. Nutrient parameters still play a prominent role in a combined criterion approach in Scenario 2 provided there is sufficient sampling over time and space. The group favored the inclusion of benthic chl-a, measured as percent cover, which could also be combined with an additional measure of peak chl-a biomass. Other indicators, such as diatom/soft algal assemblage, benthic algal AFDW, and diel DO fluctuations were included. Group 3 reasoned that including these different indicators would provide the type of coverage over space and time that would facilitate the detection of nutrient pollution and its potential impacts.

Group 3 suggested a binary decision tree in which each parameter is independently applied when determining attainment or impairment. However, the group also contemplated an alternative assessment framework in which an intermediate zone (“grey zone”) could be identified. That intermediate zone could be defined for each parameter (e.g., TN) or across all the parameters when there is conflicting information. Group 3 identified areas for further exploration, such as for which parameters an intermediate zone would be appropriate, the size of the intermediate zones, and how the intermediate zones would be defined quantitatively.

#### Group 4

For Scenario 2, Group 4 suggested a set of indictors that would be applied across all sites. They include nutrients, visual percent cover of benthic algae, DO (not diel), chl-a, and algal assemblage. Group 4 suggested including an invertebrate and/or fish IBI as a set of more general indicators to ensure that aquatic life uses are maintained. Inclusion of such an indicator would be conditioned on a measurable response to nutrient stress. If a response is not directly associated, work might be needed to develop nutrient-specific measures that will improve IBI response.

Group 4 suggested applying the same decision matrix methodology as its combined criterion approach in Scenario 1. In addition, Group 4 observed that the decision matrix and associated response indicator thresholds might differ between ecoregions, stream type, or other classifications schemes. In cases where an intermediate grade is assigned for any of the parameters (e.g., “B” in the table below), a set of site-specific indicators could be used to add information in the assessment; these indicators include enzymes, diel DO, and nutrient uptake rates. An example of Group 4’s decision matric for Scenario 2 is provided in Table 8.

**Table 8. Group 4 Example Matrix for Combined Criterion Approach in Scenario 2**

| | A | B | C |
|---|---|---|---|
| Nutrients (TP) (ug $L^{-1}$) | 0–29 | 30–60 | > 60 |
| DO (mg $L^{-1}$) | > 9.0 | 6.0–9.0 | < 6.0 |
| Chl-a (ug $L^{-1}$) | 0–3 | 3–6 | > 6 |
| Invertebrate/Fish IBI | 0–7.5 | 7.5–5.01 | < 5.0 |
| Average Assessment | Non-impacted | Stressed, site-specific | Impaired |
| | | | |
| | | | |

### Group 5

For Scenario 2, Group 5 favored the following indicators: TN, TP, chl-a and/or AFDW, percent benthic algal cover, diel DO changes, algal assemblage, and nutrient uptake velocity. The group assumed that each indicator would represent an explicit parameter in a combined criterion approach in Scenario 2 that would have an established threshold. This threshold value might differ as a function of stream type. Also, Group 5 assumed that there would be appropriate data on each parameter (i.e., robust spatial and temporal sampling).

Group 5 focused its discussion on the construction of a decision framework reflecting these indicators and assumptions. Group 5 devised a multiplicative index in which each parameter would score from 0 to 2, with 0 reflecting an impaired condition due to nutrients. This “multimetric multiplicative index (MMI)” approach would result in an index score ranging from 0 to 64 (the latter reflecting the maximum score, or highest quality water, for each contributing parameter). Total scores would then guide or prioritize water quality management action for the stream.

Group 5 subsequently modified this approach to reflect a bifurcation in the total score. The bifurcation would direct the stream to a sequence of water quality management actions. For example, if a stream’s total score was below some threshold, no further water quality assessments would be conducted and the stream would be prioritized for remediation. A stream whose total score was above the threshold would be subjected to additional monitoring to determine whether the stream should be subject to remediation actions.

## Breakout Session 3: Summary and Synthesis

Breakout Session 3 reaffirmed many of the individual views on the effectiveness of different indicators to detect nutrient pollution in streams. For instance, many individuals and groups reaffirmed caution over interpreting discrete nutrient concentration data that are infrequently distributed over space, time, or both. The groups expressed a preference for greater sampling density over space and time to estimate the true nutrient environment. This accommodation alone might still be ineffective in gauging nutrient pollution impacts in unique situations (e.g., low nutrient, high *Didymo* biomass). The effectiveness of different algal biomass indicators was also widely discussed. Many participants expressed the limitations in interpreting phytoplankton chl-a as a reliable indicator of nutrient pollution in certain stream environments; as an alternative, benthic coverage was offered as a more reliable and accurate indicator. Individuals and groups continued to express support for more detailed algal taxonomy indicators, such as nutrient-sensitive or -tolerant diatom taxa, provided that they are well calibrated to the stream environments that would be monitored and assessed. Algal taxonomic indicators appeared to be an emergent area of potential for water quality management purposes, but some individuals expressed the need for additional research and user technical capacity-building if these indicators are to be tailored for numeric nutrient criteria development.

The two scenarios in Breakout Session 3 also revealed common views on how a combined criterion approach might be constructed and operate in water quality monitoring and assessment. In Scenario 1, some groups emphasized different indicators over others and several unique decision frameworks accompanied the combined criterion approach. A common theme, however, was that the indicators in Scenario 1 (and in Scenario 2 as well) are less likely to exhibit thresholds in response to nutrients; rather, their measures will occur across a continuous gradient as a function of nutrients and other co-variables. The groups also made some of the same underlying assumptions in each scenario. For example, appropriately classified streams (perhaps classified by stream order or by region) and the access to robust (spatially and temporally dense) data were common assumptions prior to a group's construction of a combined criterion approach. Perhaps the most common theme across the groups in either scenario was the need for any combined criterion approach to have strong quantified relationships between nutrients and the indicators.

The two scenarios also stimulated unique views and differences in terms of ideal indicators and the way in which they should be monitored and assessed. The most obvious differences between groups were the different indicators in Scenario 2 and the structure of how the combined criterion approach would be assessed (e.g., binary decisions, compensatory, or multiplicative). However, many groups converged on the same indicators, such as TN, TP, percent benthic algal cover, and algal taxonomic indicators. Some individuals and groups expressed the need for better analytical work describing the links between stream trophic levels. Others identified the potential for user perception to play a larger role in quantitatively relating nutrient pollution to indicators when developing a combined criterion approach. Nutrient biogeochemistry was an area of interest for some participants, specifically the influence of terrestrial carbon sources on stream nitrogen and phosphorus dynamics, as well as nutrient uptake rates in streams. Microbial processes, which mediate stream nutrient biogeochemistry, were also identified as an area of potential in terms of detecting nutrient pollution within the context of a combined criterion approach.

During the larger workgroup discussions following the breakout sessions discussing Scenarios 1 and 2, many participants remarked on some of the challenges of developing combined criterion approaches. Some commented on the challenge of identifying thresholds and interpreting change across those thresholds as an impact on designated uses. Others emphasized the need to actively work on meeting the assumption of having robust data for criteria development and monitoring and assessment. This could be catalyzed and facilitated through the sharing of water quality data and other relevant environmental data. Communication and collaboration between the practitioners (i.e., EPA HQ and regions, state scientists, academics, consultants) was also a common view within the workgroup.

## Breakout Session 4: Discussion Outline

On the final day of the workshop, participants were asked to further develop and refine combined criterion approaches in their breakout groups. More specifically, their charge was as follows:

> Develop an outline of your group's best combined approach for each scenario. Refine your approach based on the workshop discussions. Please describe your rationale and decision-making process. Use the outline and guiding questions to organize your writing.
>
> For this write-up, your thought processes are very important to EPA. Please detail how you came to your conclusions and why you decided to include/exclude certain indicators.

As output, each group was to produce as detailed an outline as possible for its particular combined approach. An outline template was provided:

*Outline Template* (provide one for each Scenario)

- Combined approach description
    - Provide a brief description of the indicators selected and the approach proposed
- Indicator description
    - Why did you choose those indicators?
    - Feel free to use many of the same criteria used for Breakout Session 2 selection of ideal indicators.
- Combined approach
    - Why did you select this particular approach? Consider the following guiding questions:
        - What are the approach's pros and cons?
        - How is your approach sensitive to nutrient pollution (sensitivity)?
        - How does the approach protect designated uses (i.e., triggers action prior to the loss of the designated use)?
        - How does the approach improve the decision agreement between elevated nutrient concentrations and impacts on biological responses?
        - How does the approach provide numeric targets for N and P for management (putting in a permit, setting a TMDL)?

### Breakout Session 4: Scenario 1 – Summary

In Scenario 1, groups were asked to provide a combined criterion approach using only the following variables: TN, TP, chl-a, DO, and an invertebrate measure of biological condition. Only Group 2 provided a combined criterion approach for this scenario. All the other groups believed that the restricted set of variables was insufficient to accurately determine whether a stream was impaired by nutrients.

Group 2 provided a set of combined criterion approaches that included only TP and chl-a; TP, TN, chl-a, and DO; and TP, TN, chl-a, DO, and invertebrate metrics. All of these approaches assumed that good data were available, that sites were accurately classified, and that stressor-response relationships that accurately characterize causal relationships were available. The combination approach proposed for all of these sets of variables was independent applicability, in which exceedance of any one of the selected variables indicates that the stream is impaired. In combined criterion schemes for the second and third set of variables, the group thought that stressor identification would be necessary with certain combinations of exceedances to establish whether nutrients were indeed the cause of impairment. For example, with the second set of variables, measurements of low TP and chl-a, and low DO, might indicate that the stream is impaired by organic pollutants but not nutrients. Stressor identification would help to refine these decisions.

#### Selected Variables

TP and chl-a were chosen as the initial set of variables because they were closest to the nutrient pollution effect and the nutrient that is causing the effect. Average TP and chl-a (and TN in the nitrogen-limited streams) computed from sufficient samples during the growing season were thought to be sufficiently accurate indicators.

In the second set of variables, DO was added because it quantifies one pathway by which invertebrates are affected by excess nutrients. The group noted that measuring diel DO is expensive.

Invertebrate metrics were added in the final set of variables because they are all related to the causal pathway linking increased nutrients to aquatic life use.

#### Combined Criterion Approach

The group believed that independent applicability was easy to communicate and implement because no grey zone was included. They also thought that the approach was protective, but not overly protective. They believed that more variables would increase their confidence in the assessment results and that there was a possibly of assessing a site as impaired when it was not.

The underlying assumptions of this approach—good data, good classification, and accurate stressor-response relationships—were also reiterated when describing the combined approach.

### Breakout Session 4: Scenario 2 – Summary

#### Group 1

Group 1 considered the multiple lines of evidence contained in a combined approach useful when faced with uncertainty in assessment. They assumed that criteria would be developed based on stressor-response relationships with sufficient data, properly classified waters, and a linkage to designated uses. The development of nutrient-specific bug and fish metrics was recommended to assist in linking criteria to designated uses. DO was also suggested as a clear signal of “harm to use.” Indicators that the group agreed should inform such an approach included TN/TP; a measure of integrated primary production, or IPP (visual survey [percent cover by periphyton and macrophytes]), chl-a, and macrophyte biomass; a measure of respiration (diel DO/pH coupled with whole-stream respiration); and an algal assemblage (diatoms at minimum, but preferably also soft algae and cyanobacteria). The approach included TN/TP thresholds for each indicator. The group considered two approaches: (1) a dichotomous approach in which each threshold is considered a binomial test and (2) a hierarchical approach in which indicator results are considered together and a “grey zone,” where more information is required to interpret mixed results or results outside the acceptable level of confidence, might exist.

### Selected Variables

Group 1 believed it is important to link all indicators to a “harm to use.” The linkage could be based on dose-response relationships and peer-reviewed literature. The group selected the following variables:

- TN/TP
- IPP—visual survey (percent cover by periphyton and macrophytes), chl-a, and macrophyte biomass
    - Individual primary production metrics were considered meaningful measures of nutrient pollution only if applied correctly. For example, chl-a was described as imprecise in systems where macrophytes or filamentous algae are dominant. Therefore, the development and use of a regionally calibrated IPP was recommended to ensure that primary productivity is accurately measured across all stream types.
- A measure of respiration (diel DO/pH coupled with whole stream respiration)
    - Pros: DO and pH were considered to be moderately sensitive to nutrient stress, reflective of the heterotrophic pathway as well as the autotrophic pathway, and an important link to designated uses that stakeholders can understand.
    - Cons: To be useful, these indicators require continuous monitoring.
- Algal assemblage (diatoms at minimum, but preferably also soft algae and cyanobacteria)
    - Pros: An algal assemblage metric was considered to be a rapid responder to nutrient pollution and, therefore, a good diagnostic tool. It was also noted that inference models can be easily developed to relate the algal community to nutrient concentrations.
    - Cons: The taxonomic expertise is not always available.

### Combined Criterion Approach

Group 1 defined a combined criterion approach in which sites are placed into one of three groups on the basis of the values of different nutrient indicators: (1) definite attainment: all indicators pass; (2) definite impairment: all indicators fail; and (3) grey zone: mixed results from different indicators. Sites placed in the grey zone are studied for a pre-specified amount of time to confirm or revise the initial indicator values. The group thought that outcomes for sites in this grey zone needed to be defined explicitly, with specific timelines for management actions.

The group noted that the grey zone could be defined based on mixed results from different indicators (a combination of passes and fails) or based on ambiguous indicator values. For example, a very high TP concentration might clearly indicate nutrient pollution-related impairment and a very low TP concentration might indicate attainment of nutrient criteria, but moderate concentrations of TP could be ambiguous.

As part of the combined criterion approach, Group 1 noted, any high measurements of TN or TP should automatically trigger assessment of downstream impacts, regardless of the values of the other indicators.

The group believed that the proposed combined criterion approach provided the means to clearly communicate the condition of the sample site and explain what is happening ecologically at the site. The approach more clearly describes the scientific basis and logic for the management decision and allows managers to prioritize their actions.

### Group 2

#### Selected Variables

To the variables included in Scenario 1 (TP, TN, chl-a, DO, invertebrate metrics), Group 2 added the following indicators: a visual assessment of algal/macrophyte cover and an estimate of TP based on diatoms. Diatom-inferred TP was thought to provide a less variable measurement of stream TP, compared to direct measurements. The group would also include a diatom measurement of aquatic life use attainment when such an index was available.

#### Combined Criterion Approach

The combined criterion approach was based primarily on independent application of different indicators with the exception of TP and diatom-inferred TP. In cases in which diatom-inferred TP was low but measured TP was high, the group thought it was likely that the high TP measurement was due to an error or a short, inconsequential pulse of TP and therefore could be ignored. All other indicators were considered independently.

### Group 3

#### Selected Variables

Group 3 defined four classes of indicators: (1) nutrient indicators, including direct measurements of TN and TP, and diatom-inferred estimates of TN and TP; (2) primary productivity measurements, defined as some combination of chl-a and visual cover estimates; (3) diel DO, measured with continuous data loggers; and (4) algal community and stream health indicators.

#### Combined Criterion Approach

Group 3 described a combined criterion approach that was based on independent applicability but also included grey zones for each variable that would be based on statistical uncertainty. The group proposed a few approaches for making a final decision about a steam that has been placed in the grey zone, including evaluating trends to determine whether conditions are improving, collecting different indicators, intensifying data collection, and defining site-specific criteria.

The group also recommended that direct nutrient measurements be underweighted because of their inherent variability. Assessment outcomes would be based on the number of indicators that fail or are in the grey zone. Any single failure of an indicator would indicate impairment; two or more indicators in the grey zone would indicate impairment; and one indicator in the grey zone would indicate that additional sampling is required.

### Group 4

#### Selected Variables

Group 4 defined a combined criterion based on universal indicators (for use at all sites) and supplemental indicators (for use when sites fall within an area of decision uncertainty). The universal indicators were TN/TP, algal assemblage (diatoms and soft bodied), visual percent cover of macrophytes and algae, and macroinvertebrate community assemblage. The group identified issues associated with each indicator. TN and TP are known to vary strongly spatially and temporally, but they are a direct measure of nutrient pollution. Algal assemblages are integrative responders to nutrients, can be predictive of responses at higher trophic levels, and can be used to infer nutrient concentrations; however, their responses can be affected by changes in flow or canopy cover. Visual percent cover is sensitive to nutrient increases, but work is required to develop a quantitative stressor-response relationship. Macroinvertebrates are affected by many factors in addition to nutrients but potentially can be tailored to nutrient pollution responses.

Supplemental indicators suggested by Group 4 include (in order of importance) diel DO, nitrogen- and phosphorus-acquiring enzymes, and nutrient uptake length. The indicators provide information on the severity of impact, extent of impact, and nutrient limitation.

#### Combined Criterion Approach

The combined criterion approach proposed by Group 4 included elements of independent applicability and a grey zone, in that exceedance of a high threshold for any one of the indicators automatically indicated impairment. Similarly, if all indicators were past a low threshold, a site was considered to be in compliance with the numeric nutrient criterion. For each indicator, the group thought that a grey zone of uncertainty was likely and that sites with indicators falling in the grey zone would require further study.

Group 4 elements for developing a combined criterion approach included:

1. Identify nutrient-response relationships.
2. Rank individual variables based on sensitivity to stressor and predictive capability.
3. Develop a decision matrix with weighting based on rankings.
4. Include the higher trophic level indicators to provide the linkage with aquatic life use. (It is acceptable to include the less sensitive measures provided all the more sensitive ones are already included.)
5. After weighting, take the weighted average condition to decide pass/fail/grey zone.
6. The universal indicators are nutrient concentrations, percent cover, DO (not diel for practical reasons), algal assemblage, invertebrate/fish IBI, and chl-a.
7. Use other site-specific indicators for the grey zone: enzymes, diel DO, and nutrient uptake.

### Group 5

#### Selected Variables

Group 5 defined two groups of variables. Variables in the first group (Level 1) were thought to be inexpensive, easy, and affordable, and therefore they could be measured with high frequency at all monitored sites. These variables are TN, TP, percent cover, and chl-a and/or AFDM. Percent cover and chl-a and/or AFDM provide biomass measures that are relatively inexpensive to measure.

The second group of variables (Level 2) require more time and resources to measure. These variables are uptake velocity, diel DO/metabolism, and diatom assemblage information. The variables were thought to be complementary integrators of biological response and ecosystem function. Diel DO captures stressors that occur at night and heterotrophic processes. Uptake velocity integrates more ecological values, including habitat heterogeneity and heterotrophic activity, and it is fundamentally related to downstream protection. The group did not believe that invertebrates should be included in the suite of variables because of their lack of responsiveness.

#### Combined Criterion Approach

Group 5 assumed that thresholds that delineate acceptable from unacceptable conditions were available for all variables. The group also assumed that robust data were available for all variables.

In their combined criterion approach, the group specified that a site failing any of the Level 1 variables should be assessed as impaired. That is, all variables were independently applied. However, further study with Level 2 variables might provide a means of refining some of the findings from the Level 1 assessment and could provide additional information for prioritization, management, and prevention of further degradation. Also, in cases in which aquatic life use support is demonstrated, the group thought Level 2 variables could be used to support a case for not assessing a site as impaired.

## Breakout Session 4: Summary

The breakout groups varied widely in the expertise of their members, but despite these differences, most breakout groups selected a similar set of variables and proposed similar combination approaches.

#### Selected Variables

All the groups selected TN and TP, measures of primary productivity (chl-a, AFDM, and/or percent cover), and DO as variables to be included when assessing for nutrient impacts. The groups differed somewhat in whether they thought invertebrate metrics would be useful. Some groups thought tailoring invertebrate metrics to select those that were most responsive to nutrients would be possible, whereas other groups did not believe invertebrates were responsive enough to be useful. All the groups noted that algal assemblage information would be valuable for assessing the direct effects of nutrients on aquatic life use, and as an alternate means of estimating nutrient pollution; however, all the groups also acknowledged that algal assemblage data were not available for many states.

### Combined Criterion Approach

All groups based their combined criterion approach on independent application of each response threshold within the criterion, but the groups differed in the extent to which they incorporated a grey zone to allow for some uncertainty in the assessment of a site relative to a fixed threshold.

### Application of Indicators

One group presented combinations of indicators and determined whether each combination of events would indicate nutrient pollution-related impairment, using a biological rationale. Other techniques used decision matrices. In cases where it is unclear whether a water body is in attainment of the indicator target, several groups suggested that collecting additional data would be necessary. There were mixed thoughts about how a water body's nutrient status would be categorized. Some groups thought that if one indicator did not meet its numeric value, the entire site should fail; other groups thought that the categorization decision should depend on the combination of variables that passed or failed. One group emphasized that metrics should not be averaged to avoid blurring the weighting of information and removing the importance of high values. Another approach was multiplicative: Each variable was scored on a scale from 0 to 2, and the ranks were multiplied to achieve the water body rank. The impairment threshold for the overall criterion would need to be determined on a scale from 0 to 64.

After reporting on the previous day's breakout session on indicator choices and development of an approach to apply indicators, the breakout groups re-gathered. In this final breakout session, the groups finalized their sets of indicators and the approaches to identifying nutrient pollution. Each group provided EPA with a typed outline of its final recommended approach.

### The Grey Zone

As the groups developed their combined criterion approaches, there was a recurring discussion about a grey zone, where there is not a single threshold value of a response parameter that clearly indicates that a water body is or is not affected by nutrients. One group supported an approach in which there is no grey zone. Under this approach, good data and methods are assumed and a water body either meets a threshold (passes) or does not (fails).

Others argued that a grey zone is necessary, noting the uncertainty associated with establishing a single threshold value. Several groups stated that if monitoring indicated that a water body was in the grey zone, this would trigger additional monitoring, including adding indicator variables to make a determination that removes the water body from the grey zone. The grey zone could be sized to reflect the uncertainty. Another suggestion was that the grey zone be viewed as a third tier, where the nutrient condition is neither good nor bad but mediocre. The response to this mediocre tier might be to take a detailed analysis of existing permits. One breakout group suggested that there be a two-year limit on the time that a water body may remain in the grey zone in order to reduce the incentive to keep a water body in that category indefinitely.

## Closing Statements

As part of the workshop wrap-up, all nutrient expert participants were asked to state what they thought were the most relevant messages from the workshop and to identify which topics required additional attention and research. The participants' comments were compiled and organized into common themes, and they are presented below.

### Nutrient-Sensitive Response Indicators

- The workshop discussions broadened the participants' perspective with regard to nutrient-sensitive indicators and their measurement, as well as potential combined criterion approaches that can be used in numeric nutrient criteria development. As part of the ongoing discussions, workshop participants discussed and identified the complexities of selecting nutrient indicators, as well as the challenges in establishing their linkage to nutrient pollution and designated uses.
- Although many indicators were identified and evaluated as potential nutrient pollution response indicators, most experts were able to identify a short list of indicators—an integrated measure of primary productivity that includes benthic chlorophyll and algal cover, diel DO and pH, and diatom assemblage measures—as appropriate for developing numeric nutrient criteria.
- Although ecological function indicators can potentially be used to develop or assess numeric nutrient criteria, the workshop discussions focused mainly on nutrient pollution-sensitive indicators of ecosystem structure.
- The use of algae as a nutrient pollution response indicator is supported by existing knowledge. However, other assemblages, such as microbes, might be more responsive to stress than invertebrates and fish. As a result, microbes should be considered as a potential nutrient pollution response indicator.
- Regionalization and classification should be taken into consideration when choosing and refining nutrient pollution response indicators.
- There is a need to draw the line on whether, where, and when nutrient indicators are attaining or non-attaining; perhaps this can be done by combining user perception surveys and field studies.

### Combined Criterion Approach

- Some experts expressed support for the use of a combined criterion approach for the development of numeric nutrient criteria; however this sentiment was not universal. Although this approach may provide flexibility to states, the lack of necessary expertise at the state level is still a concern with respect to developing and implementing this approach.
- The Biological Condition Gradient was proposed as a resource for helping incorporate aquatic life use indicators into a combined criterion approach.
- Workshop participants expressed interest in seeing any potential combined criterion approach, and the resulting numeric values, be closely linked to assessment methodologies.

### National and State Perspectives on Numeric Nutrient Criteria

- We are facing a communication challenge: We need to get state dischargers and the public interested, engaged, and excited about discussing nutrient pollution.

- It may be easier to identify and provide justification for a range of values to be used for a given nutrient pollution response indicator than to identify one threshold value given the use of real-world data.
- Although a grey area in the development and assessment of numeric nutrient criteria is not desirable, nutrient pollution response indicators such as enzymes, nutrient uptake, and diel DO seem promising in helping to narrow the grey zone.
- Representatives of environmental agencies at the state level thought the workshop discussions affirmed the approaches used and progress achieved in successfully implementing numeric nutrient criteria by some states. Likewise, state representatives thought the workshop empowered them to address nutrient pollution in future discussions.
- Participants realized through the ongoing workshop discussions that science is easy; criteria, hard; implementation, harder.
- It was noted that although most diatoms show an increasing abundance with nutrient pollution, *Didymo* behaves differently. This species blooms as a response to decreased phosphorus.

### Research and Resource Needs

### Chlorophyll-a

- Chl-a is a known and useful indicator, but there is a need for research to determine the applicability and relevance of chl-a as a nutrient indicator in all flowing systems. For example, algal communities may vary depending on watershed area: most streams are dominated by benthic algae; most rivers are dominated by sestonic algae. Watershed characteristics such as substrate and flow may also affect the usefulness of chl-a as an indicator of nutrient pollution in some systems.
- There is a need for research on how to incorporate benthic chl-a (algae and diatoms), algal, and submerged aquatic vegetation community structure into an integrated measure of primary productivity.
- A synthesis of existing studies is needed to link chl-a to higher trophic levels as a potential way to support the development of conceptual pathways for a combined criterion approach.

### Synthesis Studies and Linkages along the Conceptual Pathway

- A synthesis and/or meta-analysis of data is needed of conceptual pathways to be used in a combined criterion approach, particularly on the trophic-level responses to nutrient pollution. An important gap is the linkage between nutrients, algal and/or plant cover, and nutrient indicators. This gap includes:
    - Showing and documenting the dose-response relationship of different indicators to nutrients and to each other.
    - Short-term fertilization studies in reference stream sites as a tool for metric confirmation.

- Research is needed on the evaluation of nutrient pollution response indicators and their incorporation into numeric nutrient criteria development including diatom indices, nutrient-specific invertebrate measures, and percent cover.
    - There is a need for calibrating diatom indices and developing nutrient-specific invertebrate and fish measures.
    - There is a need for methods for to establish common/general thresholds for percent cover, species tolerance, and changes in community structure as a response to nutrient pollution.

### Resource Needs for the Use of Diatoms as Nutrient Pollution Indicators

- Resources are limited, i.e., funding and technical support are needed to help states develop nutrient pollution response indicators. Some of these gaps include:
    - Tools and funding to develop novel techniques (particularly for algae and diatom assemblages) that measure and document confirmatory responses to nutrient pollution.
    - Building regional diatom-based models to understand the effect of their variability on application and cost.
    - Developing diatom indices and setting thresholds.

### Functional Indicators

- Research is needed on the linkage between nutrient pollution and ecosystem function (e.g., respiration, decomposition, and acidification).
- Research is needed to expand knowledge on potential functional indicators of nutrient pollution such as metabolism, enzymes, and leaf decomposition.
- In addition to informing the current discussion of nutrient pollution response indicators, molecular genetics is a promising tool to define and refine indicators in the future.

### Aquatic Life Use and Nutrient Pollution

- The use of the Biological Condition Gradient Framework to relate aquatic life use variables to nutrients when using a combined criterion approach (e.g., to develop species-specific tolerance to nutrient pollution) requires further work.
- There is a need for engagement with the social science community to propose, evaluate, and support novel indicators of nutrient pollution; for example, studies evaluating whether stakeholders think nutrient retention is an aquatic life use.

### Data Analysis

- There is a need for collective efforts for analysis that integrates data on stressors (i.e., nutrients) as well as multiple response indicators (e.g., algae, invertebrates, fish). Filling such gaps could include:
    - Data compilation followed by an exploratory analysis exercise. During such an exercise, states would be expected to share their data and use the Biological Condition Gradient as a framework for data analysis.
    - Consideration of states' expectations in planning such an effort. Potential outcomes of an exercise like this might range from helping states choose appropriate indicators of

  nutrient pollution to evaluating preliminary thresholds at a regional/national level to identifying state-specific thresholds.

- Exploratory data analysis is an imperative step to determine how data on multiple response indicators can be combined into a single nutrient criterion.
- Monitoring activities need to be coordinated and the resulting data used for developing thresholds and/or numeric criteria for nutrient pollution response indicators.
- Development of numeric nutrient criteria for streams calls for a strategy framework that includes application, testing, and adaptation of efforts, as well as adoption of novel approaches to account for differences in responses.

### Data Quality

- Explicit definitions for “good data” are required (i.e., the characteristics of data appropriate for numeric nutrient criteria development), given the assumption that good data are needed to evaluate a combined criterion approach.

### Alternatives to Move Forward with Criteria Development

- Political will is important and necessary in getting states to move forward on the development of numeric nutrient criteria. The lack of competitive funding for universities and other entities/groups is constraining progress in numeric nutrient criteria development.

## Appendix A. Workshop Participants

| Name | Organization |
|---|---|
| **Michelle Baker** | Utah State University |
| **Candice Bauer** | U.S. EPA Region 5 |
| **Max Bothwell** | Environment Canada |
| **Don Charles** | Academy of Natural Sciences |
| **Betty Fetscher** | Southern California Coastal Water Research Project |
| **Stuart Findlay** | Cary Institute of Ecosystem Studies |
| **Terry Fleming** | U.S. EPA Region 9 |
| **Steve Francoeur** | Eastern Michigan University |
| **Evelyn Gaiser** | Florida International University |
| **Jim Hagy** | U.S. EPA Office of Research and Development |
| **Anne Hershey** | University of North Carolina - Greensboro |
| **Lisa Huff** | Alabama Department of Environmental Management |
| **Ryan King** | Baylor University |
| **Tina Laidlaw** | U.S. EPA Region 8 |
| **Mark Munn** | US Geological Survey |
| **Greg Pond** | U.S. EPA Region 3 |
| **Steven Rier** | Bloomsburg University |
| **Bob Sinsabaugh** | University of New Mexico |
| **A.J. Smith** | New York State Department of Environmental Conservation |
| **Nathan Smucker** | U.S. EPA Office of Research and Development |
| **Jan Stevenson** | Michigan State University |
| **Mike Suplee** | Montana Department of Environmental Quality |

## Appendix B. Workshop Agenda

United States
Environmental Protection
Agency

# Expert Workshop: Nutrient Enrichment Indicators in Streams - April 16–18, 2013

**(**One Potomac Yard 2777 S. Crystal Drive, Arlington, VA 22202**)**

## AGENDA

**Goal:** To identify nutrient pollution indicators that are both sensitive to nutrient stress and predictive of impacts to higher trophic levels, and to use these indicators to develop criteria that are protective of aquatic life use in lotic systems.

### April 16th, 8 - 5pm: Sensitive Indicators

- 8 – 9am: Gather for coffee (BYOC)
- 9 – 930am: Welcome
    - 10 min: Introductions
    - 10 min: Opening remarks
    - 10 min: Review of agenda
- 930 – 1130am: Indicator category presentations/discussion (15 min presentation/5 min clarifying questions) by six groups:
    - Nutrient measures
    - Algal biomass indicators
    - Algal assemblage indicators
    - Algal/heterotroph physiological indicators
    - Higher trophic level indicators
    - Ecosystem function measures
- 1130 – 1145am: Break
- 1145 – 1230pm: Discussion of indicator category presentations
- 1230 – 130pm: Networking Lunch
- 130 – 2pm: Discussion of the “best” indicators
- 2 – 330pm: Break out session 1
    - Groups will discuss and rank a set of indicators based on their sensitivity to nutrient pollution and predictability of effects on higher trophic levels and designated uses
- 330 – 5pm: Groups report out and discuss “best” indicators

### April 17th, 8 - 5pm: Sensitive Indicator Writing and Combined Indicators

- 8 – 830am: Gather for coffee (BYOC)
- 830-9am: Re-cap from day 1 /prep for Breakout Session 2
- 9am – 11am: Breakout Session 2

  - Develop annotated outlines for the most sensitive and predictive indicators based on day 1 indicator discussions
- 11– 12pm: Combined Criterion Approaches
- 12 – 1pm: Working lunch
- 1 – 5pm: Breakout Session 3
  - Develop ideas, options, and feedback on combined criterion approaches

### April 18th, 9 - 5pm: Combined Indicator Outline

- 8 – 9am: Gather for coffee (BYOC)
- 9 – 11am: Groups report out and discuss combined criterion approaches identified in Breakout Session 3
- 11 – 3pm: Breakout Session 4 (Working lunch)
  - Outline the elements of a successful combined criterion approach
- 3 – 430pm: Next steps
- 430 – 5pm: Closing remarks