EVALUATION OF BIOLOGICAL ASSESSMENT DATA AND PROTOCOLS FOR TMDL REPORTS

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In the mid-1960s, it was recognized that physical and chemical monitoring of water do not adequately describe the adverse effects of water contamination on aquatic life (Jackson & Brungs, 1966). John Cairns, a pioneer in aquatic biology, suggested that biological monitoring[1] could be a useful supplement to (but not a substitute for) physical and chemical monitoring of water (Cairns et al., 1973). The focus of this paper is on the use of benthic macroinvertebrates[2] as a bioassessment tool and their relevance to establishing water impairments and total maximum daily load (TMDL) reports. Bioassessments are used to describe the benthic condition of a surface waterbody compared to an undisturbed condition. If a waterbody is classified as impaired because of its benthic condition, it is included in the "303(d) list," and a TMDL plan is required for the listed segment. State agency scientists have used the tool to implement mandates of the Clean Water Act since its inception in 1972 (Barbour & Burton, 2002).

Benthic macroinvertebrate bioassessments use the number, type, and sensitivity to certain pollutants (or tolerance) of benthic macroinvertebrates to calculate a series of metrics and provide an overall water quality rating. The presence, absence, abundance, and diversity of these organisms in a waterbody, when compared to a regional reference condition,[3] are used to classify the status of the waterbody as "impaired" or "non-impaired" owing to the benthic condition.

The objective of this article is to discuss the applicability and limitation of benthic macroinvertebrate bioassessments as a tool to develop benthic TMDL reports. The article contains an overview of the impact of stresses (called stressors) on benthic macroinvertebrates, a listing of bioassessment protocols, a discussion of the advantages to using benthic bioassessments and the challenges involved in developing benthic TMDL reports, and recommendations for developing practical benthic TMDL reports.

STRESSOR IMPACT ON BENTHIC CONDITION

The community of benthic macroinvertebrates in a waterbody is affected by the habitat structure,[4] water quality, and other environmental factors. Factors that negatively impact the benthic community population and diversity are called stressors. For water quality management purposes, it is important to distinguish between natural and anthropogenic stressors. Natural stressors include but are not limited to high winds, low and high rainfall, frost action, snowfall and intense sunshine. Anthropogenic stressors include hydraulic alterations; the impact of point and non-point sources of pollution such as sediment, organic, and chemical (e.g., heavy metals and pesticides) loads; changes in pH and water temperature; and predation or competition by introduced species. TMDL studies are only needed for streams with benthic impairments owing to anthropogenic effects. Natural stressors are considered as background effects. A brief overview of various anthropogenic stressors is given below. Details can be found in various publications (e.g. Hellawell, 1986; Minshall, 1984).

HYDRAULIC ALTERATION RELATIVE TO THE UNDISTURBED SITE

Changes in stream water depth and velocity (flow volume and rate) can negatively impact the benthic community. These changes can be caused by increased surface-runoff from adjacent lands (including flooding), sustained drought, discharges from municipal and industrial outfalls, and pooling behind man-made dams. Low flows reduce the available habitat capacity for aquatic organisms and have higher water temperatures. High flows can scour the substrate, move rocks and other valuable habitat areas downstream, and often carry higher loads of sediment and other pollutants.

SEDIMENT LOAD RELATIVE TO UNDISTURBED SITE

Sediment from non-point sources in surface-runoff and solids in municipal and industrial discharges can have multiple effects on the stream water environment. These effects include increased turbidity that reduces light penetration, increased water temperature, and reduced dissolved oxygen levels. The solids can interfere with the respiration of benthic macroinvertebrates by injuring the gills. Deposited sediment that fills interstitial spaces of the substrata reduces the available habitat for some macroinvertebrate species. Solids in water reduce the photosynthesis of aquatic plants, which are the food source for some benthic macroinvertebrates, and may clog the feeding nets of other benthic macroinvertebrates. Solids reduce the visibility in the water and can thus lower the success rate of predatory macroinvertebrates in capturing prey.

ORGANIC LOAD RELATIVE TO UNDISTURBED SITE

Organic enrichment, which leads to high dissolved organic carbon levels and associated high biochemical oxygen demand, results in low dissolved oxygen concentrations in water. The consequences of low dissolved oxygen (DO) levels include: a decrease in the number of oxygen-sensitive organisms, an increase in low-DO tolerant organisms, and therefore changes in the macroinvertebrate community composition. Deposited organic sludge forms a blanket over the benthic macroinvertebrates resulting in the loss of interstitial organisms. Sludge deposition in low velocity waters can release methane and hydrogen sulfide into the water and may result in the elimination of an entire community of benthic macroinvertebrates.

CHEMICAL LOAD RELATIVE TO UNDISTURBED SITE

Chemical inputs into waterbodies can originate from industrial, agricultural, and urban sources. The effects of chemical inputs on benthic organisms vary and can be detrimental to some species. For example, high ammonia concentrations in water can be toxic to certain benthic organisms and may cause a total elimination of or a decrease in the population of these species. Other toxins may have lethal effects on different species and cause changes in the benthic community structure and diversity.

CHANGES IN PH RELATIVE TO UNDISTURBED SITE

Stream water pH can be affected by long-term acid precipitation, acid mine drainage, and acids in effluent from industrial plants. Under acidic conditions, benthic macroinvertebrates that employ carbonate shell structures (e.g., crayfish, snails, clams, and mussels) are unable to properly build shell material. Acid conditions also indirectly affect benthic macroinvertebrates because the toxicity of some pollutants (e.g., ammonia) is more intense under acidic conditions. Toxic metals such as aluminum, manganese, and mercury become more mobile under acidic conditions and therefore become more likely to impact the benthic community.

WATER TEMPERATURE RELATIVE TO UNDISTURBED SITE

The riparian canopy and streambank vegetation influence stream water temperatures. For instance, the cutting of trees and tall vegetation along the streambank allows in more direct sunlight, which heats the water. The discharge of high temperature waters from industrial sources also influence the stream water temperatures. Changes in the benthic macroinvertebrate community can be expected under conditions of higher water temperatures. One of the most obvious effects of water temperature change concerns its effect on the amount of available dissolved oxygen, with warmer waters containing less dissolved oxygen. Water temperature regime also effects developmental and growth characteristics of benthic macroinvertebrates.

BIOASSESSMENT PROTOCOLS

Rapid Bioassessment Protocols (RBPs) for using benthic macroinvertebrates as indicators of biological integrity of waters were initially developed in the 1980s as a cost-effective screening tool. Since then RBPs have evolved into three protocols (RBPI, RBPII, RBPIII) that incorporate different levels of rigorousness. The benthic macroinvertebrate protocols (RBPI, RBPII, RBPIII) differ in the level of effort, taxonomic identification level, expertise required to perform them, and in the usefulness of obtained data.

RBPI Sampling procedures are not standardized; family-level taxonomic identification is carried out in the field; assessment decision is based on "best professional judgment."

RBPII Sampling procedures are standardized; family-level taxonomic identification is carried out in the field or in the laboratory; assessment decision is based on numerical data.

RBPIII Sampling procedures are standardized; genus/species level taxonomic identification is carried out in the laboratory; assessment decision based on numerical data.

RBPI and RBPII are useful approaches for setting priorities but are less rigorous than RBPIII. Because RBPIII involves organism identification to the lowest practical level (genus or species), it is the most laborintensive approach but gives relatively detailed information for trend analysis. The RBPII and RBPIII data are used to calculate a variety of values or metrics. A metric is a calculated term or enumeration that represents some aspect of the biological assemblage structure, function, or other measurable characteristics that changes in some predictable way in response to environmental influences (including human influences). A multimetric approach aggregates metrics into an overall assessment of the biological condition.[5] Each calculated metric is assigned a score based on a comparison to the reference (undisturbed) condition. Scores for all metrics are then summed and compared to the total metric score for the reference condition. The percent comparison between the total scores provides a final evaluation of the biological condition.

The U.S. EPA has suggested minimum requirements for state biological assessment programs as part of the Section 305(b) reporting requirements. These requirements are based upon existing state programs and when followed ensure greater accuracy and consistency in state biological assessment and criteria development efforts. Suggested requirements include the use of multiple assemblages,[6] multiple metric indices, habitat structure assessment, regional reference conditions, index periods,[7] standard operating procedures, and a quality assurance program. Details of the Rapid Biological Assessment (i.e., sampling techniques, taxonomic identification methods, water quality rating and scoring techniques) are described in U.S. EPA publications (Barbour et al., 1999; U.S. EPA, 1997a and 1997b) and the EPA Website: http://www.epa.gov/owowwtr1/monitoring/rbp/index.html.

States are in various stages of integrating different levels of bioassessments into their water quality management programs. Most states use benthic macroinvertebrates with RBPII protocols and the multimetric approach as their primary bioassessment tool. Some states use multiple assemblages in their assessment to reduce uncertainty and some (e.g., Maine) are using multivariate approaches. Multivariate analysis typically involves selecting reference sites through clustering methods that group sites of similar macroinvertebrate composition. Details of state programs can be found on the EPA's Website: http://www.epa.gov/owow/monitoring/newmon/bio/section4.htm.

Advantages to Using Benthic Bioassessments

Advantages of using benthic macroinvertebrates as a bioassessment tool are listed as follows (Barbour et al., 1999):

- Macroinvertebrates assemblages are good indicators of localized environmental conditions. Because many benthic macroinvertebrates have limited migration patterns or a sessile mode of life, they are particularly well suited for assessing site-specific impacts.
- Macroinvertebrates integrate the effects of short-term environmental variations. Most species have a complex life cycle of approximately one year or more. Sensitive species respond quickly to environmental stress while the overall community responds more slowly.
- Macroinvertebrates are relatively easy to identify to family; many taxa can be identified to lower taxonomic levels with ease. An experienced biologist can easily detect a degraded condition with an examination of the benthic macroinvertebrates assemblage.
- Benthic macroinvertebrates assemblages are made of species that constitute a broad range of trophic levels and pollution tolerances, thus providing strong and graded information for interpreting cumulative effects.
- Sampling benthic macroinvertebrates is relatively easy, requires few people and inexpensive equipment, and has minimal detrimental effect on the resident biota.
- Benthic macroinvertebrates serve as a primary food source for fish, including many recreationally and commercially important species.[8]
- Benthic macroinvertebrates are abundant in most streams. Many small streams (1st and 2nd order), which normally support a diverse macroinvertebrate fauna, only support a limited fish fauna.
- Many state water quality agencies have more expertise with invertebrates than fish. Therefore, most state water quality agencies that routinely perform biosurveys focus on macroinvertebrates.

Challenges to Using Benthic Bioassessments

Benthic degradation or impairment is particularly challenging for use in TMDL reports because the benthic condition is not the cause of a problem but merely an indicator of problems. Other stream impairments such as nutrient and sediment impairments are the direct cause of the degradation. Nutrients and sediment loads can be determined if concentrations and flow conditions are known. Impairments owing to benthic degradation are more similar to dissolved oxygen impairments, i.e., they are a symptom of multiple external effects, and a linkage between cause and effects is needed to propose any remediation action. In the case of dissolved oxygen, however, the impairment can simply be linked to oxygen demand.

Benthic measurements are more complicated and may be linked to a number of the stressors described earlier.

In this paper, for the sake of discussion, we present three scenarios with different levels of complexity and challenges to developing a TMDL report based on benthic condition. Scenario 1 is a stream designated as impaired because of the benthic condition with an obvious point source discharge speculated as the sole stressor. Scenario 2 is a stream designated as impaired owing to the benthic condition with an obvious point source as well as some nonpoint sources speculated as the stressors. Scenario 3 is located in a diverse landuse watershed with cumulative and chronic point and nonpoint source impacts.

<u>Scenario 1: Point stressor</u>

In this scenario, the benthic impairment is caused by a point source discharge as the sole stressor. This scenario is often observed immediately below the discharge point of municipal wastewater or industrial plants. Under this scenario, the discharge water quality is usually well documented because of permit requirements. Pollutant loads can be easily determined using flow rates, which are generally uniform over time. These data can be used to calculate pollutant load reductions that could meet TMDL requirements. However, a cause-response relationship between the benthic condition and stressors needs to be developed. Biologists have amassed data to link benthic degradation conditions for some specific pollutants. For example, the impact of some pesticides, metals (e.g., cadmium and copper), and other toxins on benthic organisms have been studied (Buikema & Voshell, 1993). To meet the TMDL requirements for aquatic life, the total pollutant load from the discharge should be reduced in the receiving (impaired) stream to a level comparable to the concentrations in the target water (reference) condition. One challenging problem in this scenario is the definition of the "mixing zone" especially with temporally nonuniform discharges described below. What is the length of a reasonable mixing zone, and where should the impaired segment begin?

The above principal applies when there is no significant variation in long-term temporal characteristics of the point discharge (such as with municipal wastewater discharges). The authors of this article, however, have encountered a challenge in estimating point discharge characteristics from aquaculture facilities (trout raising farms) to meet TMDL requirements. In aquaculture facilities, the effluent concentrations change with the various activities that occur: fish feeding, fish harvesting, and settling basin cleaning. Likewise, the different amounts of feed provided throughout the year, as required to meet the changing needs of the fish, influence the characteristics of the effluent. Also, the type of fish feed used affects the effluent because some feeds have higher residual content than others. Fish feeding, harvesting and settling basin cleaning disturb the settled solid wastes and change the effluent characteristics. Because the facilities do not operate in a consistent manner, intensive, year-long monitoring of each specific facility is needed to have a better grasp of the pollutant loads. Unlike municipal treatment facilities, year-round and continuous monitoring of small aquaculture facilities is not practical and is cost prohibitive. Therefore, limited intensive monitoring is performed and the results are extrapolated to determine the total loads to the receiving stream, which is then compared to the target water quality conditions as described earlier.

A second, and perhaps more critical, issue encompasses the selection of the target water condition. The water sources for the study aquaculture facilities are spring waters. The geologic formation from which a spring emerges influences its water chemistry and natural water quality. It is rather difficult to locate reference conditions of similar water chemistry and flows that are pristine or minimally influenced by surface contaminants. The following questions need to be addressed: Should TMDL implementation plans for the impaired segments below the aquaculture facilities be developed to restore the water quality and consequently the benthic condition comparable to the facility headwaters (the original natural condition) or to the reference condition? Is it realistic that a stream segment be restored to a condition comparable to a regional reference (undisturbed) condition? Or is it more practical to define a designated use for the stream segment and then strive to meet the designated use.

<u>Scenario 2: Mixed point and non-point stressors</u>

Scenario 2 is a stream designated as impaired because of the benthic condition with an obvious point source as well as some nonpoint sources speculated as the stressors. The difficulties and problems associated with point discharges were discussed in the previous section. Here we focus on the linkage between nonpoint sources and benthic degradation.

To evaluate nonpoint source effects on benthic degradation or restoration, the concept of using "reference watersheds" has been proposed. A reference watershed should meet the requirements of the regional reference condition defined earlier (see footnote 3). The challenge is to find a reference watershed of similar size and characteristics comparable to the impaired watershed. Uncertainty will be introduced due to the differences in the watersheds, and complex models are needed to compare the loads of the watersheds. Another problem that should be mentioned is the fact that undisturbed or pristine streams are not necessarily situated in undisturbed watersheds. A disturbance in a remote section of a watershed may not noticeably affect a stream reach but may skew the nonpoint source calculations for the reference watershed. Therefore nonpoint source impact calculations for a reference watershed could be skewed.

Establishing a linkage or relationship between a specific stressor and the benthic condition is difficult, especially under mixed landuse conditions when pollutant loads originate from nonpoint sources. Few studies have attempted to establish a relationship between nonpoint stressors and benthic conditions, and results for those studies are either inconclusive or indicate high uncertainty in the relationship (Frondorf, 2001). Predicting nonpoint source impacts on the benthic condition requires a 2-step model. In step 1, the nonpoint source impact on the stream water quality and stream habitat are evaluated, and in step 2, a linkage model is developed between the stream water quality and the benthic condition. Lack of spatially and temporally synchronized long-term water monitoring and bioassessment data to develop and verify the model makes the evaluation of nonpoint source effects on benthics impractical and uncertain.

Scenario 3: Cumulative point and non-point stressors

Scenario 3 is an impaired stream segment located in a diverse landuse watershed with cumulative and chronic point and nonpoint source impacts. This scenario is illustrated in the following example, which is typical of many watersheds designated impaired because of the benthic condition. In this scenario, the impaired stream originates from springs located in a town and flows through urban and agricultural areas. The benthic impairment is speculated to be nonpoint source pollution from increased urbanization of the upper portion of the watershed and agricultural activity. The reference stream is a pristine freshwater stream in a rural, forested area.

In this scenario, major land use changes have occurred within the impaired watershed during the past one-hundred years, including increased residential and agricultural development that could have chronic effects on the stream benthic condition. The town population has increased from a few hundred in 1900 to more than 50,000 residents in 2000. Historic influences include coal mining along the stream from the 1800s to mid 1930s, residential straight pipe effluent from the 1800s to mid-1900s, effluent from a sewage treatment plant in operation from the 1940s to the 1980s, faulty septic systems, agricultural and urban runoff, and wildlife. Current influences on the stream include a few straight pipes, faulty septic systems, agricultural and urban runoff, and wildlife. Urban stormwater runoff presently constitutes the greater portion of the stream flow immediately above the designated impaired segment.

Several questions have been raised about the appropriateness of using a reference stream for such conditions as listed above. Does one expect a stream that originates in an urban environment to exhibit the same benthic condition as a pristine mountain stream? How can the cumulative, long-term effects on stream benthics from multiple land uses be separated, understood, and quantified? Will a TMDL implementation be feasible and effective for the restoration of a stream with so many long-term, diverse, and cumulative impacts? What is a desired reference condition? Should the reference condition be pristine or "the best available" for the region? Could the reference condition be perhaps lower in quality than a pristine condition but still able to meet the designated use of the stream? Perhaps the appropriate response to the above questions is that this is a regulatory and hence political/public decision that should be based on science, economics and other factors. A clear statement of desired and expected TMDL outcome is needed for the TMDL process to be successful.

DISCUSSION

The application of bioassessments to water quality management and TMDL reports should be approached with caution. The major premise of the TMDL concept is to develop practical and cost-effective approaches for water quality improvements and restoration of impaired segments.

A recent National Research Council Report (NRC, 2001) raised many questions about problems associated with the TMDL reports and implementation plans. The NRC report, however, endorses the basic concept of the TMDL process. The NRC report recommends that biological criteria should be used in conjunction with physical and chemical criteria to determine whether or not a waterbody is meeting its designated use. The NRC report emphasizes that appropriate designated uses of the waterbody should be considered before listing it as impaired. The application of Use Attainability Analysis (UAA) appears suitable for benthic impaired waters before they are included in the 303(d) list. The UAA determines if the impairment is caused by natural contaminants or conditions, or nonremovable physical conditions. The UAA can consider the benefits and costs of meeting different levels of water quality standards. If the UAA concept were applied, the need for a TMDL report might prove unnecessary.

The authors of this article summarize the problems associated with the benthic macroinvertebrate bioassessment application to TMDL reporting and implementation plans as follows:

- the selection of the reference condition,
- the consideration for the designated use of water,
- the lack of an evaluation method because of restoration lag time,
- the economy of restoration,
- the lack of spatially and temporally consistent biological and chemical/physical monitoring data.

Selection of a reference condition (stream or watershed) is perhaps the most critical component of a bioassessment program. It should be noted that originally the use of a reference condition was intended to represent a pristine (undisturbed) or minimally disturbed site as a scientific yardstick for comparative purposes. However, currently under the TMDL program, the use of the reference benthic condition has been unintentionally defaulted to a water quality standard without consideration of the designated uses of the waterbody.

An ideal reference condition is a shifting goal as the pristine and minimally influenced waters continue to decrease in number because of intensive development and other anthropogenic activities. If and when an ideal reference condition is found, is it realistic to aim for a water quality standard or restoration that is not practical or economical? For example, is it economically advisable to restore the biological integrity of an urban stream comparable to that of a pristine rural stream? It makes more sense to consider that the designated uses of urban streams are different from rural mountain streams. It can be expected that urban streams be pathogen free and clean to the extent possible but not be required to sustain aquatic life comparable to a pristine mountain stream.

Determining the impacts of nonpoint sources necessitates a detailed examination of land use and land management practices in the watershed. Most nonpoint source models can predict the pollutant input from land use activities from far reaches of the watershed. However, steam water quality is more critically affected by the conditions of the riparian zone (Tufford et al., 1998). Canopy and vegetation within the riparian zone can affect sedimentation and water temperature in the stream. It appears that a detailed stream corridor assessment combined with a simple erosion prediction model will be more appropriate for benthic TMDL reports, especially for small stream segments.

The lag time for the restoration of aquatic life in an impaired stream can range between five to fifty years depending on the level of restoration desired and the degree of initial degradation (Benfield, 2001). This uncertainty in lag time makes measuring the success of the TMDL implementation plan very difficult. However, a long restoration time does not invalidate the potential need for, or benefits of, restoration.

Despite the fact that many states initiated bioassessment programs several years ago and much bioassessment data are available, these data have been collected parallel to, but not in coordination with, the physical and chemical monitoring of waters. This lack of coordination makes the available data less useful for establishing statistically valid analysis of bioassessment data such as the application of multivariate analysis (Jones, 2001), and developing models that more effectively link environmental stressors to biological responses.

RECOMMENDATIONS FOR DEVELOPING BENTHIC TMDL REPORTS

In the absence of clear-cut guidelines for designated uses of certain impaired waterbodies and a lack of the use of the UAA, under the current law, TMDL reports and implementation plans need to be developed for stream segments designated as impaired because of the benthic condition. Here, the authors attempt to make suggestions to meet the TMDL requirements for aquatic life restoration in an economically feasible and practical way within the boundaries of the current laws and regulations.

The authors propose the nine steps outlined below to address benthic impairments. A full TMDL report is to be developed only if Steps 1 to 3 below do not resolve the problem.

1. Check the validity of the reference condition. Is the impaired segment compatible with the reference condition in terms of water source (water chemistry) and other characteristics as defined under the regional reference condition? If the original biosurvey did not use a compatible reference condition, the status of the impairment should be reevaluated using a compatible reference condition.
2. Most stream segments are designated as impaired using the RBPII protocol. Reevaluate each impaired segment using the RBPIII protocol. If the RBPIII method reconfirms the status as impaired, proceed

with developing the TMDL report. Consequently, because the taxonomic identification of the RBPIII method is performed at the genus level, it may serve as an indicator of the stressors or pollutant sources.

3. Establish a relationship between benthic macroinvertebrates and their food resources and predators. For example, many fish are predators of benthic organisms, and their presence may have contributed to lower or different populations of benthics in the stream.
4. Conduct a comprehensive stream corridor survey for the riparian zone and landuse assessment for both the impaired segment and the reference condition watershed.
5. Initiate water sampling and analysis for both the impaired segment and the reference condition (NPDES permit data may not be adequate). Water sampling regime and parameter coverage should be consistent with variability regime and parameters of suspected stressors.
6. Identify stressors for both the impaired segment and the reference condition using data from Steps 4 and 5.
7. Compute stressor loads (point and nonpoint sources) for both the impaired segment and the reference condition. The riparian zone should be considered as the critical zone in terms of nonpoint source contribution.
8. Make recommendations to reduce pollutant loads in the impaired segment to equivalent or lower loads found in the target condition.
9. Make recommendations for pollutant load allocations and management practices that could achieve the objective of Step 8.

Because of a lack of synchronized spatial and temporal data for bioassessments and water chemistry monitoring and the high degree of uncertainty about the stressor impacts on biota, conventional models are not applicable to developing benthic TMDL implementation plans. The authors of this article believe the probabilistic modeling concept and professional judgment suggested by Stow et al. in this issue of *Water Resources Update* can be applied to develop adaptive management practices for water segments impaired because of the benthic condition.

SUMMARY

Designation of a waterbody as impaired leads to its inclusion in the 303(d) list and the requirement to develop a total maximum daily load (TMDL) plan for the listed waterbody. The focus of this article was on issues associated with preparing TMDL reports for benthic impairments. The article contains an overview of the impact of stressors on benthic macroinvertebrates, bioassessment protocols, advantages and challenges to using benthic bioassessments, and recommendations for developing practical benthic TMDL reports.

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ENDNOTES

[1] A broad definition of biological monitoring includes toxicity testing; ecological characterizations such as population surveys of periphyton, benthic macroinvertebrates, and fish (biological surveys); bioaccumulation analysis of contaminants; and biological indicators such as overall fish health and reproductive status (WEF 1997).

[2] Benthic macroinvertebrates are organisms that inhabit the bottom substrates (sediment, debris, logs, macrophytes, filamentous algae, etc.) of freshwater habitats for at least part of their life cycle. These organisms inhabit all types of running waters and include the larval or nymph forms of insects (e.g., stoneflies, mayflies, dragonflies), crustaceans (e.g., crayfish, isopods), snails, mussels and clams, worms, and leeches. These organisms are visible with the naked eye and can be retained by mesh sizes $\geq$ 200 to 500 µm.

[3] The regional reference condition is based on data collected from pristine or minimally-impaired sites representing regions of similar physical characteristics such as climate, soils type, physiography and vegetation (e.g., ecoregions) and further stratified by drainage area, stream order, size, and/or subecoregions.

[4] Habitat structure refers to the physical characteristics of the stream (channel morphology, floodplain shape and size, channel gradient, instream cover material, substrate types and diversity, riparian vegetation, canopy cover, and bank stability).

[5] Multimetric indices are recommended in order to strengthen data interpretation and reduce error based on isolated indices. Most multimetric indices for aquatic systems comprise 8 to 12 metrics.

[6] Multiple assemblages: the use of more than one *organism group* (e.g., benthic organisms and/or fish and/or periphyton).

[7] Index period: a defined time period during which data are collected; minimizes effects of year-to-year variability, reduces seasonal variability, and provides optimal accessibility of the target assemblages, and maximizes the efficiency of sampling equipment.

[8] Note that some streams may be designated as impaired in part because of predation of benthic macroinvertebrates by fish (based on the authors' experience).