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# Comparisons of Boating and Wading Methods Used to Assess the Status of Flowing Waters

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# Notice

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# Executive Summary

## Introduction

This document has been designed to provide an overview of the biological, physical and chemical methods of selected stream biomonitoring and assessment programs. It was written to satisfy the need to identify current methods that exist for sampling large rivers. The primary focus of this document is the boating methods used to assess flowing waters, but both boat-based and wading methods are included. The target audiences are individuals tasked:

1. to work with data generated from one or more of these programs;
2. to design or improve a bioassess- and monitoring program;
3. to conduct field work using methods (or based on methods) reviewed in this text;
4. to conduct field comparisons among these methods to determine the extent of their comparability and when each method is best employed.

This document is useful to these individuals in that it brings together relatively obscure literature from a wide variety of sources and it presents current and developing methods in a comprehensive context. These features allow this document to serve as a guide for comparing the methods used by various agencies for assessing large rivers.

Much of the included text has been largely adapted and modified from the agency documents from which it was derived. This has been done purposefully to reduce the risk of misinterpretation.

## Research Approach

The primary focus of this document is the boating methods used to assess flowing waters. However, both boat-based and wading methods are included in this document for several reasons. First, most wading methods were developed before boating methods and boating methods are often derivations of the wading methods that preceded them. Often, the methods used while in boatable waters simply call for the wading methods to be used in shallow areas (e.g., near the shore) or in the boat without any additional modifications. The inclusion of the original (wading) method as well as the derived (boating) method may also help illustrate how methods can be modified in order to meet the specific requirements of a sampling agency. Another reason that both sets are included is that it may be necessary to use both wading and boating methods among sampling sites or within a single reach when a river has varying depths. Finally, the inclusion of both sets of methods may help agencies or individuals analyze data sets that were collected using both wading and boating methods.

The information regarding the boating and wading methods reviewed in this document was derived from the available literature, the Internet, personal experience and personal communications with research scientists from respective agencies. Although some methods may have been modified or reduced since their conception, methods are presented in their entirety so as to not diminish their original intention. Where necessary, appendices are included to aid understanding of or differences among methodologies.

## Major Findings and Significance

Methods employed by the reviewed bioassessment and monitoring programs varied greatly. Differences included, but were not limited to: overall site selection (random, non-random), number and location of samples collected within the selected site, index or sample period, stream length sampled, time needed to execute methods in the field, time required to process samples in the field, type of sample collected (qualitative, semi-quantitative, or quantitative), equipment required to execute methods, expertise required to execute methods successfully, and subjectiveness of method. These differences may help individuals choose the methods appropriate to their sampling needs. Summary tables are included throughout the document that aid in understanding the differences between the methods used by the various agencies.

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# Acknowledgments

Programs reviewed in this document include the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program for Surface Waters (USEPA-EMAP-SW), U.S. Geological Survey's National Water-Quality Assessment program (USGS-NAWQA), U.S. Environmental Protection Agency's Rapid Bioassessment Protocol (USEPA-RBP), Ohio Environmental Protection Agency's flowing waters program (Ohio EPA), and Maryland's Department of Natural Resources's Maryland Biological Stream Survey program (MDNR-MBSS).

Numerous individuals at various agencies were contacted to aid in the interpretation and understanding the methods reviewed. Their continued assistance and cooperation is greatly appreciated.

Appreciation is especially extended to Marc Smith (Ohio EPA), James Lazorchak (USEPA) and Marty Gurtz (USGS-NAWQA) for the review comments they provided. These comments were important assets in the preparation of this document.

# Acronyms and Abbreviations

AFDM Ash-Free Dry Mass
AI Autotrophic Index
APA Acid/Alkaline Phosphatase Activity
ANC Acid Neutralizing Capacity
B-IBI Benthic Index of Biotic Integrity
BEST Biomonitoring of Environmental Status and Trends
BOD Biological Oxygen Demand
CC Conservation Commission
COD Chemical Oxygen Demand
CP Coastal Plains
DELT Deformities, Eroded Fins, Lesions, and External Tumors
DIC Dissolved Inorganic Carbon
DO Dissolved Oxygen
DTH Depositional-Targeted Habitat
DEP Department of Environmental Protection
DOC Dissolved Organic Carbon
*E. coli* *Escherichia coli*
EAV Emergent Aquatic Vegetation
EMAP Environmental Moni-toring and Assessment Program
EPT Ephemeroptera, Plecoptera, and Trichoptera
GIS Geographic Information Systems
H' Shannon-Weiner Diversity Index
HBI Hilsenhoff Biotic Index
IBI Index of Biotic Integrity
ICI Invertebrate Community Index
ID Identification
Iwb Index of Well-Being
LWD Large Woody Debris
MAH Mid-Atlantic Highlands
MAIA Mid-Atlantic Integrated Assessment

# Acronyms and Abbreviations (cont.)

| | |
|---|---|
| MANTA | Monitoring and Non-Tidal Assessment |
| MBSS | Maryland Biological Stream Survey |
| MDNR | Maryland Department of Natural Resources |
| NAWQA | National Water-Quality Assessment program |
| NCP | Non-Coastal Plains |
| NERL | National Exposure Research Laboratory |
| Ohio EPA | Ohio Environmental Protection Agency |
| PCA | Principal Component Analysis |
| PHab | Physical Habitat |
| $PS_c$ | Percent Community Similarity |
| PTI | Pollution Tolerance Index |
| QHEI | Qualitative Habitat Evaluation Index |
| QMH | Qualitative Multi-habitat |
| RBP | Rapid Bioassessment Protocols |
| RHA | Rapid Habitat Assessment |
| RTH | Richest-Targeted Habitat |
| SAV | Submerged Aquatic Vegetation |
| SBII | Stream Benthos Integrity Index |
| SG-92 | Scum-Getter 92 |
| SOC | Suspended Organic Carbon |
| SW | Surface Waters |
| TDS | Total Dissolved Solids |
| TNDT | Total Number of Diatom Taxa |
| TOC | Total Organic Carbon |
| TSS | Total Suspended Solids |
| USEPA | United States Environmental Protection Agency |
| USGS | United States Geological Survey |

# Section 1
# Introduction

by
Joseph E. Flotemersch

This document has been designed to provide an overview of the biological, physical and chemical methods of selected stream biomonitoring and assessment programs. The target audiences are those individuals tasked with working with the data generated from one or more of these programs, yet unfamiliar with the basics of the sampling procedures themselves. Other tasks that may be aided by this document are the design or improvement of a bioassessment and monitoring program, conducting field work using methods reviewed in this text, or conducting field comparisons among these methods to determine the extent of their comparability and when each method is best employed. However, this document is not intended to serve as a substitute for the protocol manuals produced by the respective agencies. Individuals intending on implementing any of these protocols should, at a minimum, obtain a copy of the agency's original protocol manual. It would also be beneficial to these individuals to contact the agencies in order to gain the insight of the scientists who developed these protocols or who utilize them on a regular basis. Such contact could provide clarification or modifications to the protocols of interest. Table 1-1 provides contact information for the five agencies that are reviewed in this document.

The reviewed biomonitoring programs differ not only in their methods for collecting samples in the field but also their methods for processing samples in the laboratory. While the different laboratory methods may create additional differences in the final data produced by the different agencies, these laboratory methods are outside the scope of this document which will focus exclusively on the field methods.

Much of the included text has been largely adapted and modified from the agency documents from which it was derived. This has been done purposefully to reduce the risk of misinterpretation.

Programs reviewed include the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program for Surface Waters (USEPA-EMAP-SW), U.S. Geological Survey's National Water-

**Table 1-1.** Contact Information for the Five Reviewed Programs

| Biomonitoring Program | Program Contact | General E-Mail Contact and Web Sites | Publications Contact |
|---|---|---|---|
| USEPA-EMAP-SW | **John Stoddard**<br>USEPA National Health and Environmental Effects Research Lab/ORD<br>Western Ecology Division<br>**Address:**<br>200 S.W. 35th Street<br>Corvallis OR 97333-4902<br>**Telephone:** 541-754-4441<br>**E-mail:**<br>Stoddard@mail.cor.epa.gov | **E-mail:** emap@epa.gov<br>**Web Site:**<br>www.epa.gov/emap | National Service Center for Environmental Publications<br>**Address:**<br>P.O. Box 42419<br>Cincinnati, OH 45242-2419<br>**Telephone:** 800-490-9198<br>**Fax Number:** 513-489-8695 |
| USGS-NAWQA | **Tom Muir**<br>Coordinator, NAWQA<br>**Address:**<br>Mail Stop 3660<br>1849 C Street, N.W.<br>Washington, D.C. 20240<br>**Telephone:** 703-648-5114<br>**E-mail:** tmuir@usgs.gov | **Web Site:**<br>www.water.usgs.gov/nawqa/nawqa_home.html | U.S. Geological Survey Earth Science and Information Center<br>Open-File Reports Section<br>**Address:**<br>Box 25286, MS 517<br>Denver Federal Center<br>Denver, CO 80225<br>**Telephone:** 800-435-7627<br>800-872-6277 |
| USEPA-RBP | **Michael T. Barbour**<br>Tetra Tech, Inc.<br>Ecological Sciences<br>**Address:**<br>10045 Red Run Road, Suite 110<br>Owings Mills, MD 21117<br>**Telephone:** 410-356-8993<br>**E-Mail:**<br>Michael.Barbour@tetratech.com | **Web Site:**<br>www.epa.gov/owow/monitoring/rbp | National Service Center forEnvironmental Publications<br>**Address:** P.O. Box 42419<br>Cincinnati, OH 45242-2419<br>**Telephone:** 800-490-9198<br>**Fax Number:** 513-489-8695 |
| Ohio EPA | **Chris Yoder**<br>Division of Surface Water/ Ecological Assessment Unit<br>**Address:**<br>4675 Homer Ohio Lane<br>Groveport, OH 43125<br>**Telephone:** 614-836-8778<br><br>**Agency Mailing Address:**<br>Lazarus Government Center<br>P.O. Box 1049<br>Columbus, OH 43216-1049<br>**Agency Telephone:** 614-644-2001 | **E-Mail:**<br>info-request@www.epa.state.oh.us<br>**Web Sites:**<br>www.web.epa. ohio.gov<br>www.epa.state.oh.us | N/A |

(continued)

**Table 1-1.** Continued

| Biomonitoring Program | Program Contact | General E-Mail Contact and Web Sites | Publications Contact |
|---|---|---|---|
| MDNR-MBSS | **Ann Smith**<br>Monitoring and Nontidal Assessment Program of the Maryland Department of Natural Resources<br>**Telephone:** 410-260-8611<br>**E-mail:** asmith@dnr.state.md.us.<br><br>**Agency Mailing Address:**<br>Tawes State Office Building<br>580 Taylor Avenue<br>Annapolis, MD 21401 | **Web Sites:**<br>www.dnr.state.md.us/streams/ mbss/mbss_methods.html<br><br>www.nt2.versar. com/mbss/mbss. html | **Paul Miller**<br>Tawes State Office Building, C-2<br>MD Department of Natural Resources<br>**Address:**<br>580 Taylor Avenue<br>Annapolis, MD 21401<br>**Telephone:** 410- 260-8610<br>**E-mail:**<br>pmiller@dnr.state.md.us. |

Quality Assessment program (USGS-NAWQA), U.S. Environmental Protection Agency's Rapid Bioassessment Protocol (USEPA-RBP), Ohio Environmental Protection Agency's flowing waters program (Ohio EPA), and Maryland's Department of Natural Resources's Maryland Biological Stream Survey program (MDNR-MBSS). While the USEPA-EMAP-SW, USGS-NAWQA and USEPA-RBP programs are concerned with assessing rivers on the National and Regional levels, the Ohio EPA and MDNR-MBSS programs are concerned with assessing the rivers in their respective states. These differences in scale are reflected in the way each program developed and currently implements their protocols.

## 1.1 Boating and Wading Methods

The depth of flowing waters can be roughly characterized as boatable or wadeable. The methods used to assess the condition of these flowing waters may vary depending on their depth status. Because it is the goal of this document to help individuals understand the differences between the ways data are collected, this document distinguishes between boating and wading methods when they occur.

The primary focus of this document is the boating methods used to assess flowing waters, however, both boating and wading methods are included in this document for several reasons. First, most wading methods were developed before boating methods and boating methods are often derivations of the wading methods that preceded them. Often, the methods used while in boatable waters simply call for the wading methods to be used in shallow areas (e.g., near the shore) or in the boat without any additional modifications. The inclusion of the original (wading) method as well as the derived (boating) method may also help illustrate how methods can be modified in order to meet the specific requirements of a sampling agency. Another reason that both sets are included is that it may be necessary to use both wading and boating methods among sampling sites or within a single reach when a river has varying depths. Also, separate protocols specifically tailored for either boatable or wadeable streams are not available for all phases of all programs. Therefore, it is necessary to include the protocols that are available even if they are not specified as protocols for boatable streams. Finally, the inclusion of both sets of methods may help agencies or individuals analyze data sets which were collected using both wading and boating methods.

## 1.2 Overall Sampling Design Of Reviewed Programs

### *1.2.1 USEPA-EMAP-SW Methods*

The USEPA has designated EMAP-SW to develop the necessary monitoring tools that can determine the current status, extent, changes and trends in the condition of our Nation's ecological resources on regional and national scales (U.S. EPA 1998). The sampling framework for this program consists of 40-$km^2$ hexagons placed over a systematic triangular grid of approximately 12,500 points for the contiguous United States. The program's national design states that approximately 800 lakes and 800 streams are chosen from one quarter of the grid hexagons each year, giving a four-year resampling cycle. The field sampling sites are selected using statistical probability methods to ensure that robust population inferences can be made and that the sites represent the spatial distribution of lakes and streams (Overton et al. 1991). Sites are randomly selected by establishing size strata, to ensure an adequate characterization of larger lakes and streams.

The sampling period, or *index* period, for USEPA-EMAP-SW varies with the location and type of project being conducted. For the Mid-Atlantic Integrated Assessment (MAIA) project, a spring (April to June) index period was selected in 1993 and 1994. In 1997 and 1998, however, a summer (July to September) index period was selected, which coincided with the low flow period of streams in this research area.

The elementary sampling unit used by USEPA-EMAP-SW for biological, physical and chemical data collection is a length of stream 40 times the channel width. This length was derived from pilot studies that indicated this sampling approach was needed to collect 90% of the species in the stream reach. In streams less than four meters wide, a length of 150 m is used as a minimum sample reach length. No maximum reach length was established for boatable or wadeable streams. Reaches are laid out so that 50% of the survey area is upstream, and 50% of the survey area is downstream of the predetermined latitude and longitude of the study site.

A designated sample reach is divided into 10 subsections delineated by 11 transects spanning the width of the stream and labeled "A" through "K". The downstream endpoint of the sample reach is transect "A". Transect "B" is that point which is 1/10 (four channel widths in big streams or 15 m in small streams) of the designated stream length upstream from the start point (transect A) [Figure 1-1 shows a member of a field crew marking a transect at the proper distance from the previous transect.] When transect "B" is determined, a roll of a die is used to determine the location along the transect where sampling of certain indicators will take place. Options are a left(L), center(C), or right(R) sampling point. After the first random selection (transect *B*), sampling locations are assigned to each transect, alternating in order as L, C, or R. This process is repeated until the upstream extent of the sample reach is located (transect *K*).

**Figure 1-1.** A field crew member ties a flag in a tree to mark the a transect at the proper distance from the previous transect.

Ecological indicators included in the stream sampling program are physical habitat, water chemistry, periphyton/phytoplankton assemblages, sediment metabolism, benthic macroinvertebrate assemblages, aquatic vertebrate assemblages, fish tissue contaminants, and sediment toxicity. This document focuses on the water chemistry, physical habitat, and assemblage indicators only.

Physical habitat data are collected from each stream reach. Stressor indicators derived from the collected data are used to help explain or diagnose stream conditions relative to various indicators. Important attributes of physical habitat in streams are channel dimensions, gradient, substrate characteristics, habitat complexity and cover, riparian vegetation cover and structure, disturbance due to human activity, and channel-riparian interaction (Kaufmann 1993).

Water chemistry data are collected from each stream in order to measure a variety of physical and chemical analytes. Information from these analyses is used to evaluate stream condition with respect to stressors such as acidic deposition (mine drainage), nutrient enrichment, and other organic and inorganic contaminants.

Periphyton samples are collected from erosional and depositional habitats located at each of the nine interior cross-sectional transects (*B* through *J*). Four different types of laboratory samples are prepared: 1) an ID/enumeration sample to determine taxonomic composition and relative abundances, 2) a chlorophyll sample, 3) a biomass sample for ash-free dry mass, and 4) an acid/alkaline phosphatase activity sample. Benthic macroinvertebrates are collected using a modified kick net. A kick net sample is collected from each of the nine interior cross-sectional transects (*B* through *J*) at the sampling point (Left, Center, or Right) assigned when the location of the sampling reach is determined. Mussels and snails, within the kick net sample points, are hand-collected. In boatable streams, drift nets are also used to collect benthic macroinvertebrates.

Fish are sampled using a single-pass electrofishing method covering the determined reach length. Each pass of the electrofishing sampling has a duration of at least 45 minutes but does not exceed three hours. Herpetofauna observed in the course of electrofishing for fish are collected and identified to the species level.

The USEPA-EMAP-SW sampling methods are detailed in Lazorchak et al. (1998) for wadeable streams and Lazorchak et al. (1999 draft version) for large rivers. Boatable methods have been tested and refined in a pilot study in Mid-Atlantic states during 1997 and 1998 and Midwestern states during 1999.

### 1.2.2 USGS-NAWQA Methods

The objectives of the USGS-NAWQA program are to: 1) describe current water-quality conditions for a large part of the Nation's freshwater streams, rivers, and aquifers, 2) describe how water quality is changing over time, and 3) improve understanding of the primary natural and human factors that affect water-quality conditions (Fitzpatrick et al. 1998). Investigations are performed on a staggered time scale in 59 of the largest and most significant hydrologic systems in the country (Gilliom et al. 1995). Individual investigations are performed in *study units* and consist of four to five years of intensive assessment, which consists of a retrospective analysis, occurrence and distribution assessment, assessment of long-term trends and changes, and case studies of sources, transport, fate, and effects.

The USGS-NAWQA sampling design is modified from an approach used by Frissel et al. (1986) and includes four spatial scales: *basin*, *segment*, *reach*, and *microhabitat*. *Basins* refer to entire stream systems. *Segments* are streams bounded by confluences or chemical/ physical discontinuities. The *reach* scale includes individual pools and riffles within stream segments. *Microhabitat* data (e.g., velocity, substrate type and depth) are collected from the locations where invertebrate and algal samples are taken. Basin and segment data are collected using Geographic Information Systems (GIS), topographic maps, and aerial photographs but reach and microhabitat sampling require site visits. Procedures for the collection of reach data are described in later sections of this document. Procedures for collecting microhabitat data are described in the USGS-NAWQA protocols for the collection of invertebrates (Cuffney et al. 1993a) and algal samples (Porter et al. 1993).

Sampling sites are chosen to represent a set of important environmental variables in the Study Unit. Basic fixed sites are placed at or near USGS gaging stations where continuous discharge measurements are available. Synoptic sites may be nongaged sites where typically one-time measurements of a limited number of characteristics are made with the objective of answering a specific question. The purpose of a synoptic site is to answer questions regarding source, occurrence, or spatial distribution. Only one sampling reach is generally used to characterize a synoptic site (Gilliom et al. 1995).

The location of each sampling reach is usually related to a durable reference point such as a stream gage or bridge pier that is used to permanently define its location (Meador et al. 1993a). Sampling reaches are located where instream and riparian habitat conditions are representative of the local area and support USGS-NAWQA study-unit objectives. For example, sampling reaches should be representative of a specific land use, agricultural practice, or reference condition. In order to meet these objectives, the sampling reach may be located upstream, downstream, or adjacent to the site location as long as the water chemistry and hydrologic data collected at the site accurately reflect conditions within the sampling reach.

Sampling is conducted during low and stable-flow periods, usually mid-June to early October. These conditions increase the likelihood that samples throughout the study unit can be collected under similar flow conditions (Gilliom et al. 1995).

The primary determinant for the length of the sampling reach is the presence of repetitions of two geomorphic channel units, such as a sequence of pool, riffle, pool, riffle (Meador et al. 1993b). Other determinants for reach length are fish sampling considerations (Meador et al. 1993a). Only those geomorphic channel units (riffle, run, and pool) that cover more than 50% of the active channel width are considered when determining the length of the reach. If repetitions of geomorphic channel units are not present or are present at intervals of greater than 1,000 m (for example, in large rivers), the length of the reach is determined to be 20 channel widths based on the width of the channel at the boundary of the reach. Theoretically, this length will represent at least one complete meander wavelength (Leopold and Wolman 1957). Regardless of the method used to establish the length of the sampling reach, the minimum and maximum acceptable reach lengths are 500 and 1,000 m, respectively, for boatable sites; 150 and 300 m, respectively, for wadeable sites; and 150 and 500 m, respectively, for wadeable sites with stream widths greater than 30 m. Typically, a single sampling reach is established at each site, however, three sampling reaches are established at a subset of sites in order to assess variability among sampling reaches.

Ecological indicators included in the USGS-NAWQA stream sampling program are water chemistry, tissue contaminants, stream habitat, benthic and sestonic algal community samples, benthic invertebrate communities, and fish communities. This document focuses on the water chemistry, physical habitat, and community indicators only.

Stream habitat data are collected at each sample site to relate habitat to other physical, chemical, and biological factors to describe water-quality conditions. Data collected at each reach include measurements and observations of channel, bank, and riparian characteristics (Meador et al. 1993b).

Water chemistry data are collected using three levels of sampling and analytical intensity. These three levels are basic fixed-site assessment, intensive fixed-site assessment, and water column synoptic studies. The basic fixed-site assessment assesses a suite of analytes using continuous monitoring supplemented by fixed-interval and extreme-flow sampling. Intensive fixed-site assessments utilize a higher-frequency sampling scheme and add pesticides to the analytes. Water-column synoptic studies are short-term investigations specifically designed for a particular study unit.

Benthic algal communities are characterized by collecting qualitative and quantitative periphyton samples at each sampling location. In boatable streams, phytoplankton may be collected from the water column to characterize the sestonic algal community. Estimates of algal biomass (i.e., chlorophyll content and ash-free dry mass) are also optional measures of water-quality conditions (Porter et al. 1993).

Benthic invertebrates are characterized to develop a list of taxa within the associated stream reach and to determine the structure of benthic invertebrate communities within selected microhabitats of each reach. Benthic macroinvertebrates are qualitatively collected with a kick net, which may be supplemented with seines, visual collections, grab samples, and/or diver operated dome samplers if required by the stream's morphology. In addition, benthic invertebrates are collected semi-quantitatively from a measurable area of natural substrate. When the natural substrate is unsuitable for collection, artificial substrates may be used (Cuffney et al. 1993a, b).

Fish communities are characterized in order to relate fish community characteristics to physical, chemical, and other biological factors. A representative sample of the fish community is collected using electrofishing and/or seining, depending on the appropriateness of each method for the particular sampling site (Meador et al. 1993a). The USGS-NAWQA sampling methods are detailed in later sections.

### 1.2.3 USEPA-RBP Methods

The primary purpose of the USEPA-RBP is to provide state and local water-quality monitoring agencies with a practical technical reference for conducting cost-effective biological assessments of lotic systems (Barbour et al. 1999). The methods included are a synthesis of methods employed by various state water resource agencies. Therefore, the protocols do not contain a set sampling design.

The USEPA-RBP methods state that for assessment and monitoring, sites can either be *targeted* sites, which are relevant to special studies focusing on potential problems, or *random* sites, which provide information of the overall status or condition of the watershed, basin, or region. In a random or probabilistic sampling regime, stream characteristics may be highly dissimilar among the sites, but will provide a more accurate assessment of biological condition throughout the area than targeted designs. Most studies conducted by state water quality agencies for identification of problems and sensitive waters are done with a targeted design. Studies for aquatic life-use determination can be done with a random or targeted design (Barbour et al. 1999).

The recommended sampling season is mid to late summer, when stream and river flows are moderate to low, and less variable than during other seasons. The USEPA-RBP suggests that stream reach designations based on a fixed or proportional distance method are acceptable, and that decisions between the two methods should be based on the results of pilot studies (Barbour et al. 1999).

Suggested ecological indicators included in the USEPA-RBP are measurements of physicochemical parameters, as well as periphyton, benthic macroinvertebrate and fish communities (Barbour et al. 1999).

The habitat assessment protocols suggested by the USEPA-RBP include 13 metrics. Three of the metrics are used only at high gradient sites and three metrics are used only at low gradient sites. Therefore, only ten metrics are used at any one site. Each metric is assigned a score that ranges from 0 to 20 points. Each metric is scored by matching observations made of the entire sample segment with one of four established ranking categories. Higher index scores are associated with more pristine habitats.

The recommended water sampling methods are intended to provide a brief and easily-obtained analysis of water chemistry that can be completed in the field. The suggested assessment includes four quantitative measurements and four estimated measurements. The four estimated parameters are each assigned to a scoring category.

The objectives of the recommended RBP for periphyton assessment include assessment of biomass, identification of species and determination of the periphyton assemblages' biological condition. During periods of stable stream flow, periphyton are collected from all available microhabitats in the sampling reach in the approximate proportion each microhabitat occurs. Algal mats or other soft-bodied algal forms can be collected from depositional areas. For chlorophyll analyses, periphyton are scraped from fixed areas onto a glass fiber filter. Periphyton can be sampled by collecting from artificial substrates (periphytometers) that are placed in aquatic habitats and colonized over a period of time. Semi-quantitative assessments of benthic algal biomass and taxonomic composition can be made rapidly with a viewing bucket marked with a grid and biomass scoring system.

The USEPA-RBP recommend benthic macroinvertebrates be sampled using either a single habitat or a multiple habitat approach. In the single habitat approach, all riffle/run areas within a 100-m representative reach are candidates for sampling macroinvertebrates. Cobble substrate is sampled where it is the predominant habitat and alternative habitats are sampled when cobble is not the dominant substrate. Sampling begins at the downstream end of the reach and proceeds upstream using a 1-m, 500-µm mesh kick net. The stream is sampled two or three times at locations of varying velocity in the riffle. In the multiple habitat approach, all habitat types in a 100-m representative reach are sampled in the approximate proportion in which they are represented in the reach. Sampling begins at the downstream end of the reach and proceeds upstream using a D-frame, 500-µm mesh dip net. A total of 20 jabs or kicks are taken over the length of the reach.

The methods suggested by the USEPA-RBP for fish involves careful, standardized field collection, species identification and enumeration, and analyses using aggregated biological attributes. The suggested fish collection procedure is a multi-habitat approach for wadeable streams, which allows the sampling of habitats in relative proportion to their local availability. The USEPA-RBP endorses electro-fishing as the most comprehensive and effective single method for collecting stream fishes. Protocols suggest that collection efforts begin at a shallow riffle, or other physical barrier at the downstream limit of the sample reach, and terminate at a similar barrier at the upstream end of the reach.

### 1.2.4 Ohio EPA Methods

In order to monitor the state's aquatic resources, Ohio EPA uses an approach in which each basin has the potential to be studied for one field season during a five-year cycle. Each five-year study focuses intensively on the biological, physical and chemical conditions found within the chosen study basins. Study segments are identified based on criteria such as their potential to be threatened by current or projected local impacts or their potential for harboring unique or critical aquatic habitat and biota. The size of the stream study segment is adjusted based on the size of the stream and whether or not the stream is boatable. In general, monitoring is based on approximately a 500-m segment if the stream or river is boatable, a 150 to 200-m segment if the stream or river is wadeable or a headwater stream (<20 $mi^2$ of drainage area). Sampling is conducted during summer low flow months (June 15 to October 15) and the study areas are visited one to three times during the field season. The number of visits to a single study site depends on a variety of factors. Typically, headwater sites or impacted sites are sampled once in a field season and wadeable and boatable sites are sampled twice during a field season. The wadeable and boatable sites may be sampled three times in a field season if resources permit (OEPA 1988).

Ecological indicators included in Ohio EPA's stream sampling program include physical habitat, water chemistry, macroinvertebrate assemblages and fish assemblages.

The characterization of physical habitat in Ohio streams has been addressed through Ohio EPA's development of the Qualitative Habitat Evaluation Index (QHEI). This index was designed to provide an evaluation or estimate of habitat attributes that generally correspond to those physical factors that affect fish communities and other aquatic organisms. Important attributes of the QHEI include substrate, instream cover, channel morphology, riparian and bank condition, pool and riffle quality, and gradient (Rankin 1989).

Water-quality sampling and analysis are conducted to provide data which can be used to interpret the quality or condition of the water under investigation. Collected samples may be discrete or integrated grabs or composites. Composite samples are preferred to insure temporally representative samples. Discrete grab samples and integrated grabs are considered satisfactory under temporally uniform conditions (OEPA 1988). An additional method used to monitor water quality are continuous monitors. The monitors are set in areas to be modeled and on an availability basis. They provide information on a river or stream's temperature, pH, conductivity and dissolved oxygen (DO) level.

Macroinvertebrates are primarily sampled using Hester-Dendy artificial substrate samplers. Samplers (n=5) are ideally placed in runs and harvested after a six-week colonization period. In addition, macroinvertebrates are sampled qualitatively by kick-net sampling and/or hand-picking natural substrates for a period of at least 30 minutes and then until no new taxa are observed.

Fish are sampled in one, two or three single electrofishing passes of each sampling segment per season (OEPA 1988, 1989). Each of these sampling methods is discussed in greater detail during later sections.

### 1.2.5 MDNR-MBSS Methods

The MDNR-MBSS approach is designed to provide three years of full coverage of the state's 18 basins that contain headwater, non-tidal, first, second, and third order streams. Approximately 300, non-overlapping, 75-m stream segments are sampled each year. The streams are defined using 1:250,000 scale base maps and the segments are randomly selected using a lattice sampling approach in which the segments are stratified by year and basin. Within a stream order, the number of segments sampled per basin is proportional to the number of stream miles in the basin. A predetermined number of segments are selected from each basin and ranked in order of selection. Extra segments are selected as a contingency to the loss of sampling segments as a result of field conditions. If a basin contains a small number of sites, additional segments are selected to increase sample size (Roth et al. 1997a, b).

In each segment, seven components are monitored. Five components, *fish*, *herpetofauna*, *macrophytes*, *mussels*, and *habitat quality*, are sampled in the summer period (June 1 to September 30) and two components, *benthic invertebrates* and *water quality* are sampled in the spring period (March 1 to May 1). Fish and habitat measurements are taken during summer low flow conditions for three reasons: 1) spawning migration of fish is minimal in the summer; 2) low flow conditions are advantageous for electrofishing, and 3) low flow conditions provide an opportunity to assess the area and type of habitat available to fish communities at a time when habitat may be limiting. Benthic sampling is conducted in the spring when, according to Plafkin et al. (1989), macroinvertebrate assemblages are good indicators of ecosystem health (Roth et al. 1997b).

The MBSS qualitative habitat assessment method consists of 13 metrics. Each metric is scored by matching observations made of the sample segment to the one of four possible ranking categories that describe possible conditions. Each of the four ranking categories has a range of possible scores. The method is designed so that higher scores indicate more pristine habitats. No total index score is computed for the MDNR-MBSS habitat assessment. In addition to the 13 qualitative habitat assessment metrics, MDNR-MBSS makes an additional six quantitative habitat assessment measurements.

Chemical water samples are analyzed following U.S. EPA's Handbook of Standard Methods for Acid Deposition Studies (U.S. EPA 1987). Parameters analyzed include *pH*, *acid neutralizing capacity* (*ANC*), *conductivity*, *sulfate*, *nitrate*, and *dissolved organic carbon* (*DOC*). These variables are believed to describe basic water quality conditions with an emphasis on changes related to acidic deposition (Roth et al. 1997b).

Invertebrates are sampled using a "D" net, sampling one-$ft^2$ areas of all available habitats, for a total area of 20 $ft^2$ per 75-m stream segment. Fish are sampled in two electrofishing passes of each 75-m segment (Roth et al. 1997b). Detailed descriptions of the sampling methods are given in later sections.

# Section 2
# Habitat Assessment Methods

by
Bradley C. Autrey and Joseph P. Schubauer-Berigan

This section summarizes and evaluates the habitat assessment protocols of five agencies, USEPA-EMAP-SW, USGS-NAWQA, USEPA-RBP, Ohio EPA, and MDNR-MBSS. It begins with a description of the origin of the habitat indices most widely used by these agencies. Then the habitat assessment methods of each agency are summarized. Finally, the methods are compared and contrasted. The USGS-NAWQA and MDNR-MBSS sections differ from the other agencies' sections because USGS-NAWQA and MBSS do not compute an index value from the recorded metrics. Instead, many metrics are used to determine whether relationships exist among the habitat variables or if any relationships exist between habitat variables and dependent variables such as fish, invertebrate, or periphyton assemblages. These relationships are then examined to determine what they indicate about stream quality.

## 2.1 Development of Habitat Assessment Methods

The methods used by the USEPA-EMAP-SW, USGS-NAWQA, USEPA-RBP, Ohio EPA, and MDNR-MBSS were each developed to meet the objectives of their respective programs. The way in which each of these protocols was developed reflects the differences and the similarities among these agencies (e.g., their spatial scales and objectives). Figure 2-1 shows a member of a field crew making a physical habitat measurement.

### *2.1.1 USEPA-EMAP-SW*

The USEPA-EMAP-SW's habitat assessment protocols were developed by Kaufmann (1993) and Kaufman and Robison (1998) for wadeable streams and Kaufmann

**Figure 2-1.** A field crew member measures canopy density by using a densiometer.

(Lazorchak et al. 1999 draft version) for boatable rivers. Both sets of protocols use a randomized, systematic spatial sampling design which minimizes bias in the placement and positioning of measurements (Lazorchak et al. 1998, 1999 draft version).

### 2.1.2 USGS-NAWQA

The USGS-NAWQA habitat assessment protocols were developed by Meador et al. (1993b) and were revised by Fitzpatrick et al. (1998). The stratification in USGS-NAWQA's habitat sampling design is a modification of Frissell et al. (1986). In addition, microhabitat assessment protocols were developed by Cuffney et al. (1993a) in conjunction with protocols for the collection of invertebrates and by Porter et al. (1993) in conjunction with protocols for the collection of algae (Fitzpatrick et al. 1998). These microhabitat assessment protocols are not addressed in this document.

### 2.1.3 USEPA-RBP

Barbour et al. (1999) state that the USEPA-RBP methods for habitat assessment are derived from the *Wisconsin Stream Classification Guidelines* (Ball 1982) and *Methods of Evaluating Stream, Riparian, and Biotic Conditions* (Platts et al. 1983).

### 2.1.4 Ohio EPA

The (QHEI) which is currently used by Ohio EPA was developed by Rankin (1989, 1991, 1995). The development of the index was based on six broad metrics: substrate, instream cover, channel morphology, riparian and bank condition, pool and riffle quality, and gradient. These metrics are used because they have been shown to be correlated with stream fish communities (Rankin 1989).

### 2.1.5 MDNR-MBSS

The MDNR-MBSS qualitative habitat assessment methods were developed by

Kazyak (1995). Initial development was based on the USEPA-RBP (Barbour and Stribling 1991) and Ohio EPA's QHEI (OEPA 1988, Rankin 1989). Additional metrics were included in order to meet the specific objectives of MDNR-MBSS (Roth et al. 1997b).

## 2.2 U.S. EPA-EMAP-SW Habitat Assessment Index

The primary habitat assessment techniques used by USEPA-EMAP-SW are the Rapid Habitat Assessment (RHA) index and the Physical Habitat (PHab) assessment. In addition to the RHA and the PHab, supplemental habitat parameters are measured which enable a more complete stream characterization. These separate sets of metrics are not combined into a single habitat assessment score (Kaufmann and Robison 1998).

### *2.2.1 USEPA-EMAP-SW RHA Index*

The RHA index contains 12 metrics (Table 2-1) which are defined in Appendix A (Kaufmann and Robison 1998). Each metric is assigned a score that ranges from 0 to 20

**Table 2-1.** The Metrics and Scoring For The USEPA-EMAP-SW RHA Index.

| Metric | Description[a] | Score |
|---|---|---|
| Instream cover | Amount and diversity of useable fish cover | 0-20 |
| Epifaunal substrate | Presence and size of riffles and amount of cobble substrate present | 0-20 |
| Velocity/depth regimes | Variety of velocity/depth regimes | 0-20 |
| Frequency of riffles | Frequency of riffles and the variety of habitat | 0-20 |
| Channel alteration | Type and amount of channel alteration | 0-20 |
| Bank condition | Bank stability and erosion | 0-20 |
| Embeddedness | Percentage of gravel, cobble, and boulders that are covered by sediment | 0-20 |
| Channel flow status | The degree to which water fills the channel | 0-20 |
| Riparian vegetation zone | Width of the riparian zone and the presence of human disturbances | 0-20 |
| Sediment deposition | Degree of bar development and effect of sedimentation on the channel | 0-20 |
| Bank vegetation protection | Percentage of stream bank surfaces covered by vegetation | 0-20 |
| Grazing/disruptive pressure | Degree of vegetative disruption by mowing or grazing on the banks | 0-20 |

[a]Complete descriptions are given in Appendix A.

points. Scores for each metric are determined by matching observations made of the entire sample segment with one of four established ranking categories. These ranking categories each contain descriptions of the respective metric and the observer chooses the category with the characteristics that most closely matches the observations. Each of the four ranking categories has a range of possible scores (e.g., Optimal 20-16; Sub-Optimal 15-11; Marginal 10-6; Poor 5-0). The index is designed so that higher scores indicate a more pristine habitat. A maximum index score of 240 points is possible.

### 2.2.2 USEPA-EMAP-SW-PHab Assessment

The PHab assessment is made up of four metrics, each with a number of sub-metrics (Lazorchak et al. 1998). Many of these sub-metrics are based on quantitative field measurements while others are based on ranked categories of field measurements (Table 2-2). All PHab metrics and sub-metrics are defined in Appendix A. The measurements made from the PHab assessment are not incorporated into an overall score.

### 2.2.3 Additional Habitat Parameters

In addition to the RHA index and PHab assessment metrics, USEPA-EMAP-SW protocols measure five supplemental habitat parameters. Two of the habitat parameters, *general assessment* and *local anecdotal information*, are text descriptions (Table 2-3). The three remaining parameters are based on ranked categories of field measurements and classified lists of field observations (Table 2-3). No scores are assigned to any of the parameters. Like the measurements for the PHab, it is unclear how these measurements are used in analysis. It is possible that the classified habitat information could be used to ground truth GIS data layers, but that is not directed by the protocols.

## 2.3 USGS-NAWQA Habitat Assessment Protocol

The goal of the USGS-NAWQA stream habitat protocol (Meador et al. 1993b) is to measure habitat characteristics that are essential in describing and interpreting water chemistry and biological conditions in the different types of streams studied by USGS-NAWQA. A basic overview of this sampling program is contained in section 1.2 of this document.

### 2.3.1 Habitat Sampling Design

The USGS-NAWQA assesses habitat conditions in four spatial scales, *basin*, *segment*, *reach*, and *microhabitat* (Fitzpatrick et al. 1998). The basin serves as a fundamental ecosystem unit and an important perspective from which to understand the characteristics of streams. A segment is a length of stream that has relatively homogeneous physical, chemical, and biological properties. A reach is a sampling unit within the segment. Physical, chemical, and biological data are collected from the reach. The microhabitat scale provides information on patterns of relations between biota and habitat with a fine-scale resolution. Procedures for collection of microhabitat data (e.g., velocity, substrate type, and depth) are described in the USGS-NAWQA protocols for the collection of invertebrate and algal samples (Cuffney et al. 1993a; Porter et al. 1993) and will not be described in this document.

**Table 2-2.** Metrics And Scoring Used In The PHab Assessment.

| Metric | Sub-metric | Scoring |
|---|---|---|
| Thalweg profile | Thalweg depth | Meters |
| | Wetted width | Meters |
| | Bar width | Meters |
| | Soft/Small sediment | Present/absent |
| | Side channel presence | Present/absent |
| | Channel unit code | 11 categories |
| | Pool form code | Seven categories |
| Large woody debris (LWD) tally | Total number of LWD | Sum |
| | Class of each LWD | 12 categories |
| Channel/riparian cross-section | Slope | Meters/kilometer |
| | Bearing | 0-360$^0$ |
| | Substrate size class | 11 categories |
| | Bank angle | 0-90$^0$ |
| | Undercut distance | Meters |
| | Wetted width | Meters |
| | Bankfull channel width | Meters |
| | Exposed mid-channel bar width | Meters |
| | Incised height | Estimated meters |
| | Bankfull flow height | Estimated meters |
| | Canopy density | Percent |
| | Dominant canopy vegetation | Five categories |
| | Areal cover class of large trees | Five categories |
| | Areal cover class of small trees | Five categories |
| | Dominant understory vegetation | Five categories |
| | Area cover of understory | Five categories |
| | Areal cover of ground cover | Five categories |
| | Type of instream fish cover | Eight categories |
| | Areal cover of fish cover | Five categories |
| | Presence of human influences | Four categories |
| | Discharge | Velocity[a] Meters/ second |

[a]The velocity-area method, timed filling method, and neutral buoyant object method are used for large, medium, and small streams, respectively.

Basin and segment assessments for fixed or synoptic sites are conducted using GIS, topographic maps, or aerial photographs (Tables 2-4, 2-5). Site visits are needed to collect the data for reach and microhabitat assessments. At a subset of fixed sites, reach data are collected from multiple reaches and during the base flow stage of different years (Table 2-6).

## 2.3.2 Basin Characterization

Basin characterization consists of geomorphic descriptors derived from USGS 7.5-minute topographic maps, climate and potential runoff characteristics, streamflow characteristics, and land-cover data from thematic maps. Climate descriptors used by USGS-

**Table 2-3.** Additional Parameters Used For The USEPA-EMAP-SW Protocols.

| Parameter | Sub-parameter | Scoring |
|---|---|---|
| Watershed activities and disturbances observed | Residential | Seven categories[a] |
| | Recreational | Four categories[a] |
| | Agricultural | Six categories[a] |
| | Industrial | Eight categories[a] |
| | Stream management | Eight categories[a] |
| Reach characteristics | Vegetation cover type | Six categories[b] |
| | Land use/type | Four categories[b] |
| | Water clarity | Four categories[b] |
| Waterbody character | Pristine | Five categories[c] |
| | Appealing | Five categories[c] |
| General assessment | Wildlife | Text |
| | Vegetation diversity | Text |
| | Forest age class | Text |
| Local anecdotal information | None | Text |

[a]Categories are examples of typical disturbances and each is recorded as *none*, *low*, *moderate*, or *high*.
[b]Each category is recorded as rare (<5%), sparse (5-25%), moderate (25-75%), or extensive (>75%).
[c]Categories are ranks, one to five, with one being the least pristine/appealing and five being the most pristine/appealing.

NAWQA include precipitation, temperature, evaporation, and runoff. At least three types of streamflow characteristics of a basin are useful: estimated peak flow, flood volume, and seven-day low flow for given recurrence intervals. Thematic maps of ecoregion, physiographic province, geology, soils, land use, and vegetation are also used to describe a basin. The *Basinsoft* computer program (Harvey and Eash 1996) has been developed by the USGS to quantify basin characteristics (Table 2-4).

### 2.3.3 Segment Characterization

The USGS-NAWQA protocols measure segment characteristics in the categories of *gradient*, *sinuosity*, and *water-management features* (Fitzpatrick et al. 1998). The parameters measured within these categories are given in Table 2-5.

### 2.3.4 Reach Characterization

The selection of the sampling reach is based on four criteria, *stream width*, *stream depth*, *geomorphology*, and *local habitat disturbance*. In general, the reach length is determined by multiplying the mean wetted channel width by 20. For boatable streams, recommended minimum and maximum stream lengths are 500 and 1,000 m, respectively. The minimum and maximum reach lengths for wadeable streams are 150 and 300 m, respectively. If possible, the reach should contain at least two examples of two habitat types from the categories of *pools*, *runs*, or *riffles*. At the beginning of data collection, the

| Table 2-4. The USGS-NAWQA Parameters Recorded For Basin Characterization. | | |
|---|---|---|
| Parameter | Description | Units |
| Drainage area | Delineated area enclosed by a drainage divide | $km^2$ |
| Average annual runoff | Average amount of water contributed through runoff | cm |
| Average annual air temperature | Average ambient air temperature | ºC |
| Average annual precipitation | Average precipitation | cm |
| Average annual evaporation | Average surface evaporation | cm |
| Basin length | Length of entire basin | km |
| Minimum elevation | Minimum elevation within the basin | m |
| Maximum elevation | Maximum elevation within the basin | m |
| Basin relief ratio | The difference between maximum and minimum elevation divided by basin length | m/km |
| Drainage shape | Drainage area divided by the square of the basin length | $km^2/km^2$ |
| Stream length | The distance from the headwaters to the site | km |
| Cumulative perennial stream length | The cumulative length of all perennial streams and canals in the basin | km |
| Drainage density | The cumulative perennial stream length divided by the basin area | $km^{-1}$ |
| Drainage texture | The number of crenulations on the most crenulated contour line divided by the basin perimeter length | contours/km |
| Entire stream gradient | Difference between elevations at 85 and 10% of the stream length divided by the distance between those points | m/km |
| Estimated flow characteristics | Estimated peak flow, flood volume, and seven-day low flow | - |

general condition of the reach is noted and 11 equidistant transects are established throughout the reach. The transects are established so that habitat characteristics are statistically represented within the reach and observer bias is eliminated. The parameters measured within the reach provide information on channel, bank, and riparian characteristics. These parameters are given in Table 2-6.

## 2.4 USEPA-RBP Habitat Assessment Index

The index suggested by the USEPA-RBP consists of 13 metrics (Barbour et al.

**Table 2-5.** The USGS-NAWQA Parameters Measured For Segment Characterization.

| Parameter | Description | Units |
|---|---|---|
| Segment length | Straight-line length of the segment | km |
| Curvilinear channel length | Length of the main channel through the segment | km |
| Upstream and downstream elevation | Elevation at upstream and downstream boundaries | m |
| Sinuosity | Curvilinear channel length divided by segment length | km/km |
| Segment gradient | Upstream elevation minus downstream elevation, divided by segment length | m/km |
| Water management feature | Type(s) of water management feature(s) likely to influence segment habitat | 21 categories[a] |
| Strahler stream order | Stream order | Numerical |
| Link | Sum of the orders for all upstream tributaries | Numerical |
| Downstream link | Sum of the orders for tributaries contributing to the next downstream segment | Numerical |
| Valley sideslope gradient | The average of three representative gradient calculations based on a cross-sectional profile of the segment valley. | m/km |

[a]The categories of water management features are *bridge, diversion, return flow, stp > 5 (more than 5 sewage treatment plants), ips > 5 (more than 5 industrial point sources), impoundment, low-head dam, natural lake, bank stabilizer, tile drain, none, channelized, feedlot, sewage treatment, gw inflow, hydropower, industrial, mining, storm sewer, thermal, and other*.

1999) (Table 2-7) (see Appendix A). Three of the metrics, *embeddedness, frequency of riffles, and velocity/depth combinations*, are used only at high gradient sites, and three of the metrics, *pool substrate, pool variability, and channel sinuosity*, are used only at low gradient sites. Therefore, only ten metrics are used at any one site. Each metric is assigned a score ranging from 0 to 20 points (Table 2-7). The metrics *bank stability, bank vegetation protection, and riparian vegetation zone width*, are assigned a score ranging from 0 to 10 points for each bank (0 to 20 points for both banks combined). Each metric is scored by matching observations made of the entire sample segment with one of four established ranking categories. The chosen categories should contain the characteristics that most closely match the observations. Each of the four ranking categories has a range of possible scores (e.g., Optimal 20-16; Sub-Optimal 15-11; Marginal 10-6; Poor 5-0). Higher index scores are associated with more pristine habitats. The maximum index score is 200.

**Table 2-6.** USGS-NAWQA Parameters For Reach Characterization.

| Parameter | Description | Units |
|---|---|---|
| **For the reach** | | |
| Stage | Water level at a fixed point | m |
| Instantaneous discharge | Flow of the stream | L/s |
| Channel modification | Any channel modification at the reach is noted | Seven categories[a] at reach |
| Mean channel width | The average of three representative measurements of wetted channel width | m |
| Curvilinear reach length | Length of reach measured through channel | m |
| Distance between transects | The reach length divided by ten | m |
| Curvilinear distance from site to reach ends | Distance along the channel from a reference location to the upstream and reference downstream reach boundaries | m |
| Reach water-surface gradient | Difference between the water surface elevations at both ends of the reach, divided by the reach length | m/m |
| Geomorphic channel units | The length of all riffles, runs, and pools that make up more than 50% of channel width are recorded | m and type |
| **For each of the 11 transects** | | |
| Habitat type | Whether the transect is located in a riffle, run, or pool | Three categories |
| Wetted channel width | Width from the left edge of the water to the right edge of the water, excluding bars, shelves, or islands | m |
| Bankfull channel width | Width from the top edge of the left bank to the top edge of the right bank | m |
| Channel features | Width of channel bars, shelves, or islands | m and type |
| Aspect | Compass heading of downstream flow | 0 to $360^0$ |
| Canopy angles | Sum of the angles from the middle of the transect to the visible horizons on the left and right banks, subtracted from $180^0$ | 0 to $180^0$ |
| Riparian canopy closure | The portion of the overhead view that includes vegetation | 0 to100% |

(continued)

[a]Choose from 1) *natural woody debris pile*, 2) *overhanging vegetation (terrestrial)*, 3) *undercut banks*, 4) *boulders*, 5) *aquatic macrophytes*, 6) *manmade structure*, 7) *too turbid to determine*, or 8) *none*.

**Table 2-6.** Continued

| Parameter | Description | Units |
|---|---|---|
| Dominant riparian land use | Land use within an approximate 30-m distance from the top bank | 12 categories[b] |
| Bank angle | Angle formed by the bank at the stream bottom | 0 to $90^{0}$ [c] |
| Bank height | Vertical distance from channel bed to the top of the bank | m |
| Bank substrate | Type of dominant bank substrate | Ten categories[d] |
| Bank vegetative cover | Visual estimation of percentage of bank covered in vegetation | 0 to 100% |
| Bank erosion | Presence or absence of bank erosion at each end of transect | Present/absent |
| Habitat cover features | Presence or absence of any mineral or organic matter that produces shelter for aquatic organisms | Present/absent in eight categories[e] |
| Depth | Water depth from water surface to stream bed | m |
| Velocity | Velocity at 60% depth when depth is less than 1 m, or average velocity at 20 and 80% depth when depth is more than 1 m. | m/s |
| Dominant bed substrate | Type of dominant bed substrate | Ten categories[d] |
| Embeddedness | The estimated portion five large substrate particles that are surrounded or covered by fine-grained sediment | 0 to 100% |
| Silt present | The presence or absence of significant amounts of silt | Present/absent |

[b] Choose from 1) *cropland*, 2) *pasture*, 3) *farmstead/barnyard*, 4) *silviculture*, 5) *urban* residential/*commercial*, 6) *urban industrial*, 7) *rural residential*, 8) *right-of-way*, 9) *grassland*, 10) *shrubs/woodland*, 11) *wetlands*, or 12) *other*.
[c] Measurement may be greater than $90^{0}$ if the bank is undercut.
[d] Choose from one of 1) *smooth bedrock/concrete/hardpan* , 2) *silt/clay/marl/muck/organic detritus*, 3) *sand (0.063-2 mm)*, 4) *fine/medium gravel (2-16 mm)*, 5) *coarse gravel (16-32 mm)*, 6) *very coarse gravel (32-64 mm)*, 7) *small cobble (64-128 mm)*, 8) *large cobble (128-256 mm)*, 9) *small boulder (256-512 mm)*, or 10) *large boulder/irregular bedrock/irregular hardpan/irregular artificial surface (>512 mm)*.

**Table 2-7.** The Metrics and Scoring used in the USEPA-RBP'S Habitat Assessment Index.

| Metric | Scoring |
|---|---|
| Epifaunal substrate/ available cover | 0-20 |
| Channel alteration | 0-20 |
| Bank stability | 0-10 (per bank) |
| Channel flow status | 0-20 |
| Riparian vegetative zone width | 0-10 (per bank) |
| Sediment deposition | 0-20 |
| Bank vegetative protection | 0-10 (per bank) |
| Velocity/Depth combinations - (high gradient) | 0-20 |
| Frequency of riffles - (high gradient) | 0-20 |
| Embeddedness - (high gradient) | 0-20 |
| Pool substrate - (low gradient) | 0-20 |
| Pool variability - (low gradient) | 0-20 |
| Channel Sinuosity - (low gradient) | 0-20 |

## 2.5 Ohio EPA'S Qualitative Habitat Evaluation Index (QHEI)

The QHEI (Rankin 1989) consists of seven metrics, six of which are made up of two to four scored sub-metrics (Table 2-8). Each sub-metric is further divided into sub-categories which are used to determine the sub-metric scores (Tables 2-8, 2-9). To compute a final score for the QHEI, the scores of the sub-metrics are summed and the scores of the seven metrics are summed. The maximum score for the QHEI is 100 (Table 2-8). A habitat quality ranking scheme has been produced by Ohio EPA based on the overall QHEI score (Table 2-10). According to Rankin (1989), three metrics, *pool quality*, *channel quality*, and *substrate quality*, are consistently correlated with the fish IBI in Ohio. In contrast, *riparian zone quality* is found to be less correlated with the fish IBI in Ohio (Rankin 1989). Because the scores among the metric categories are different, the overall index score is weighted to give different metrics varying importance. The metrics *substrate* and *instream cover*, by virtue of the way they are designed, can have a maximum value greater than 20 points. If, as a result of the field measurements they are scored above 20 points, the final scores must be truncated to 20. Nine additional observations that are either not scored or not used in the final cumulative scoring, are recorded while performing a QHEI. These additional observations are given in Table 2-11.

## 2.6 MDNR-MBSS Habitat Assessment Method

The habitat assessment methods used by MDNR-MBSS include a habitat assessment protocol very similar to the USEPA-RBP's habitat assessment protocol and the USEPA-EMAP-SW RHA. It also includes a group of nine, generally quantitative, additional measurements that are similar to a number of those performed for the USEPA-EMAP-SW PHab (Table 2-2). Currently, no method exists for incorporating these separate measurements into a single habitat assessment score.

### *2.6.1 Qualitative Habitat Assessment*

The MBSS qualitative habitat assessment method (Roth et al. 1997b) consists of 13

**Table 2-8.** The Metrics, Sub-metrics, and Scoring Ranges for the Ohio EPA'S QHEI.

| Metric | Sub-metric | Sub-metric scoring range | Maximum metric score |
|---|---|---|---|
| Substrate | Type | 0 to 22 | 20[a] |
| | Quality | -7 to 4 | |
| Instream cover | Type | 0 to 10 | 20[a] |
| | Amount | 1 to 11 | |
| Channel Morphology | Sinuosity | 1 to 4 | 20 |
| | Development | 1 to 7 | |
| | Channelization | 1 to 6 | |
| | Stability | 1 to 3 | |
| Riparian zone/bank erosion | Flood plain width | 0 to 4 | 10 |
| | Flood plain quality | 0 to 3 | |
| | Bank erosion | 1 to 3 | |
| Pool/Glide Quality | Pool maximum depth | 0 to 6 | 12 |
| | Current type | -4 to 4 | |
| | Pool morphology | 0 to 2 | |
| Riffle/run quality | Depth | 0 to 4 | 8 |
| | Substrate stability | 0 to 2 | |
| | Embeddedness | -1 to 2 | |
| Gradient (scaled by ft/mi) | | 2 to 10 | 10 |
| QHEI Overall | | | 100 |

[a]If the sum of the sub-metric scores exceeds 20, the metric score is truncated to 20.

**Table 2-9.** An Example of the Metric Scoring Method used by the QHEI.

| Composite metric | Sub-metric | Scoring categories | Scores |
|---|---|---|---|
| Riffle quality | Riffle/ run depth | Generally, >10 cm deep, >50-cm maximum depth | 4 |
| | | Generally, >10 cm deep, <50-cm maximum depth | 3 |
| | | Generally, 5-10 cm deep | 2 |
| | | Generally, <5cm deep | 1 |
| | Riffle/run substrate | Stable (e.g., cobble, boulder) | 2 |
| | | Moderately Stable (e.g., pea gravel) | 1 |
| | | Unstable (e.g., gravel, sand) | 0 |
| | Embeddedness | None | 2 |
| | | Moderate | 1 |
| | | Low | 0 |
| | | Extensive | -1 |

**Table 2-10.** Habitat Quality Rankings Developed by the Ohio EPA for QHEI Score Evaluation.

| Habitat quality ranking | QHEI score range |
|---|---|
| Very Poor | 0 - 40 |
| Poor | 41-50 |
| Fair | 51-60 |
| Good | 61-70 |
| Very Good | 71-80 |
| Excellent | 81-90 |
| Extraordinary | 91-100 |

**Table 2-11.** Observations Recorded in Addition to the QHEI Parameters.

| Observation | How recorded |
|---|---|
| Additional comments/ pollution impacts | Text |
| Sampling gear/ distance sampled | Type of fishing gear used/length of sampling reach |
| Water clarity | Clear, stained, or turbid |
| Water stage | Meters |
| Canopy | Percent of sampling site not shaded or covered by woody bank vegetation |
| Gradient | Very low, low, low-moderate, moderate, moderate-high, high, or very high |
| Length, width, and maximum depth at sampling sites | Meters |
| Stream diagram: cross sections | Two or three drawings of the stream cross section |
| Stream map | Sketch of the entire sampling section |

metrics (Table 2-12, Appendix A). Each metric is scored by matching observations made of the sample segment to one of four possible ranking categories that best describes observed conditions. Each of the four ranking categories has a range of possible scores. The method is designed so that higher scores indicate more pristine habitats. Nine of the metrics are evaluated in this fashion and assigned a score ranging from 0 to 20 points. However, three of the metrics, *embeddedness*, *channel flow status*, and *shading* are given percentage scores and one of the metrics, *riparian buffer*, is given a score in meters (Table 2-12). No total index score is computed for the MDNR-MBSS habitat assessment. In addition to the qualitative habitat assessment metrics (Table 2-12), MDNR-MBSS makes these quantitative habitat assessment measurements:

- Maximum depth
- Stream gradient
- Wetted width
- Straight-line segment length
- Overbank flood height
- Discharge

## 2.7 Differences and Similarities Between the Habitat Assessment Methods

The methods of the various agencies differ in the type, number, and scoring of metrics. This section addresses these differences and the similarities among the five methods.

**Table 2-12.** Metrics used in the MDNR-MBSS Qualitative Habitat Assessment Method.

| Metric | Description | How scored |
|---|---|---|
| Instream habitat structure | Perceived value of habitat based on its type and structure | 0-20 |
| Epifaunal substrate | Amount/variety of hard, stable substrates for benthic invertebrates | 0-20 |
| Velocity/depth diversity | Variety of velocity/depth regimes | 0-20 |
| Pool/glide/eddy quality | Variety and spatial complexity of slow or still water habitat | 0-20 |
| Riffle quality | Complexity and functional importance of riffle/run habitat | 0-20 |
| Channel alteration | Degree and type of channel alteration | 0-20 |
| Bank stability | Presence of vegetation or other bank stabilizing material | 0-20 |
| Aesthetic rating | Visual appeal of site, presence of human refuse, degree of channelization, and vegetation disturbance | 0-20 |
| Remoteness rating | Presence of detectable human activity and accessability of site | 0-20 |
| Embeddedness | Percentage of stream gravel, cobble, and boulder surface area not surrounded by fine sediment | 0-100% |
| Channel flow status | Percentage of stream channel that has water | 0-100% |
| Shading | Percentage of the site that is shaded | 0-100% |
| Riparian buffer | Minimum width of vegetated buffer (50 m maximum) | meters |
| Total | | 0-180 |

### 2.7.1 The USEPA-EMAP-SW RHA and the USEPA-RBP Habitat Assessment Indices

The USEPA-EMAP-SW RHA and the USEPA-RBP indices are very similar in their composition. Ten of the 12 RHA index metrics are either very similar or directly comparable to USEPA-RBP metrics.

These ten metrics are:

- epifaunal substrate
- velocity/depth regimes
- frequency of riffles
- channel alteration
- bank condition or stability
- embeddedness
- channel flow status
- riparian vegetation zone
- sediment deposition

• bank vegetation protection

The RHA index has two metrics, *instream cover* and *grazing/disruptive pressure*, that are not included in the USEPA-RBP index and the USEPA-RBP index has three metrics, *channel sinuosity*, *pool variability*, and *pool substrate*, that are not used by the RHA index. The criteria used to evaluate the two metrics, *instream cover* and *epifaunal substrate*, by the RHA index are combined into one metric, *epifaunal substrate*, by the USEPA-RBP index. Whereas all 12 of the RHA index metrics are scored for every sample stream segment, only ten of the 13 USEPA-RBP index metrics are scored for a sample segment. Three of the USEPA-RBP metrics, *embeddedness*, *frequency of riffles*, and *velocity/depth combinations*, are used only at high gradient sites, and three of the USEPA-RBP metrics, *pool substrate*, *pool variability*, and *channel sinuosity*, are used only at low gradient sites. Finally, one major difference between the USEPA-RBP index and the overall USEPA-EMAP-SW habitat assessment methods is that the USEPA-EMAP-SW habitat assessment method includes two additional components, the PHab and additional assessment parameters (Tables 2-2, 2-3). These additional elements provide quantitative measurements of parameters such as channel sinuosity and discharge that are qualitatively assessed by the USEPA-RBP index.

### 2.7.2 The MDNR-MBSS Qualitative Habitat Assessment Protocols and the Other Programs

Maryland's MBSS qualitative habitat assessment protocols were partially derived from the USEPA-RBP index and are, therefore, similar to both the RHA and USEPA-RBP indices (Table 2-12). The MDNR-MBSS qualitative habitat assessment protocols have seven metrics, *epifaunal substrate*, *velocity/depth diversity*, *channel alteration*, *bank stability*, *embeddedness*, *channel flow status*, and *riparian buffer*, with similar or identical evaluation criteria to USEPA-RBP metrics. Six metrics, *instream cover*, *pool/glide/eddy quality*, *riffle quality*, *shading*, *aesthetic rating*, and *remoteness rating*, are included in the MDNR-MBSS qualitative habitat assessment protocols, but not in the USEPA-RBP index. Also, the USEPA-RBP index contains six metrics, *pool substrate*, *pool variability*, *frequency of riffles*, *sediment deposition*, *bank vegetation protection*, and *channel sinuosity*, that are not used in the MDNR-MBSS qualitative habitat assessment protocols. As with the RHA index, the MDNR-MBSS qualitative habitat assessment separates the evaluation criteria used in USEPA-RBP *epifaunal substrate* metric into two metrics, *instream cover* and *epifaunal substrate*, and all of the metrics are scored for every stream segment, regardless of the gradient level. Unlike the RHA and the USEPA-RBP, which only evaluate the riparian buffer to 18 m on each bank, the MDNR-MBSS qualitative protocols measure the riparian zone to a distance of 50 m on each bank. The MDNR-MBSS protocols, like those of the USEPA-EMAP-SW, make a number of additional quantitative measurements of the stream segment physical features (Section 2.6.1) as well as categorizing the adjacent land use. The data from the two components of the MDNR-MBSS protocols are not incorporated into an overall habitat score.

MBSS is unique in that it is the only program that identifies instream submerged aquatic vegetation (SAV), emergent aquatic vegetation (EAV), and riparian vegetation to species (USEPA-EMAP-SW uses vegetation categories and the Ohio EPA QHEI only addresses vegetation in terms of percent cover). Aquatic plants are also not sampled concomitantly with the standard Ohio EPA stream habitat and biotic assessment sampling.

### 2.7.3 Ohio EPA QHEI and the Other Programs

The Ohio EPA QHEI is the most unique of the indices reviewed. Substantial differences exist between the scoring system and metric definitions in the QHEI and in the other four indices. The scoring categories of the QHEI metrics are not grouped like the other indices, but rather individual scores are assigned to numerous scoring categories which are part of metrics or sub-metrics (Table 2-8). Each metric and sub-metric is uniquely designed and consists of varying numbers of scoring categories. The individual scoring categories range in the number of points assigned to each category and are, therefore, not equally weighted. Some of the QHEI metrics can have total scores greater than the maximum scores permitted for those metrics. If the total exceeds the maximum score for the metric, the score is truncated to the maximum score value. The QHEI is similar to the USEPA-RBP, RHA methods, and the MDNR-MBSS assessment methods in that it qualitatively assesses some of the major features of stream structure related to the quality of stream habitat. These structural features include *substrate*, *instream cover*, *physical channel features*, and *flow regime*. Unlike the other protocols, the QHEI has established habitat quality ranking standards based upon index scores.

### 2.7.4 USGS-NAWQA and the Other Programs

One of the primary differences between the methods used to characterize habitat for the USGS-NAWQA and those used by the other four agencies is that NAWQA has extensive characterization of the habitat on four spatial scales, *basin*, *segment*, *reach*, and *microhabitat* (Tables 2-4, 2-5, and 2-6). The protocols for USGS-NAWQA are unique also because there is no formal index score calculated. The program instead focuses on the use of repeatable, quantitative data in order to produce nationally-consistent stream quality evaluations and the use of additional qualitative data for the generation of qualitative indices where applicable (Fitzpatrick et al. 1998).

### 2.7.5 Broad Scale Differences Among the Habitat Assessment Methods Used by the Five Reviewed Programs

Contrasting the assessment methods used by USEPA-RBP and USEPA-EMAP-SW and those used by Ohio EPA and MDNR-MBSS reveals a number of differences between these sampling methods. Differences exist at the broad scale in dealing with study site identification and assessment of the status of the aquatic resources. Also, differences exist at the local scale in the methods used to collect data. At the broad scale, identification of the MDNR-MBSS and USEPA-EMAP-SW sampling sites is accomplished using statistically-based sampling designs. However, no statistical designs are used by Ohio EPA or USEPA-RBP to identify the study segments. In its first nine-year cycle, USGS-NAWQA used a common sampling design for 59 of the most environmentally significant watersheds in the nation. It uses a design on four spatial scales, but is not statistically based.

The USEPA-EMAP-SW sampling framework consists of hexagons placed over a grid map of the contiguous United States with 12,500 points. Using statistical probability methods, approximately 800 lakes and 800 streams are chosen from 25% of the grid hexagons each year. Therefore, this method has a four-year sampling cycle (Overton et al. 1991). In order to ensure an adequate characterization of larger lakes and streams, sites are randomly selected from established size strata.

MDNR-MBSS uses a similar approach which is designed to provide full coverage of the state's 18 drainage basins over a period of three years. Approximately 300 non-overlapping stream segments are randomly selected using a lattice sampling method and are sampled each year. Within a stream order, the number of segments sampled per basin is proportional to the number of stream miles in the basin.

In contrast to the USEPA-EMAP-SW and MDNR-MBSS methods, Ohio EPA uses a five-year cycle to monitor Ohio's aquatic resources. Each year of the five-year cycle focuses intensively on the biological, chemical, and physical habitat data found within a chosen basin. Study sites are identified based on criteria such as the potential to be threatened by local impacts or their potential for harboring unique or critical aquatic habitat or biota. Unlike the method used by the Ohio EPA, the methods used by USEPA-EMAP-SW and MDNR-MBSS, allow robust population inferences to be made and ensure that the sites represent the spatial distribution of lakes and streams within the study areas.

### 2.7.6 Local Scale Differences Among the Habitat Assessment Methods used by the Five Reviewed Programs

At the local scale, a number of differences exist between the sampling methods used by the reviewed programs. The sampling reach length for the USEPA-EMAP-SW assessment is generally 40 times the stream channel width and in the USGS-NAWQA sampling method, the reach length is generally 20 times the stream channel width. In contrast, the USEPA-RBP, Ohio EPA and MDNR-MBSS procedures use fixed sampling reach lengths. USEPA-RBP and MDNR-MBSS uses a sampling reach of 75 m for wadeable streams. The sampling reach length for Ohio EPA is generally a 500-m segment if the stream is boatable or a 150 to 200-m segment if it is a wadeable stream.

Quantitative thalweg profile measurements are made using the USEPA-EMAP-SW and MDNR-MBSS protocols. Quantitative measurements of reach average and maximum depth, and pool/glide/riffle/run length, width, and depth are made using the Ohio EPA method. Between 100 and 150 individual thalweg profile measurements are made along the sample reach using the USEPA-EMAP-SW protocol, as opposed to 3, (one each at 0- 25, 50, and 75 m along the sample segment), for the MBSS index and 11 sets of thalweg measurements per sample reach using the USGS-NAWQA protocol. Clearly, the sampling density for quantitative measurements is much greater for the USEPA-EMAP-SW index than for the other programs' indices. Also, depending on the index used, the specific habitat and location sampled, the assessment made by the USEPA-EMAP-SW may be based on a larger segment of the stream than the assessments made by the other programs.

## 2.7.7 Sampling Season

Sampling season is an important factor to consider because of the influence it can have on the scoring of metrics associated with all of the assessment methods. For instance, life history traits such as fish spawning and insect emergence or changes in stream flow associated with seasonal or short term patterns of precipitation, can dramatically influence the presence or absence of organisms and affect other estimates and evaluations based on the timing of single measurements of physical and chemical parameters.

# Section 3
# Water Chemistry Assessment Methods

by
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This section summarizes and evaluates the surface water column chemistry assessment methods for USEPA-EMAP-SW, USGS-NAWQA, USEPA-RBP, Ohio EPA, and MDNR-MBSS. The basic objective of surface water column chemistry assessment is to characterize surface water quality by measuring a suite of analytes. Water chemistry data are measurements of chemical concentrations and physical properties of streamwater. Because each program has a unique set of objectives, each suite of analytes is also unique. A summary of the analytes used by the five reviewed programs is presented in Table 3-1. Figure 3-1 shows a member of a field crew filling a cubitainer with a water sample that will be used in water chemistry analysis.

In addition to surface water column samples, the USEPA-EMAP-SW and USGS-NAWQA programs have additional protocols which are used to analyze the quality of ground water and use bed sediment and tissue analyses to further assess surface water quality. These additional analyses are important for the programs' understanding of water quality and an integral part of their water quality assessment programs. However, only surface water column sampling and analyses are addressed in this document.

## 3.1 USEPA-EMAP-SW Water Chemistry Assessment

The objectives of the USEPA-EMAP-SW water chemistry protocols are to determine the acidity/alkalinity of the water, to characterize the trophic condition of the stream, to ascertain the presence or absence of chemical stressors, and to classify the water chemistry type. At each sampling reach, water chemistry measurements are made *in situ* and water samples are collected for laboratory analysis (Table 3-1). One 4-L cubitainer and two 60 ml syringes are filled from a flowing portion of the stream, labeled, and stored in a cooler with ice. These samples are shipped to the analysis laboratory within 24 hours of collection (Herlihy 1998).

The in situ measurements include *specific conductance*, *dissolved oxygen*, and *tem-*

**Table 3-1.** Water Chemistry/Water Quality Measurements made by USEPA-EMAP-SW, USGS-NAWQA, USEPA-RBP, Ohio EPA and MDNR-MBSS in Conjunction with Monitoring and Assessment[a]

| Analytes | USEPA-EMAP-SW | USEPA-RBP | USGS-NAWQA[b] | Ohio EPA[c] | MDNR-MBSS |
|---|---|---|---|---|---|
| **Physical analytes** | | | | | |
| Color | L | | | | |
| Conductivity/Specific conductance | F | F | F, L | F, L | F, L |
| Dissolved oxygen (DO) | F | F | F | F | F |
| Residue (total, filtered, non-filtered) | | | | L | |
| Stream type | | F[d] | | | |
| Temperature (C) | F | F | F | F | F |
| Total dissolved solids (TDS) | | | L | | |
| Total suspended solids (TSS) | L | | | L | |
| Turbidity | L | F[d] | F | | |
| Water odors | | F[d] | | | |
| **Demand analytes** | | | | | |
| Biological Oxygen Demand (BOD) | | | L | | |
| Chemical oxygen demand (COD) | | | | L | |
| **Nutrient analytes** | | | | | |
| Acid neutralizing capacity (ANC) | L | | | | L |
| Alkalinity | L | | F, L | | |
| Bicarbonate | L | | F | | |
| Carbonate | L | | F | | |
| Chlorine, residual | | | | F | |
| Dissolved inorganic carbon (DIC) | L | | | | |
| Nitrogen as ammonia | L | | L | L | |
| Nitrogen as nitrate ($NO_3$) | L | | L | L | L |
| Nitrogen as nitrite ($NO_2$) | | | L | | |
| Nitrogen as nitrate-nitrite $NO_3$-$NO_2$ | | | L | L | |
| Nitrogen, total | L | | L | L | |
| pH | L | F | F, L | F, L | L, F |
| Silica | L | | L | | |
| Sulfate | L | | L | L | L |
| Phosphorus, ortho | | | L | | |
| Phosphorus, total | L | | L | L | |
| Phosphorus, total dissolved | | | L | | |
| **Organic analytes** | | | | | |
| Dissolved organic carbon (DOC) | L | | L | | L |
| Suspended organic carbon (SOC) | | | L | | |
| Total organic carbon (TOC) | | | L | L | |

(continued)

[a] L indicates analysis takes place in the laboratory, and F indicates analysis takes place in the field.
[b] These are the analytes used in USGS-NAWQA's basic fixed-site analysis.
[c] These analytes were derived from those taken to assess stream quality in Ohio EPA (1995).
[d] These are estimated measurements.

**Table 3-1[a].** Continued

| Analytes | USEPA-EMAP-SW | USEPA-RBP | USGS-NAWQA[b] | Ohio EPA[c] | MDNR-MBSS |
|---|---|---|---|---|---|
| **Organic waste analytes** | | | | | |
| Water surface oils | | F[d] | | | |
| Oil and grease | | | | L | |
| Phenolics, total | | | | L | |
| **Metal analytes** | | | | | |
| Aluminum, total/dissolved | | | L | | |
| Aluminum, inorganic monomeric | L | | | | |
| Aluminum, PCV reactive | L | | | | |
| Arsenic | | | L | L | |
| Barium | | | L | | |
| Beryllium | | | L | | |
| Boron | | | L | | |
| Cadmium | | | L | L | |
| Calcium | L | | L | L | |
| Chromium | | | L | L | |
| Cobalt | | | L | | |
| Copper | | | L | L | |
| Hardness | | | L | L | |
| Iron | L | | L | L | |
| Lead | | | L | L | |
| Lithium | | | L | | |
| Magnesium | L | | L | L | |
| Manganese | L | | L | L | |
| Mercury | | | L | L | |
| Molybdenum | | | L | | |
| Nickel | | | L | L | |
| Selenium | | | L | L | |
| Silver | | | L | | |
| Strontium | | | L | | |
| Vanadium | | | L | | |
| Zinc | | | L | L | |
| **Bacteria analytes** | | | | | |
| *E. coli* | | | | L | |
| Fecal coliforms | | | L | L | |
| Fecal streptococci | | | L | L | |
| **Ionic analytes** | | | | | |
| Anion Deficit (C-A) | L | | | | |
| Anions, estimated organic | | | | | |

(continued)

[a] L indicates analysis takes place in the laboratory, and F indicates analysis takes place in the field.
[b] These are the analytes used in USGS-NAWQA’s basic fixed-site analysis.
[c] These analytes were derived from those taken to assess stream quality in Ohio EPA 1995.
[d] These are estimated measurements.

**Table 3-1[a].** Continued

| Analytes | USEPA-EMAP-SW | USEPA-RBP | USGS-NAWQA[b] | Ohio EPA[c] | MDNR-MBSS |
|---|---|---|---|---|---|
| Anions, sum | L | | | | |
| Cations, base sum | L | | | | |
| Cations, sum | L | | | | |
| Chloride | L | | L | L | |
| Fluoride | | | L | | |
| Ionic strength | L | | | | |
| Potassium | L | | L | L | |
| Sodium, total | L | | L | L | |
| **Radio-chemicals** | | | | | |
| Gross alpha | | | L | | |
| Gross beta | L | | | | |
| Radium-226 | L | | | | |
| Tritium | L | | | | |
| Uranium | L | | | | |

[a] L indicates analysis takes place in the laboratory, and F indicates analysis takes place in the field.
[b] These are the analytes used in USGS-NAWQA's basic fixed-site analysis.
[c] These analytes were derived from those taken to assess stream quality in Ohio EPA 1995.

**Figure 3-1.** A member field crew member fills a cubitainer with water that will be used in water chemistry analysis.

*perature*. The samples from the two 60 ml syringes are used to measure *pH*, *dissolved inorganic carbon (DIC)*, and *monomeric aluminum species*. The bulk 4-L sample is used to measure the *major ions*, *nutrients*, *total iron*, *total manganese*, *turbidity*, and *color* (Herlihy 1998).

## 3.2 USGS-NAWQA Water Chemistry Assessment

The USGS-NAWQA program has three basic levels of water chemistry analyses, *basic fixed-site* assessment, *intensive fixed-site* assessment, and *water column synoptic* studies. The intensity of sampling and the analytes measured differ among these three levels.

### 3.2.1 Basic Fixed-Site Assessment

Data from *basic fixed-site* sampling are used for assessing temperature, specific conductance, suspended sediment, major ions and metals, nutrients, and organic carbon. The sampling strategy at each *basic-fixed site* consists of three types of sampling activities, *continuous monitoring*, *fixed-interval sampling*, and *extreme-flow* sampling, each of which is conducted for at least two years.

*Continuous monitoring* is conducted by automated gaging stations for the entire sampling period. *Fixed-interval sampling* is the collection of samples at regular time intervals for laboratory analyses. The minimum and most common sampling frequency is monthly during the minimum two-year period of operation. *Extreme flow sampling* usually consists of four to eight supplemental samples per year. Although *fixed-interval sampling* provides data for the most common flows and concentrations, high and low flows and concentrations that occur less often during the two-year sampling period have a small chance of being sampled. All samples are flow weighted and cross-sectionally integrated by standard USGS methods. Complete descriptions of sample collection and processing methods are provided by Shelton (1994).

Each time a *basic-fixed site* is sampled, field measurements (e.g., water temperature, pH, conductivity, DO) are made, and samples are submitted to the laboratory for analyses of a national target list of suspended sediments, dissolved solids, major ions and metals, nutrients, and dissolved and suspended organic carbon. These analytes (Table 3-1) are selectively augmented in some study units as required to meet specific local needs (Gilliom et al. 1995).

### 3.2.2 Intensive Fixed-Site Assessment

*Intensive fixed-site* assessments are conducted for one year and are the same as *basic fixed-site* assessments except for more frequent sampling and the addition of dissolved-pesticide analyses (Table 3-2). The goal of *intensive fixed-site* sampling is to accurately assess the dissolved pesticides in the stream through relatively high-frequency sampling at a few carefully chosen sites during key periods (Gilliom et al. 1995).

### 3.2.3 Water-Column Synoptic Studies

*Water-column synoptic studies* are short-term investigations designed to address water-quality issues specific to a study unit or region (two to three study units). Every *water-column synoptic study* is custom designed to provide more specific water-quality infor-

**Table 3-2.** Dissolved Pesticides Analyzed by USGS-NAWQA in Addition to Basic Fixed Site Analytes in Conducting Intensive Fixed-Site Assessment.

| Category[a] | Pesticides |
|---|---|
| Amides | Alachlor, Metolachlor, Napropamide, Pronamide, Propachlor Propanil |
| Carbamates | Aldicarb, Aldicarb sulfone [b], Aldicarb sulfoxide [b], Butylate, Carbaryl, Carbofuran, 3-Hydroxy [b], EPTC, Methiocarb, Methomyl, Molinate, Oxamyl, Pebulate, Propham, Propoxur, Thiobencarb, Trillate |
| Chlorophenoxy herbicides | 2,4-D (acid), Dichlorprop (2,4-DP), 2,4-DB, MCPA, MCPB, Silvex (2,4,5-TP), 2,4,5-T, Triclopyr |
| Dinitroanilines | Benfluralin, Ethafluralin, Oryzalin, Pendimethalin, Trifluralin |
| Organochlorides | Chlorothalonil, Dacthal (DCPA), p,p'-DDE, Dichlobenil, Dieldrin, alpha-HCH [b], gamma-HCH |
| Organophosphates | Azinphos-methyl, Chlorpyrifos, Diazinon, Disulfoton, Ethoprop, Fonofos, Malathion, Methyl parathion, Parathion, Phorate, Terbufos |
| Pyrethroids | cis-Permethrin |
| Triazine herbicides | Atrazine, desethyl [b], Cyanazine, Metribuzin, Prometon, Simazine |
| Uracils | Bromacil, Terbacil |
| Ureas | Fenuron, Diuron, Fluometuron, Linuron, Neburon, Tebuthiuron |
| Miscellaneous | Acifluorfen, Bentazon, Bromoxynil, Chloramben, Clopyralid, Dicamba, 2,6-Diethylaniline [b], Dinoseb, DNOC, 1-Napthol [b], Norflurazon, Picloram, Propargite |

[a] Some of the analytes listed may be deleted or qualified depending on method performance for ambient samples.
[b] Degradation products

mation than fixed-site data. Most *water-column synoptic studies* are conducted in the second and third years of the three-year intensive data-collection phase. This is done after initial results from the first year of sampling can be combined with existing data to guide the study design (Gilliom et al. 1995).

## 3.3 USEPA-RBP Water Chemistry Assessment

The objective of the USEPA-RBP is to recommend water sampling methods which will provide a brief and easily-obtained analysis of water chemistry. The protocols recommend a water-quality assessment that can be made entirely in the field. The suggested assessment includes four quantitative measurements, *temperature*, *dissolved oxygen*, *pH*, and *conductivity* and four estimated measurements, *stream type*, *water odors*, *water surface oils*, and *turbidity* (Table 3-1). The four estimated parameters are each assigned to a category. The categories for these parameters are given in Table 3-3 (Barbour et al. 1999).

## 3.4 Ohio EPA Water Chemistry Assessment

The objective of the Ohio EPA water sampling guidelines is to provide data which can be used to interpret the quality or condition of the stream being sampled. The analytes measured by the Ohio EPA are given in Table 3-1. Because water quality characteristics are not uniform between sites, the Ohio EPA considers the mixing conditions of the stream when designing a sampling regime. The Ohio EPA makes a series of conductivity and temperature measurements to check the mixing conditions in the stream and those mixing conditions determine the types of samples that will be taken (OEPA 1988).

**Table 3-3.** Categories Available for Scoring the Estimated Parameters of the USEPA-RBP'S Recommended Water Quality Assessment

| Parameter | Categories |
|---|---|
| Stream type | Coldwater<br>Warmwater |
| Water odors | Normal<br>Sewage<br>Petroleum<br>Chemical<br>None<br>Other (with notation) |
| Water surface oils | Slick<br>Sheen<br>Globs<br>Flecks<br>None |
| Turbidity[a] | Clear<br>Slightly turbid<br>Turbid<br>Opaque |

[a] In addition to the given categories, the color of the water is also noted for this parameter.

## 3.4.1 Sample Types

The Ohio EPA uses two primary types of samples, *grabs* and *composites*. *Grab* samples are individual samples gathered over a period of time not exceeding 15 minutes. If a stream is evenly mixed, the *grab* samples can be integrated. *Integrated grab* samples can be either *horizontally integrated* samples or *vertically integrated* samples. The *horizontally integrated* samples are mixtures of *grab* samples gathered from different points across the width of the stream and *vertically integrated* samples are mixtures of *grab* samples gathered from different depths of the stream.

Composite samples are mixtures of discreet samples taken at equal time intervals. These samples allow variable water quality characteristics to be averaged over a period of time. The length of time is determined by factors such as the intended use of the data and the specific characteristics of the stream being sampled (OEPA 1988).

## 3.4.2 Procedures for Collecting Grab Samples

Before *grab* samples are taken, the mixing condition of the stream is determined. If the mixing condition cannot be determined, samples are taken near the stream sample where the velocity and turbulence are the greatest. If the stream is very wide/deep or if it is incompletely mixed, *integrated grab* samples must be taken.

The individual collecting the water sample should wade into the stream or, if collecting from a bridge, use a bucket and a rope. The collecting bucket should be rinsed with ambient water. Water is collected while facing upstream and from the top 40% of the water column. Enough water is collected to fill two one-quart cubitainers and a one-gallon cubitainer. Before the cubitainers are filled, they are expanded and rinsed with a small amount of the sample. After they are filled, they are labeled, excess air is removed, and they are stored at $4^0$ C until preserved. Samples are preserved by adding an ampule of sulfuric acid, nitric acid and sodium hydroxide (OEPA 1988).

## 3.4.3 Procedures for Collecting Composite Samples

Composite samples are taken from a single point in the stream and can be collected with automatic samplers or manually. Automatic samplers are preferred because they can increase the frequency and regularity of the samples taken. Samples can either be collected directly into a composite jar or collected as aliquots. If collected as aliquots, samples are mixed in a compositor that has been rinsed with stream water and transferred into cubitainers. If it is not possible to set an automatic sampler, manual samples are taken. Manual samples are collected using the same basic procedure as grab samples. The samples are collected in aliquots that are the proportion of the total sample needed. For example, if 1,000 ml are being collected in eight aliquots, each aliquot should be 125 ml (OEPA 1988).

### 3.4.4 Parameters Requiring Special Collecting and Handling Procedures

When sampling water for bacteria analysis, a sample is collected in four one-ounce bottles containing sodium thiosulfate crystals and topped with foil-lined screw caps. When sampling water to test for oil and grease, a sample is collected in a 1-L widemouth glass jar with a Teflon or aluminum foil lined screw cap. When sampling near an area that may exceed limits for acidity/alkalinity within a given time period, measurements should not come from composite samples (OEPA 1988).

## 3.5 MDNR-MBSS Water Chemistry Assessment

During the spring, water samples are collected from each site and analyzed for *pH*, *ANC*, *conductivity*, *sulfate*, *nitrate*, and *DOC*. At each site, a grab sample is collected in a 1-L bottle for all analytes except pH. A water sample for pH is collected in a syringe so that air bubbles can be expunged. Samples are stored on ice and shipped to the analysis laboratory within 48 hours. Chemical analyses are conducted as described in the *Handbook of Methods for Acid Deposition* (U.S. EPA 1987). The exception is that the sample for ANC analysis, is reduced in volume to 40 ml for easier handling (Roth et al. 1997b).

During the summer, *in-situ* measurements are made of *DO*, *pH*, *temperature*, and *conductivity*. These additional measurements are made in order to further characterize water quality conditions that may influence biological communities. These measurements are taken at an undisturbed portion of the stream using calibrated electrode probes (Roth et al. 1997b).

## 3.6 Comparisons Between Programs

### 3.6.1 Sampling Methods

Of the five programs reviewed, all except USEPA-RBP collect water samples for laboratory analyses in addition to making water-chemistry measurements in the field. The USEPA-RBP recommends field measurements of eight parameters only and no laboratory analyses. This allows the USEPA-RBP to meet its objective of suggesting methods for the rapid assessment of stream quality. The USGS-NAWQA program uses automatic samplers at gaging stations. Therefore, that program is able to take a large number of samples over fixed increments of time. The remaining programs rely heavily on samples gathered during a small number of visits to the field. Based on sampling methods and including pesticide analysis, the USGS-NAWQA program conducts the most thorough evaluation of water chemistry.

### 3.6.2 Analytes Sampled

Of the 60 total analytes measured, only four, *conductivity*, *DO*, *pH*, and *temperature*, are common to all five programs.

The Ohio EPA and USGS-NAWQA monitoring programs measure more contaminants than the other programs. Ohio EPA monitors bacteria (i.e., fecal coliforms and fecal strep) and USGS-NAWQA monitors for the presence of a suite of pesticides. The USEPA-EMAP-SW measures a large number of analytes, including several ionic analytes not measured by other programs. The MDNR-MBSS and the USEPA-RBP each measure only eight analytes. Measuring a small number of analytes allows these programs to quickly, if not thoroughly, assess the chemical and physical properties of the streamwater.

# Section 4
# Comparison Of Periphyton And Phytoplankton Assessment Methods

by
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The term *periphyton* refers to the protozoa, fungi, bacteria, mosses, and algae that are attached to or are in close proximity to the substrata of an aquatic system. However, periphyton surveys that are used to assess stream quality deal primarily with microscopic algae (microalgae) assemblages (Rosen 1995). Periphyton are useful indicators of stream quality because they reproduce rapidly, have short life cycles and their assemblages are therefore very responsive to disturbances. In addition, most periphyton taxa can be identified to species by experienced phycologists, and tolerance or sensitivity to specific changes in environmental condition are known for many species (Rott 1991, Dixit et al. 1992).

*Phytoplankton* are microalgae that are buoyantly suspended in the water column of aquatic systems. They are passively transported by currents and turbulent mixing, and reflect water quality conditions of the water mass in which they occur (Clesceri et al. 1989). Phytoplankton are especially valuable as indicators of water quality when large areas are assessed, when resources are limited, or when phytoplankton are an important part of the ecosystem being studied.

*Diatoms* are a type of microalgae that are often the focus of phytoplankton and periphyton assessments. They are useful indicators of biological condition because they are found in all aquatic habitats.

## 4.1 USEPA-EMAP-SW Periphyton Assessment Program

The USEPA-EMAP-SW program defines periphyton as algae, fungi, bacteria, protozoa, and associated organic matter affiliated with the channel substrates (Hill 1998). Periphyton are useful indicators of environmental conditions because they respond rapidly and are sensitive to a number of anthropogenic disturbances, including habitat destruction and contamination by nutrients, metals, herbicides, hydrocarbons, and acids. Periphyton indices of stream condition are being developed based on the composite indices for biotic integrity, ecological sustainability, and trophic condition (Hill 1999). The composite indices will be calculated from measured or derived indices that include species richness, species diversity, cell density, ash free dry mass (AFDM), chlorophyll content, and enzyme activity acid/alkaline phosphatase activity (APA), which individually indicate ecological condition in streams. The metrics associated with the periphyton indicators are summarized in Table 4-1 (Hill 1998).

### 4.1.1 Sample Collection

At each stream reach, *composite index* samples are collected from *erosional* and *depositional habitats* located at each of the nine interior transects (transects B through J; See Section 1.2.1). Samples are collected from the sampling point assigned (left, center, or right; section 1.1) during the layout of the reach. In *erosional habitats*, a sample of rock or wood substrate is removed from the stream. Attached periphyton are dislodged from a 12-cm$^2$ area on the upper surface of the substrate with a stiff-bristled toothbrush for 30 seconds. Figure 4-1 shows a member of a field crew dislodging periphyton using the EMAP technique. Dislodged periphyton are then washed into a 500-ml bottle using stream water. In *depositional habitats*, a 12-cm$^2$ area of soft sediment is defined and the top 1 cm from that area is vacuumed into a 60-ml syringe. The *erosional habitat* samples from the nine transects are compiled into an *erosional habitat* composite index sample and the *depositional habitat* samples from the nine transects are compiled into a *depositional habitat* composite index sample (Hill 1998).

**Table 4-1.** USEPA-EMAP Proposed Periphyton Indicators Of Stream Condition And Associated Parameters

| Indicator and Description | Associated Parameters |
|---|---|
| Species composition | Species diversity, evenness, autecological indices |
| Cell density (cells/cm$^2$) | Abundance |
| Chlorophyll (µg./cm$^2$) | Standing crop, productivity, trophic status, autotrophic index |
| Standing stock (mg AFDM/cm$^2$) | Productivity, trophic status |
| Phosphatase activity (mmol/g AFDM) | Community activity (function) |

### 4.1.2 Sample Processing and Methods

Four different types of laboratory samples are prepared from each of the two composite index samples, an ID/enumeration sample, a chlorophyll sample, a biomass sample, and an acid/alkaline phosphatase activity (APA) sample.

ID/enumeration samples are used to determine taxonomic composition and relative abundances. These samples are preserved in 10% formalin. Chlorophyll samples are prepared by filtering a 25-ml aliquot of each composite index sample through a 0.4 to 0.6-µm glass fiber filter. Biomass samples are used for AFDM analysis. The preparation of filters for biomass samples is the same as for chlorophyll samples except that the filters have been combusted, desiccated, rehydrated, dried and weighed. The APA samples are used to measure enzymatic activity. They are prepared by freezing 50-ml subsamples of each

**Figure 4-1.** A member of a field crew dislodges attached periphyton using the EMAP-SW method.

composite index sample. Analytical methodologies are summarized in Table 4-2 (Hill 1998).

## 4.2 USGS-NAWQA Algae Assessment Program

Benthic algae and phytoplankton communities are characterized in the USGS-NAWQA program as part of an integrated physical, chemical, and biological assessment of the Nation's water quality (Porter et al. 1993).

### *4.2.1 Sample Collection*

Periphyton may be collected from natural substrates by scraping, brushing, siphoning, or other methods appropriate to each microhabitat. Porter et al. (1993) describe methods for collecting periphyton from microhabitats. The collection of phytoplankton samples, or the use of artificial substrates for collecting periphyton samples, are listed as options for collection efforts in large boatable streams and rivers to meet specific program objectives. Estimates of algal biomass (chlorophyll content and ash-free dry mass) are optional measures that may be useful for interpreting water-quality conditions. The character of periphyton microhabitats in the sampling reach determines the types of sampling devices and methods used for collecting representative algal samples. Relevant site information, sampling information, and microhabitat characteristics are recorded on data sheets. Table 4-2 list the measurements made during the USGS-NAWQA periphyton and phytoplankton analyses.

#### 4.2.1.1 Natural Substrates

Periphyton samples are collected from the surfaces of natural substrates in relation to the presence of microhabitats in the sampling reach and the selection of habitats for benthic invertebrate sampling (Section 5.3). Sampling is conducted at locations chosen to

**Table 4-2.** USEPA-EMAP Analytical Methodologies used for Periphyton

| Sample Type and Measurement | Expected Range and/or Units | Summary of Methods | References |
|---|---|---|---|
| **ID/Enumeration** | | | |
| Species composition, Relative density | species/ sample, cells/ml, or cells/$cm^2$ | Quantitative sample collected and preserved; Soft algae analysis by Palmer cell counts (200 organisms) using either strip count or random field technique; Diatom analysis using permanent slides mounted in Naphrax (500 frustules) using a strip count. | Weitzel (1979); APHA (1991) |
| **Chlorphyll**: | | | |
| Chlorophyll *a* | 1 to 100 µg/$cm^2$ | Quantitative filtration; Extraction of filter into acetone; Analysis by spectrophotometry (monochromatic) | APHA 10200 H-2; APHA (1991) |
| **Biomass** | | | |
| AFDM | mg/$cm^2$ | Quantitative filtration; Gravimetric analysis | APHA (1991) |
| **APA** | | | |
| Enzymatic activity | mmol/g, AFDM mmol/$cm^2$ | Spectrophotometric determination | Sayler et al. (1979) |

represent combinations of natural and anthropogenic factors that are important in influencing the water quality at local, regional, and national scales (Porter et al. 1993). An overview of the sampling design can be found in section 1.2. Each sampling reach is characterized using a combination of qualitative and quantitative periphyton samples.

#### 4.2.1.1.1 Qualitative Multihabitat Periphyton Samples

Qualitative periphyton samples are collected to document taxa richness in all available periphyton microhabitats present in the sampling reach. This qualitative multihabitat (QMH) periphyton sample is prepared by compositing collections of periphyton from all instream microhabitat types present in the sampling reach (Porter et al. 1993). The possible microhabitats that are targeted by the QMH sampling are listed in Table 4-3.

#### 4.2.1.1.2 Quantitative Targeted-Habitat Periphyton Samples

The goal of quantitative periphyton sample collection is to measure relative abundance and density of taxonomically-representative periphyton within: (1) a richest-targeted habitat (RTH), which supports the taxonomically richest assemblage of organisms within

**Table 4-3.** Microhabitats Used By The USGS-NAWQA Periphyton Collection Protocol And Methods Used For The Qualitative Survey

| Microhabitat | Description | Collection Methods |
|---|---|---|
| Epilithic | Submerged rocks, bedrock or other hard surfaces | Rocks are removed from the water. The attached algal material is removed by hand or scraped into a sample container. Bedrock may be sampled using a PVC pipe sampler. |
| Epidendric | Submerged tree limbs, roots or other wood surfaces | Woody material is removed from the water. The algal material is removed by hand or scraped into a sample container. |
| Epiphytic | Submerged plants or macroalgae | The plant or macroalgal material is removed from the water. The attached algae is scraped or brushed into a sample container. The liquid contents are squeezed from algal mats or aquatic vascular plants into the same sample container. |
| Epipelic | Fine streambed sediments | The top 5-10 mm of pigmented fine sediment is collected using a disposable pipette and bulb, a similar suction device, or a spoon or scoop. |
| Epipsammic | Coarse streambed sediments (e.g., sand) | The top 5-10 mm of pigmented coarse sediments are collected using a disposable pipette and bulb, a similar suction device, or a spoon or scoop. |

a sampling reach, and (2) a depositional-targeted habitat (DTH), where organisms are likely to be exposed to sediment-borne contaminants for extended periods of time. Typical RTH areas include riffles in shallow, coarse-grained, high-gradient streams or woody snag habitats in sandy-bottomed coastal streams. For the RTH portion of the quantitative collection, periphyton are normally collected from five locations within the sampling reach. At each location, periphyton samples are taken from five representative substrates (25 total samples). When available, epilithic (see Table 4-3) samples are taken. If epilithic substrates are not available, then epidendric samples are taken. If there are no epilithic or epidendric substrates, then epiphytic samples are taken. The SG-92 sampling device is used to quantify the size of the sampled area. The SG-92 is a syringe barrel fitted with a rubber o-ring on one end. The end with the rubber o-ring is placed flat on the substrate surface so that a seal is formed. A periphyton brush is then placed through the syringe barrel and used to dislodge the attached periphyton from the surface of the substrate. The sample area is then washed with a squirt bottle and the dislodged periphyton are rinsed into the sample collection container. Figure 4-2 shows a member of a field crew using a SG-92 and a brush to dislodge periphyton from a substrate. If the substrate surface is irregular so that the rubber o-ring cannot form a seal, the periphyton can be brushed from the entire substrate and the entire substrate is then fitted with aluminum foil. The

**Figure 4-2.** A member of a field crew dislodges attached periphyton from its substrate using the USGS-NAWQA method with the SG-92.

substrate is discarded and the foil is returned to the laboratory so that the surface area of the substrate can be determined. If bedrock is to be sampled, then a PVC pipe sampler is used. The periphyton from all 25 samples are composited into the same sample collection jar.

An example of a DTH area is an organically-rich depositional area such as a pool. If epilithic or epidendric (see Table 4-3) substrates are available in the DTH area, then periphyton should be collected in the same manner as they are collected from the RTH areas. However, if these substrates are not present, then epipelic or epipsammic microhabitats should be sampled. In order to sample epipsammic or epipelic habitats, the top half of a disposable 47-mm plastic petri dish is gently pushed into the streambed sediment. Then, a small sheet of Plexiglas or spatula is slipped under the petri dish top so that the sediment is trapped inside. The contents are then rinsed into a sample jar. Because the volume of the petri dish top can be measured, then the sample can be quantified. Five sediment samples are taken for the entire reach. All DTH samples are composited into a single sample jar.

The quantitative periphyton samples should be obtained prior to collecting qualitative algae and benthic invertebrate samples unless there are sufficient personnel and space within the sampling reach to ensure that the two sampling activities do not interfere with one another (Porter et al. 1993).

#### 4.2.1.2 Using Artificial Substrates to Collect Periphyton

When natural substrates cannot be sampled because of inaccessibility of the microhabitats, cost of sample collection, or safety issues associated with the collection of representative samples, artificial substrates can be used in sampling reaches These limitations are more likely to occur in large rivers and should be duly considered when designing a sampling program for this type of system. Samples obtained from artificial substrates may have reduced heterogeneity compared to those obtained from natural substrates but can be used to compare water quality among streams with disparate periphyton microhabitats. However, data from artificial substrates cannot be compared with data from natural substrates. If artificial substrates are used for one or more stream reaches in a basin, it is recommended that they be used at all sites so that meaningful water-quality interpretations can be made. The advantages and limitations of artificial substrates are discussed in Porter et al. (1993).

#### 4.2.1.3 Quantitative Phytoplankton Samples

Phytoplankton are more reflective of conditions in the open water column than periphyton which are truly benthic indicators and represent conditions at the sediment/substrata-water interface. Quantitative phytoplankton samples are obtained by collecting a representative whole-water sample. A sample volume of 1 L is sufficient for samples collected from productive, nutrient-enriched rivers as indicated by water color, but a larger sample volume is required for samples collected from unproductive, low-nutrient rivers as indicated by water transparency. Phytoplankton samples taken in conjunction with water-chemistry sampling are taken with a depth-integrating sampler. Alternatively, quantitative phytoplankton samples can be collected with a water-sampling bottle or with a pump. If chlorophyll is not to be determined, the entire sample is preserved with buffered formalin. For chlorophyll determinations, an unpreserved subsample is withdrawn from the phytoplankton sample, and the aliquot is filtered onto a glass-fiber filter. The filtered subsample volume should be sufficient to ensure that adequate algal biomass is retained on the filter. Filters are then wrapped in aluminum foil, placed into a sample bottle or container, and immediately stored on dry ice (Porter et al. 1993). Figure 4-3 shows a member of a field crew filtering a phytoplankton sample for chlorophyll analysis.

**Figure 4-3.** A field crew member filters a periphyton sample for chlorophyll analysis.

### 4.2.2 Sample Processing and Methods

Algal samples are labeled in the field. Optional algal samples for the determination of chlorophyll concentrations or ash-free dry mass are processed in the field, placed on dry ice, and submitted for analyses. Both the periphyton and phytoplankton samples can be used for the chromatographic-fluorometric and spectrophotometric analyses of chlorophyl a and chlorophyl b. The periphyton samples can additionally be used for the determination of biomass through both dry weight and ash weight analyses. Samples for the identification and enumeration of algal taxa are preserved with buffered formalin and shipped to a laboratory for analysis (Porter et al. 1993).

## 4.3 USEPA-RBP Periphyton Assessment Protocols

The USEPA-RBP recognizes benthic algae as primary producers that integrate physical and chemical disturbances to the stream reach and that are sensitive indicators of environmental conditions (Barbour et al. 1999). The objectives of the RBP for periphyton assessment include, but are not limited to: 1) assessment of biomass, 2) identification of species, and 3) determination of the periphyton assemblages' biological condition. The methods endorsed by the RBP are a composite of the techniques used in Kentucky, Montana, and Oklahoma (Kentucky DEP 1993, Bahls 1993, Oklahoma CC 1993). Periphyton assemblages serve as good biological indicators because they generally exhibit high species richness and respond rapidly to exposure but also recover quickly when the insult is removed. In addition, most periphyton taxa can be identified to species by experienced biologists, and tolerance values to specific environmental conditions are known for many species (Rott 1991; Dixit et al. 1992). Diatoms are particularly useful indicators of biological condition because they are found in all lotic systems.

### 4.3.1 Sample Collection

Three basic periphyton collection techniques for wadeable streams are reviewed and

**Table 4-4.** Summary of RBP Collection Techniques for Periphyton from Wadeable Streams

| Substrate Type | Collection Technique |
|---|---|
| **Hard removable substrate**<br>gravel, pebbles, cobble, and woody debris | Remove representative substrates from water; brush or scrape representative area of algae from surface and rinse into sample jar. |
| **Soft removable substrate**<br>mosses, macroalgae, vascular plants, root wads | Place a portion of plant into a sample container with water, shake vigorously; remove plant. |
| **Large non-removable substrates**<br>boulders, bedrock, logs, trees, roots | Place PVC pipe with a neoprene collar at one end on the substrate so that the collar is sealed against the substrate. Dislodge algae in the collar with a brush or scraper and retrieve them with a pipette. |

summarized in Table 4-4 (Plafkin et al. 1989; Barbour et al. 1999).

### 4.3.1.1 Natural Substrates

For an accurate assessment of the assemblage, samples should be collected during periods of stable stream flow. High flows can scour the stream bed and flush the periphyton downstream.

Peterson and Stevenson (1990) recommend a three-week delay following high, bottom-scouring stream flows to allow recolonization and succession to a mature periphyton community (Plafkin et al. 1989; Barbour et al. 1999).

The collection procedures have been adapted from Kentucky and Montana protocols (Kentucky DEP 1993, Bahls 1993). Periphyton should be collected from all available microhabitats in the sampling reach. Composite qualitative samples are collected from microhabitats in the approximate proportion each microhabitat occurs. Both riffles and pools are sampled if available. Algal mats or other soft-bodied algal forms can be collected from depositional areas with forceps, a suction bulb and disposable pipette, a spoon or an eyedropper.

All samples should be placed in watertight, unbreakable, wide-mouthed containers. A 4-oz (125-ml) sample is usually sufficient for analysis (Bahls 1993). Lugol's solution (potassium iodide), buffered 4% formalin, ethanol or other preservatives may be used to preserve samples.

For chlorophyll analyses, periphyton are scraped from fixed areas onto a glass fiber filter. Filters are wrapped in foil and frozen for transportation to the laboratory (Plafkin et al. 1989; Barbour et al. 1999).

### 4.3.1.2 Artificial Substrates

Periphyton can be sampled by collecting from artificial substrates that are placed in aquatic habitats and colonized over a period of time. This procedure is particularly useful in boatable streams, rivers with no riffle areas, wetlands, or the littoral zones of lentic environments. Both natural and artificial techniques are useful in monitoring and assessing waterbody conditions, and have corresponding advantages and disadvantages (Stevenson and Lowe 1986, Aloi 1990).

The methods summarized here are a composite of those specified by Kentucky (Kentucky DEP 1993), Florida (Florida DEP 1996), and Oklahoma (Oklahoma CC 1993). The RBP endorses the use of periphytometers. Periphytometers are sampling devices that can either be deployed as floating or benthic. They are fitted with glass slides, glass rods, clay tiles, plexiglass plates, or similar substrates and deployed at the sampling location for two to four weeks. A minimum of three periphytometers are placed at each site to account for spatial variability, depending upon the research design and hypothesis being tested. Samples can be composited or analyzed individually. After the incubation period, slides are collected and subsampled for chlorophyll *a* and taxonomic analysis. Storage containers for chlorophyll *a* are filled with deionized water and those for taxonomic analysis are filled with ambient water. Microslides for taxonomic analysis are scraped and samples are preserved. Samples should be stored in a dark refrigerator until they are processed. Microslides for chlorophyll analysis should be scraped and rinsed with deionized water onto a glass-fiber filter. Filters with captured algal cells are wrapped in foil and frozen to await extraction and analysis (Plafkin et al. 1989; Barbour et al. 1999).

## 4.3.2 Methods for Semi-Quantitative Assessments of Benthic Algal Biomass and Taxonomic Composition

Semi-quantitative assessments of benthic algal biomass and taxonomic composition can be made rapidly with a viewing bucket marked with a grid and biomass scoring system (Stevenson and Lowe 1986). The advantage of using this technique is that it enables rapid assessment of algal biomass over large areas. This technique is a survey of the natural substrate that does not require laboratory processing, and may be an alternative screening technique to other RBP methods (Plafkin et al. 1989; Barbour et al. 1999).

At least three transects across the habitat are established. Riffles or runs in which benthic algal accumulation is readily observed and easily characterized are preferred locations for establishing the transects. Three locations are selected objectively on each transect. Algae in each location are characterized by observing the stream through the bottom of the viewing bucket and counting the number of dots covered by macroalgae. The maximum length of the macroalgae is measured and recorded. If two types of macroalgae are present, information for each type of macroalgae is measured and recorded separately. While viewing the same area, the number of dots under which substrate occur that are of a suitable size for microalgae accumulation is recorded. The type of microalgae (usually diatoms and blue-green algae) is determined and the density under each dot estimated using the scale in Table 4-5. The density of algae on the substrate is characterized by calculating the average percent cover of the habitat by each type of macroalgae, the maximum length of each type of macroalgae, the mean density of each type of microalgae on suitable substrates, and the maximum density of each type of microalgae on suitable substrates (Plafkin et al. 1989; Barbour et al. 1999).

## 4.3.3 Periphyton Metrics

The periphyton metrics summarized in the RBP manual are in use by several states (Kentucky DEP 1993, Bahls 1993, Flordia DEP 1996) (Table 4-6). Two metrics are measurements of taxa richness (total taxa and Shannon diversity); these are estimated from the count of taxa encountered in a target number of cells (500 cells). If the cell counts vary

**Table 4-5.** Scale Used to Score the Density of Microalgae in the RBP Semi-quantitative Method

| Microalgal Density | Score |
|---|---|
| Substrate rough with no evidence of microalgae | 0 |
| Substrate slimy, but no accumulation of microalgae is evident | 0.5 |
| A thin layer of microalgae is evident | 1 |
| Accumulation of microalgal layer from 0.5-1 mm thick is evident | 2 |
| Accumulation of microalgal layer from 1-5 mm thick is evident | 3 |
| Accumulation of microalgal layer from 5-20 mm thick is evident | 4 |
| Accumulation of microalgal layer from >20 mm thick is evident | 5 |

**Table 4-6.** Diatom and Non-diatom Metrics Summarized in the RBP Manual

| Diatom Metrics | Non-diatom Metrics |
|---|---|
| Total number of diatom taxa (TNDT) | Taxa richness of non-diatoms |
| Shannon diversity (for diatoms) | Indicator non-diatom taxa |
| Percent community similarity (PSc) of diatoms | Relative abundance of all taxa |
| Pollution tolerance index for diatoms | Number of Divisions represented all taxa |
| Percent sensitive diatoms | Chlorophyll *a* |
| Percent motile diatoms | Ash-free dry-mass (AFDM) |
| Percent *Achnanthes minutissima* | |

See Appendix B for details.

by more than 20% from 500, then it may be necessary to adjust the taxa richness estimate with a rarification formula (Barbour and Gerritsen 1996). Periphyton metrics are described in Appendix B.

## 4.4 Indices

The amount of pollution present can shift the structure of the natural community of diatoms (Patrick 1963, 1964; Patrick et al. 1954; Patrick and Hohn 1956; and Hohn 1959). The methods of water quality assessment using diatoms can be classified into three main types. The first method is the *saprobic* system and its derivatives in which diatom assemblages are characterized by their tolerance to organic pollution (Kolkwitz and Marsson 1908, Liebmann 1962, Sladecek 1973). A second method is based on the classification of diatoms according to their sensitivity to all types of pollution (Fjerdingstad 1950, 1960; Coste 1974). Fjerdingstad (1950, 1960) classified diatom species according to their ability to withstand varying amounts of pollution and then described communities in terms of dominant and associated species. A third category of methods is based on the diversity of diatom communities. These methods include plotting the number of species against the number of individuals per species (Patrick 1964) as well as calculating diversity indices (review by Archibald 1972).

### 4.4.1 The Pollution Tolerance Index (PTI)

An example of a water-quality assessment method based on the pollution tolerance of diatom assemblages is the Pollution Tolerance Index (PTI), which is used by the Kentucky Department of Environmental Protection (DEP). The PTI is most similar to that of Lange-Bertalot (1979) and resembles the Hilsenhoff Biotic Index (HBI) for macroinvertebrates (Hilsenhoff 1987). Lange-Bertalot distinguished three categories of diatoms according to their tolerance to pollution, with the most tolerant taxa being assigned a value of 1 (e.g., *Nitzschia palea* , *Gomphonema parvulum*) and sensitive taxa being assigned a value of 3. For the PTI, Lange-Bertalot's categories were expanded to four. Therefore the resulting PTI diatom pollution tolerance values range from 1 (most tolerant) to 4 (most sensitive). The formula used to calculate PTI is:

$$PTI = \Sigma \frac{n_i t_i}{N}$$

Where $n_i$ is the number of cells counted for species i, $t_i$ is the tolerance value of species *i* (1-4), and *N* is the total number of cells counted. Tolerance values have been generated from several sources, including Lowe

(1974), Patrick and Reimer (1966, 1975), Patrick (1977), Lange-Bertalot (1979), Descy (1979), Sabater et al. (1988), Bahls et al. (1992), and Oklahoma Conservation Commission (1993).

### 4.4.2 Percent Community Similarity ($PS_c$)

An example of a water-quality assessment method based on the diversity of diatom assemblages is percent community similarity ($PS_c$) by Whittaker (1952). The$PS_c$ was chosen for use in diatom bioassessments because it shows community similarities based on relative abundances, and in doing so, gives more weight to dominant taxa than to rare ones. $PS_c$ should only be used when comparing a study site to a control site, or when conducting multivariate cluster analysis. If the emphasis is comparing a study site to a regional reference condition (i.e., a composite of sites), $PS_c$ should not be used. $PS_c$ values range from 0 (no similarity) to 100%.

The formula for calculating percent community similarity is:

$$PS_c = 100 - 0.5 \sum_{i=1}^{s} a_i - b_i$$

Where $a_i$ is the percentage of species $i$ in sample A, and $b_i$ is the percentage of species $i$ in sample B.

### 4.4.3 The Autotrophic Index

Because periphyton are found on or in close proximity to the substrate, Ash Free Dry Mass (AFDM) values are used as tools to assess their assemblages. AFDM is used as an estimate of total organic material accumulated on the substrate. This organic material includes all living organisms (periphyton and macroinvertebrates) as well as non-living detritus. AFDM values are used in conjunction with chlorophyll *a* as a means of determining the trophic status of streams through the use of the Autotrophic Index (AI). The formula used to calculate the AI is:

$$AI = AFDM\ (mg/m^2) / Chlorophyll\ a\ (mg/m^2).$$

High AI values (>200) indicate the community is dominated by heterotrophic organisms, and can indicate poor water quality (Weber 1973, Weitzel 1979, Matthews et al. 1980). This index should be used with discretion, because non-living organic detritus can artificially inflate the AFDM value.

The USEPA-RBP (Barbour et al. 1999) recommends that the AI be modified to:

$$AI = Chlorophyll\ a\ (mg/m^2) / AFDM\ (mg/m^2)$$

In this form, the index is positively related to the autotrophic proportion of the assemblage instead of the heterotrophic proportion. Also, the modified index would have better statistical properties as a proportion or percent (normally, chlorophyll *a*/AFDM values are approximately 0.1%) than the original index.

## 4.5 Summaries of the Periphyton Assessment Programs of the USEPA-EMAP-SW, USGS-NAWQA, and USEPA-RBP

Because they do not evaluate periphyton/phytoplankton in their assessments of stream quality, no methods for the Ohio EPA or MDNR-MBSS are reported in this section. Table 4-7 summarizes the assessment methods used by the USEPA-EMAP-SW, USGS-NAWQA and USEPA-RBP. The USEPA-EMAP-SW program assesses algal assemblages using a quantitative method to sample erosional or depositional habitats. They use the periphyton samples for four types of analyses: *ID/enumeration*, *chlorophyll*, *biomass*, and *APA*.

The USGS-NAWQA program uses both qualitative and quantitative methods to sample natural substrates. In addition, artificial substrates and samples from the water column can be used to further quantify the conditions of the periphyton and phytoplankton assemblages.

The USEPA-RBP recommends the qualitative collection of periphyton from natural substrates as well as a quantitative assessment from artificial substrates. In addition, the USEPA-RBP suggests a rapid semi-quantitative method for assessing the macroalgae.

**Table 4-7.** Methods used by the Three Reviewed Programs for the Collection and Assessment of Periphyton and Phytoplankton Assemblages

| Methods | USEPA-EMAP-SW | USGS-NAWQA | USEPA-RBP |
|---|---|---|---|
| **Collection methods** | | | |
| Periphyton from natural substrates - quantitative | X | X | |
| Periphyton from natural substrates - qualitative | | X | X |
| Periphyton from artificial substrates | | X[1] | X |
| Periphyton from natural substrates - semi-quantitative | | | X |
| Phytoplankton | | X | |
| **Analysis methods** | | | |
| ID/enumeration | X | X | X |
| Chlorophyll | X | X | X |
| AFDM | X | X | |
| APA | X | | |

[1]This method is an option for the USGS-NAWQA program, but it is not typically used (Gurtz, personal communication 1999).

# Section 5
# Comparison of Benthic Macroinvertebrate Sampling Methods

by
Bradley C. Autrey

This section compares the benthic macroinvertebrate sampling methods from the three federal programs, the USEPA-EMAP-SW, the USGS-NAWQA program, and the USEPA-RBP, as well as the two state programs, the Ohio EPA and MDNR-MBSS. The differences among the methods from these five programs reflect their regional differences, the divergent ecological interests in sampling benthic macroinvertebrates, and the various habitats sampled.

Most water quality agencies that routinely collect water quality data study benthic macroinvertebrates (Southerland and Stribling 1995). Several factors contribute to the high utilization of benthic macroinvertebrates as indicators of stream condition:

- benthic macroinvertebrates are present in a variety of habitats,
- sampling is relatively easy to conduct and it has a limited detrimental effect on the resident biota,
- benthic macroinvertebrates are relatively sedentary,
- benthic macroinvertebrates are sensitive to a wide range of chemical stressors,
- assemblages are often made up of species that have a broad range of pollution tolerances,
- the response of benthic macroinvertebrates to physical and chemical stressors has been widely described and
- many states have background benthic macroinvertebrate data.

Combined, these factors allow for the cumulative chemical and physical attributes of aquatic ecosystems to be effectively assessed through the evaluation of their benthic macroinvertebrate assemblages (BEST 1996).

## 5.1 USEPA-EMAP-SW Macroinvertebrate Assessment

The USEPA-EMAP-SW benthic macroinvertebrate protocol (Klemm et al. 1998) is used to evaluate the overall condition of and detect the relative stress levels in wadeable and boatable streams. Sampling protocols for wadeable streams are based on the USEPA-RBP III - Benthic Macroinvertebrates (Plafkin et al.1989) with the modification of a one person kick net procedure developed for the USGS-NAWQA program (Cuffney et al. 1993a) replacing USEPA-RBP's original two-man kick net procedure. In boatable streams, benthic macroinvertebrates are sampled with drift nets in addition to the modified kick net procedure. Figure 5-1 shows a modified kick net.

### *5.1.1 Wadeable Streams: Riffle/Run and Pool/Glide Sampling*

When sampling riffle/run habitats in wadeable streams, a 595/600 µm modified kick net is used to collect organisms at the nine interior transects, at either the left, right, or middle points of each transect as determined by the role of a die (see section 1.1). The sampler is held securely on the stream bottom while kicking the substrate vigorously for 20 seconds in an area of about 0.5 $m^2$ in

**Figure 5-1.** A modified kick net (left) such as is used in the USEPA-EMAP-SW protocols and a D-frame kick net (right) such as is used in the USGS-NAWQA protocols.

front of the net. Heavy organisms (such as mussels and snails), in the sample area are hand-picked and placed into the net. At the end of the 20-second period, with the net still being held in place, any organisms found on rocks in the delimited area are placed in the net. The net contents are then rinsed into the *riffle* bucket that is half filled with water. All riffle samples are combined into a single composite *riffle* bucket.

When sampling pool/glide habitats in wadeable streams, a 595/600 µm modified kick net is used to collect samples at the interior transects where very slow water is present. Heavy organisms on the stream bottom are hand picked and placed into the net. A 0.5 $m^2$ area of substrate is disturbed by vigorous kicking. A 20-second sample is collected by dragging the net repeatedly through the disturbed area just above the bottom while vigorously kicking. The net is kept moving in order to prevent collected organisms from escaping. After 20 seconds, organisms found on loose rocks in the sample area are placed into the net. Net contents are placed into the *pool* bucket that is half filled with water. All pool samples are combined into a single composite *pool* bucket. If there is too little water to use the kick net, the substrate is stirred with gloved hands and a US Standard #30 sieve is used to collect the organism from the water for 20 seconds in the same way the net was used in larger pools.

The contents of the *riffle* and *pool* buckets are individually poured through a US Standard #30 sieve (Figure 5-2). The buck-

**Figure 5-2.** A fieldcrew member processes a benthic macroinvertebrate sample before it is transported to the laboratory for analysis.

ets are rinsed with stream water in order to ensure that all organisms are evacuated. Large objects are rinsed with stream water and discarded. The sieve is thoroughly rinsed and its contents are washed into a jar that is labeled with sampling information and designated as "riffle" or "pool". In order to preserve the sampled organisms, 95% ethanol is added to each jar until a final concentration of at least 70% is obtained. Each jar is capped and sealed until the samples are analyzed (Klemm et al. 1998).

### 5.1.2 Boatable Streams

In boatable streams, kick net sampling is conducted the same way as in wadeable streams with the exceptions that all 11 transects are sampled, instead of 9, and all samples are combined into a single composite sample, instead of separate composite samples for riffle/run habitats and pool/glide habitats. Also, in boatable streams, benthic macroinvertebrates are additionally sampled using drift nets. Each drift net consists of a nylon or nylon monofilament bag (595-600 µm) that is 1 m in length at the closed end. The open end is 30.48 cm X 45.72 cm. At each sampling location, two drift nets are set at the downstream end of a sample reach (transect A). If possible, one drift net is set about 25 cm from the bottom substrate and one drift net is set about 10 cm below the surface of the water. In systems with stronger currents, both nets may be set 10 cm below the surface of the water. Nets can be set with stainless steel rods, but are usually deployed using two *floating drift net assembly* devices (Wildco 15-D10), one of which may be outfitted with a deep-deep drift attachment (Wildco 15-D12).

Drift nets are set for three to four hours and only in streams with currents greater than 0.05 m/s. Once the drift nets are set in the stream, the water velocity at each net opening is measured and recorded. After the nets have been set for three to four hours, the water velocity is again measured at each net opening and recorded. The nets are then removed from the stream and the samples are combined and sieved using a sieving bucket (595 µm-mesh/standard #30). After being cleared of macroinvertebrates, large debris from the sample is discarded. The composite sample is then transferred to a collection jar and preserved with 95% ethanol.

The results of the drift net benthic macroinvertebrate collection are reported per unit of time and flow (Allan and Russek 1985, Klemm et al. 1998).

## 5.2 USGS-NAWQA Macroinvertebrate Assessment Program

USGS-NAWQA utilizes several types of sampling equipment and techniques for the collection of benthic macroinvertebrates. The proper type of sampling equipment and technique depends on the morphology of the stream or river being sampled as well as the objectives of the study (Cuffney et al. 1993a, 1993b).

### 5.2.1 Qualitative Multihabitat (QMH) Sampling Methods

The purpose of qualitative multihabitat sampling is to obtain the most complete list of invertebrate taxa possible during approximately one hour of sampling. This is accomplished by sampling as many habitat types within the sampling reach as is possible with approximately equal intensity. The primary sampling device used in wadeable streams is a D-frame kick net equipped with a 210- µm mesh net. Kicking, dipping, or sweeping motions, as appropriate, are used to collect samples from the substrate. Figure 5-1 shows a D-frame kick net.

Visual detection and seines are used to collect firmly attached and highly-motile invertebrates, respectively. Visual collection involves manually collecting large rocks, coarse debris, or other substrates and visually locating and removing any associated organisms. This method is useful for collecting sessile organisms and organisms that burrow into hard substrates. Figure 5-3 shows a member of a field crew brushing attached benthic macroinvertebrates from a rock into a sieve. Seining with a 3.2 µm mesh can be used to collect larger, highly motile organisms, such as amphipods, decapods and freshwater prawns.

The choice of collection methods for QMH samples from boatable habitats depends upon the depth of the water, current velocity, and bed material. Grab samplers are suitable for sand or fine gravel substrates in moderate-current conditions and waters of medium depths. Shipek and Van Veen samplers are useful in extremely deep and fast rivers with sand or fine gravel bottoms. A diver-operated dome sampler is used in deep rivers when the bed material is composed of large gravel, cobble, boulder, or bedrock (Cuffney et al. 1993a).

### 5.2.2 Semi-Quantitative Targeted-Habitat Sampling Methods

The purpose of semi-quantitative targeted-habitat sampling is to obtain representative samples of benthic invertebrate commu-

**Figure 5-3.** A field crew member uses a stiff-bristled brush to remove the attached benthic macroinvertebrates from a rock.

nities from two instream habitat types: 1) a habitat supporting the most taxonomically diverse community of benthic invertebrates (richest-targeted habitat, RTH), usually a fast-flowing, coarse-grained riffle; and 2) a fine-grained, organically rich depositional habitat (depositional-targeted habitat, DTH), usually a pool. Semi-quantitative sampling methods usually characterize the structure of invertebrate communities in terms of the relative abundances of each taxon. The type of sampler used to collect a semi-quantitative sample depends upon the depth, velocity, and substrate within the instream habitat that is to be sampled. Artificial substrates are used in situations where natural substrates cannot be sampled due to inaccessibility of the habitat, cost of sample collection, or safety concerns. Under certain conditions, such as a large, deep river with cobble, boulder, or bedrock substrate, artificial substrates may offer the only viable means of obtaining benthic macroinvertebrate samples.

All nets and screens used in the collection of semi-quantitative samples have a mesh size of 425 µm. Samples are washed, sieved, and split in the field to reduce the bulk of the composite sample to less than 0.75 L. Samples collected and processed in this manner are preserved in 10% formalin (Cuffney et al. 1993a).

#### 5.2.2.1 Wadeable Coarse-Grained Substrates

Disturbance-removal sampling techniques are the most appropriate method for sampling wadeable coarse-grained substrates with current velocities greater that 5 cm/s. These techniques involve defining a specific area, disturbing the substrate within that area to dislodge invertebrates into a sampler, and then removing the larger substrate elements to acquire any specimens that are adhering tightly to the rocks. Hess samplers, Surber samplers, stovepipe corers, and box samplers are examples of samplers that can be used in these situations (Cuffney et al. 1993a).

#### 5.2.2.2 Boatable Coarse-Grained Substrates

Coarse substrates in boatable streams (water deeper than approximately 0.50-0.75 m) cannot be effectively measured using most disturbance-removal type samplers. A diver-operated dome sampler, artificial substrates, and stovepipe samplers (for water less than 0.75 m deep) can be used in these situations. Nets with 425- µm mesh are used in each case, to catch organisms dislodged or suspended in the sampler (Cuffney et al. 1993a).

#### 5.2.2.3 Wadeable Fine-Grained Substrates

Grab samplers are appropriate for sampling in shallow, fine-grained riffles or pools. All screening on the grab should have mesh openings of 425 µm or smaller (Cuffney et al. 1993a).

#### 5.2.2.4 Boatable Fine-Grained Substrates

Grab samplers can be used from boats to obtain samples from deep rivers with fine-grained substrates. A hand or power winch is recommended for sampling in deep waters or using weighted grab samplers. All screening on the grab sampler should have mesh openings of 425 µm or smaller (Cuffney et al. 1993a).

#### 5.2.2.5 Woody Snags and Macrophytes

When snags are used in the semi-quantitative RTH portion of the macroinvertebrate survey, they are sampled by removing sections of tree limbs with a saw or lopping shears and collecting the attached invertebrates by hand picking and brushing the limb's surface and cavities. The loss of motile or loosely attached organisms can be minimized by placing a net downstream from the limb to catch dislodged organisms. The lengths and diameters of the sampled snags are recorded in order to estimate the surface areas.

When macrophyte beds are used in the semi-quantitative RTH portion of the macroinvertebrate survey, they can be sampled with disturbance-removal samplers. Net samplers can be used if there is sufficient current to wash the dislodged plant and animal material into the net. A knife or trowel can be used to dislodge the plant material from the substrate. Stovepipe samplers may prove more effective and should be used when the macrophytes are too tall to allow use of a dredge. The macrophytes that are removed should be inspected carefully for invertebrates that are attached and for those that burrow into stems (Cuffney et al. 1993a).

## 5.3 USEPA-RBP Macroinvertebrate Assessment

The current USEPA-RBP methods (Plafkin et al. 1989; Barbour et al. 1999) emphasize the sampling of a single habitat in wadeable streams, preferably those having riffles/runs, because macroinvertebrate diversity and abundance are usually highest in these habitats. When some streams lack the riffle/run habitats, a method suitable to sampling a variety of habitats is desired. The proposed multi-habitat sampling approach is designed to sample major habitats in proportional representation within a sampling reach.

### 5.3.1 Single Habitat Approach

A 100-m reach that is representative of the stream is selected. All riffle/run areas within the 100-m reach are candidates for sampling macroinvertebrates because macroinvertebrate diversity and abundance are usually highest in cobble substrate. Where cobble substrate is the predominant habitat, this sampling approach provides a representative sample of the stream reach. In cases where cobble substrate represents less than 30% of the sampling reach, alternative habitats (such as snags, vegetated banks, submerged macrophytes, and sand) will need to be sampled.

Sampling begins at the downstream end of the reach and proceeds upstream. Using a 1-m, 500-µm mesh kick net, the stream is sampled two or three times at locations of various velocity in the riffle. A kick in the single habitat approach is a stationary sampling accomplished by positioning the net and disturbing 1 $m^2$ upstream of the net. Large substrate particles are gathered and the attached organisms are removed. The sample is then transferred to sample containers and preserved in 95% ethanol (Barbour et al. 1999).

### 5.3.2 Multi-Habitat Approach

A 100-m reach that is representative of the stream is selected. Different types of habitat are to be sampled in the approximate proportion in which they are represented in the reach. Sampling begins at the downstream end of the reach and proceeds upstream. A total of 20 jabs or kicks are taken over the length of the reach. A jab consists of forcefully thrusting the net into the habitat for 0.5 m. A kick in the multi-habitat approach is a stationary sampling accomplished by positioning the D-frame, 500 µm mesh dip net and disturbing the substrate for a distance of 0.5 m upstream of the net. The jabs or kicks collected from the multiple habitats are combined to obtain a single homogeneous sample. The sample is transferred to sample containers and preserved in 95% ethanol (Barbour et al. 1999).

## 5.4 Ohio EPA Macroinvertebrate Assessment

Assessments of the ambient macroinvertebrate community by the Ohio EPA (OEPA 1988, 1989) consists of two types: 1) intensive surveys of stream or river reaches using multiple sites in upstream to downstream longitudinal or synoptic sub-basin configurations, and 2) multiple-year sampling at a specified fixed station on a stream or river. Sampling sites are located based on the characteristics of the stream or river, and in accordance with the survey objectives.

### *5.4.1 Artificial Substrate*

The primary sampling equipment used for quantitative sampling is the modified Hester-Dendy artificial substrate sampler. It is constructed of 0.125-inch tempered hardboard cut into three $in^2$ plates and 1.0 $in^2$ spacers. A total of eight plates and twelve spacers are used for each sampler. Plates and spacers are placed on a 0.25-inch eyebolt so there are three single spaces, three double spaces, and one triple space between the plates. The total surface area of the sampler, excluding the eyebolt, is 145.6 $in^2$ (approximately 1.0 $ft^2$). Figure 5-4 shows a Hester-Dendy sampler in place at a sampling location.

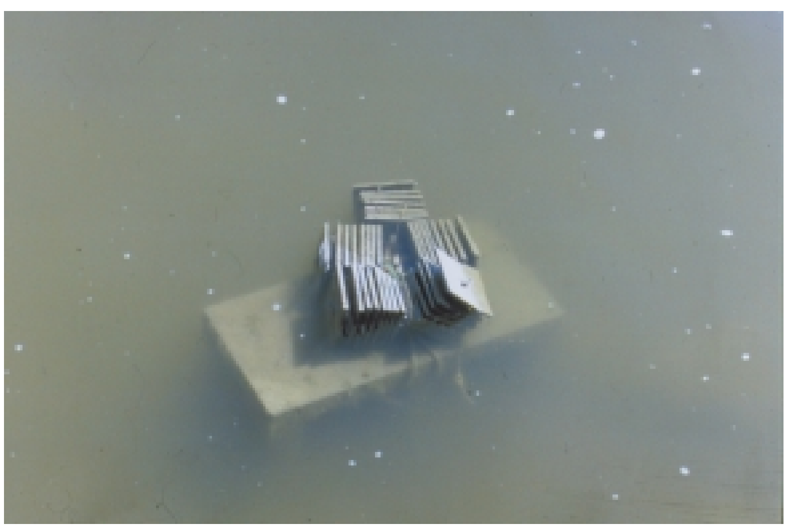

Figure 5-4. A Hester-Dendy sampling device placed in a river. Note: This sampler was set in a more shallow area for photographic purposes. Hester-Dendy samplers are normally set approximately 1 meter below the water's surface.

Before the samplers are placed in streams, they are tied to concrete construction blocks in order to anchor them in place. Whenever possible, samplers are placed in runs rather than in pools or riffles, so that a steady flow of water is running through the sampler and an attempt is made to place all samplers in habitats that are as similar to each other as possible. At each sampling site, a set of five artificial substrate samplers are exposed for a six-week period, usually between June 15 and September 30.

Retrieval of the samplers is accomplished by separating them from the concrete block and placing them in one-quart plastic containers while still submersed. Enough formalin is added to each container to approximate a 10% solution (OEPA 1989).

### *5.4.2 Natural Substrate*

For the purpose of metric development, qualitative samples of macroinvertebrates inhabiting the natural substrates are also collected at the same time that the artificial substrate sampler is retrieved. All available habitat types are sampled and voucher specimens are retained for laboratory identification. In shallow waters, forceps and a triangular ring frame with a US Standard #30-mesh (595-600 µm) dip net are used. Grab samplers can be used in deep waters. The qualitative sampling continues until, as determined by gross examination, no new taxa are taken.

When only qualitative samples are collected, an attempt is made to sample a riffle, run, margin, and pool habitat at each station. Stations should be sampled in order, moving from upstream to downstream, to detect any changes between sites. Sample areas should be physically similar among the different sites. Collections are made for a minimum of 30 minutes. Once the 30 minute minimum sampling time has been met, sampling is continued until no new taxa are collected.

In addition, quantitative samples of macroinvertebrates inhabiting the natural substrates can also be optionally collected. This is accomplished by using a Surber square-foot sampler, with # 30-mesh netting, and a hand cultivator with two-inch tines. Standing on the downstream side of the sampler, the collector works the substrate using the hand cultivator. For large rocks, a brush can be used. Three to five Surber samplers are taken at each site (OEPA 1989).

## 5.5 MDNR-MBSS Macroinvertebrate Assessment

For this program benthic macroinvertebrates are collected to provide a qualitative description of the community composition at each sampling site (Janicki et al. 1993). Sampling is conducted in the spring index period (between March 1 and May 1) in wadeable streams (Roth et al. 1997b).

### *5.5.1 Sampling Methods*

A 600-µm mesh D net is used to collect organisms from habitats with the highest probable taxonomic diversity; thus, riffle areas are preferred, because macroinvertebrate abundance and diversity are usually highest in riffle areas. Other habitat types include rootwads, woody debris, leaf packs, macrophytes and undercut banks. A variety of techniques are used for collection, such as kicking, jabbing, and gently rubbing hard surfaces by hand to dislodge organisms. Each jab covers one $ft^2$. For every 75-m segment, 20 sites are sampled.

Combined substrates from each segment are preserved in 70% ethanol (Roth et al. 1997b).

## 5.6 Origin of Benthic Macroinvertebrate Indices

The four primary benthic macroinvertebrate indices used by these programs to determine water quality conditions are the Invertebrate Community Index (ICI), the Hilsenhoff Biotic Index (HBI), the Benthic Index of Biotic Integrity (B-IBI), and Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness. EPT richness is a simple index (Lenat 1987) that incorporates three orders of macroinvertebrates which are generally intolerant to poor water conditions. Also reviewed is the Stream Benthos Integrity Index (SBII), which is currently under development by the National Exposure Research Laboratory (NERL) for the USEPA-EMAP-SW.

### 5.6.1 The ICI

Development of the ICI was a result of the 1983-84 Ohio Stream Regionalization Project, a cooperative pilot venture between Ohio EPA and USEPA/ERL-Corvallis (Whittier et al. 1987). It is now the primary tool used by the Ohio EPA for measuring the condition of macroinvertebrate communities (DeShon 1995). Table 5-1 shows the metrics included in the Ohio EPA's ICI and their expected responses to disturbances. These ten metrics are scored and summed to obtain an ICI value.

**Table 5-1.** Metrics used in the Ohio EPA's ICI and Their Expected Responses to Disturbance

| Metric | Expected response to disturbance |
|---|---|
| Total number of taxa | Decrease |
| Total number of Ephemeroptera taxa | Decrease |
| Total number of Trichoptera taxa | Decrease |
| Total number of Dipteran taxa | Increase |
| Percent Ephemeroptera composition | Decrease |
| Percent Trichoptera composition | Decrease |
| Percent Tanytarsini midge composition | Increase |
| Percent other Dipteran and non-insect composition | Increase |
| Percent tolerant organisms | Increase |
| Total number of qualitative EPT taxa | Decrease |

### 5.6.2 The HBI

The USEPA-EMAP-SW, USEPA-RBP, and MDNR-MBSS use the HBI. Hilsenhoff (1977) refined the index first proposed by Chutter (1972) in developing the HBI. Resh and Jackson (1993) found the HBI to be an effective measurement discriminating between impaired and unimpaired sites in California. A North Carolina study found that both the EPT and the HBI are good indicators of stream water quality (Wallace et al. 1996). The HBI attempts to summarize the overall pollution tolerance of the benthic macroinvertebrate community. Its value is calculated using the following formula:

$$HBI = \sum (n_i \, x \, a_i) / N$$

Where $n$ is the number of individuals in each taxon, $a$ is the tolerance value assigned to that taxon and N is the total number of individuals in the sample. Tolerance values for individual taxa are listed in Hilsenhoff (1988). Tolerant organisms are those frequently associated with gross organic contamination and are generally capable of thriving under anaerobic conditions (given a score of 4 or 5). Facultative organisms are those having a wide range of tolerance that frequently are associated with moderate levels of organic contamination (given a score of 2 or 3). Intolerant organisms are those that are usually not found associated with organic contaminants and are generally intolerant of even moderate reductions in dissolved oxygen (given a score of 0 or 1). Organisms not listed in Hilsenhoff (1988) are given a value of 5, unless available information suggests otherwise.

#### 5.6.2.1 Scoring of the HBI

An HBI value is calculated using the pollution tolerance values for the represented taxa (Hilsenhoff 1988) and the equation given in section 5.6.2. The resulting value can be used as an indicator of water quality. The water quality categories indicated by the respective HBI scores are given in Table 5-2.

### 5.6.3 The B-IBI

The MDNR-MBSS developed two versions of the Benthic Index of Biotic Integrity (B-IBI) for the Monitoring and Non-Tidal Assessment (MANTA) Division of the MDNR. One version is for the coastal plains (CP) region of Maryland and the other is for the non-coastal plains (NCP) region (Table 5-3). These indices were modeled after Karr et al.'s (1986) Index of Biotic Integrity (IBI). While the IBI was developed to estimate the condition of an aquatic ecosystem based on its fish community, the B-IBIs will allow the MDNR to more accurately assess the condition of its streams by surveying their benthic macroinvertebrates (Roth et al. 1997b). Definitions of metrics used in the B-IBI and scoring parameters may be found in Appendix C.

### 5.6.4 The SBII

The Stream Benthos Integrity Index (SBII) was developed by the NERL for

**Table 5-2.** Water-Quality Levels Indicated by Different Ranges of HBI Scores.

| Range of HBI Scores | Indicated Water Quality |
|---|---|
| 0.00-3.75 | Excellent |
| 3.76-4.25 | Very Good |
| 4.25-5.00 | Good |
| 5.01-5.75 | Fair |
| 5.76-6.50 | Fairly Poor |
| 6.51-7.25 | Poor |
| 7.26-10.00 | Very Poor |

**Table 5-3.** Metrics used for the CP B-IBI and the NCP B-IBI

| Metric | CP B-IBI | NCP B-IBI |
|---|---|---|
| Total Number of Taxa | X | X |
| Number of EPT Taxa | X | X |
| Number of Ephemeroptera Taxa | | X |
| Number of Dipteran Taxa | | X |
| Percent Ephemeroptera | X | X |
| Percent Tanytarsini of Chironomidae | X | |
| Percent Tanytarsini | | X |
| Number of Intolerant Taxa | | X |
| Percent Tolerant Individuals | | X |
| Beck's Biotic Index | X | |
| Number of Scraper Taxa | X | |
| Percent Collectors | | X |
| Percent Clingers | X | |

USEPA-EMAP-SW, specifically for the Mid-Atlantic Highlands (MAH) region. The SBII is a multimetric index developed using a stepwise process to evaluate candidate metrics and best professional judgement for final selection of metrics. Seven metrics were selected for inclusion in the SBII (Table 5-4), with the score of each metric ranging from 0 to 1 on a continuous scale. Scoring of metrics is based on the fraction of the "best attainable value" observed at a site, where the "best attainable value" is established using the 95$^{th}$ (metrics that decrease in response to stress) or 5$^{th}$ (metrics that increase in response to stress) percentile of the overall distribution of each metric. Two of the metrics are adjusted for watershed size prior to scoring. The SBII ranges from 0 to 7, with 3 condition categories and 2 transition ranges (Table 5-5), based on a power analysis.

**Table 5-5.** The USEPA's SBII Condition Categories and Associated Score Ranges

| Condition | Range of Scores |
|---|---|
| Good | 5 to 7 |
| Good-Fair transition | >4.5 to <5 |
| Fair | 2.5 to 4.5 |
| Fair-Poor transition | >2 to <2.5 |
| Poor | 0 to 2 |

## 5.7 Indices and Metrics used by the Programs for Analysis of Benthic Macroinvertebrate Communities

This section contains the metrics and indices used by the programs to analyze benthic macroinvertebrate data. The analytical techniques used by USGS-NAWQA are not presently available and are, therefore, not included in this section.

**Table 5-4.** Metrics used in the USEPA's SBII and Their Expected Responses to Disturbance

| Metric | Expected Response to Stress |
|---|---|
| Number of taxa | Decrease |
| Number of EPT taxa | Decrease |
| % Intolerant taxa | Decrease |
| % Plecoptera taxa | Decrease |
| Hilsenhoff Biotic Index | Increase |
| % Oligochaetes and leeches | Increase |
| % Chironomid taxa | Increase |

### *5.7.1 USEPA-EMAP-SW Benthic Macroinvertebrate Analysis*

The USEPA-EMAP-SW protocols utilize three indices to analyze the metrics gathered from the survey of benthic macroinvertebrates and are currently developing a fourth index (Table 5-6). Together, these indices allow the USEPA to thoroughly evaluate the relative health of its rivers and streams (Klemm et al. 1998).

### *5.7.2 USEPA-RBP Benthic Macroinvertebrate Analysis*

In addition to the metrics in Table 5-7, the USEPA-RBP also suggests the calculation of the HBI (section 5.6.4) which weighs the relative abundances of taxa with their tolerances to pollution (Barbour et al. 1999).

### *5.7.3 Ohio EPA Benthic Macroinvertebrate Analysis*

Ohio EPA evaluates benthic community fitness using the Invertebrate Community In-

| **Table 5-6.** Indices used by the USEPA-EMAP-SW Protocols | | |
|---|---|---|
| Index | Definition | Expected Response to Perturbation |
| Percent EPT | Number of individuals in each order of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) in each sample divided by the total number of individuals in the sample | Decrease |
| Shannon Diversity Index | Incorporates both richness and evenness in a measure of general diversity and composition; H' = © (N log – n log n))/N, where n is the total number of individuals of $i^{th}$ species, N is the total number of individuals, and © is 3.321928 which converts base 10 log to base 2 log. H' ranges from 0 to 3.321928 log N | Decrease |
| Hilsenhoff Biotic Index | Uses relative abundance weighted by pollution tolerances to evaluatewater quality. HBI = (( n x a)/N), where n is the total number of individuals in the $i^{th}$ taxon, a is the tolerance value assigned to that taxon, and N is the total number of individuals in the sample. | Increase |
| Stream Benthos Integrity Index* | Integrates 10 macroinvertebrate population or community metrics into a single biological integrity index score using specimens that have been identified to genera and/or species levels of identification. | Increase |

*Currently under development by USEPA-EMAP-SW.

| **Table 5-7.** Metrics Recommended by the USEPA-RBP | | |
|---|---|---|
| Metric | Definition | Expected Response to Perturbation |
| Total number of taxa | Measures the overall variety of the macroinvertebrate assemblage | Decrease |
| Number of EPT taxa | Sum of the number of taxa in the insect orders Ephemeroptera, Plecoptera, and Trichoptera | Decrease |
| Percent dominant taxon | Measures the dominance of the single most abundant taxon | Increase |
| Ratio of Hydropsychidae/ Trichoptera | Number of individuals in Hydropsychidae family divided by the number of individuals in class Trichoptera | Increase |
| Ratio scrapers/ (scrapers + filterers) | Number of individual scrapers divided by the sum of the number of individual scrapers and filterers | Decrease |
| % shredders | Relative abundance of the shredder functional feeding group | Decrease |

dex (ICI). The ICI consists of 10 structural community metrics, each with four scoring categories of 6, 4, 2, and 0. The point system evaluates a sample against a database of 247 relatively undisturbed reference sites throughout Ohio. Each metric was visually examined to determine if any relationship existed with drainage area. When it was decided if a direct, inverse, or no relationship existed, the appropriate 95% line was estimated and the area beneath quadrisected as determined by the distribution of the reference points. Some percent abundance and taxa richness categories were not quadrisected, since the data points showed a tendency to clump at or near zero. In these situations, a quadripartite method was used in which one of the four scoring categories included zero values only, and the remaining scoring categories were delineated by an equal division of the reference data points.

Six points are given if a metric has a value comparable to those of exceptional stream communities; 4 points, if comparable to typical good communities; 2 points, if slightly deviating from the expected range of good values, and 0 points for metric values strongly deviating from the expected range of good values. The summation of the individual metric scores results in the ICI value (OEPA 1989). Definitions of metrics and justification for inclusion in the ICI can be found in Appendix D.

### *5.7.4 MDNR-MBSS Benthic Macroinvertebrate Analysis*

The MDNR-MBSS calculates the EPT (section 5.1), the HBI (section 5.1.4), and the B-IBI (section 5.1.3) to characterize the benthic community status. The B-IBI consists of seven metrics for the CP region, and nine metrics for the NCP region. The point system evaluates a sample against a database of 37 reference sites in Maryland. For each sampling location, metrics are developed and scores (1, 3, or 5) assigned according to the thresholds ($10^{th}$, $50^{th}$, or $90^{th}$ percentiles, respectively) established during the indicator development process. Raw index scores for the CP and NCP indices ranged from 7 to 35 and 9 to 45, respectively. These scores were adjusted to a common scale ranging from 1 to 5, to be consistent with the MDNR-MBSS fish IBI. On this scale, a score of 4-5 indicates good stream quality, 3-3.9 indicates fair stream quality, 2-2.9 indicates poor stream quality, and 1-1.9 indicates very poor stream quality (Roth et al. 1997b; Stribling et al. 1998).

## 5.8 Comparison of Benthic Macroinvertebrate Indices

Primarily, programs that conduct benthic macroinvertebrate surveys have the objective to assess the overall quality of the studied stream based on its benthic macroinvertebrate community. Also, most programs have similarities in their preferred methods for conducting the surveys. For example, all programs sample within a defined length of stream, all programs use multimetric indices in the analyses of macroinvertebrate data, and all programs compare the index values from individual sites to reference conditions. However, because each program has its own subset of objectives which reflect the needs of the region it serves, each program has its own subset of methods to meet those objectives (Table 5-8).

### 5.8.1 Indices

The USEPA-EMAP-SW uses three indices, EPT, Shannon Diversity (H'), and HBI, and is currently developing a fourth index, the SBII. USEPA-RBP suggests using two indices, EPT and HBI. MDNR uses EPT, HBI, and B-IBI. Ohio EPA uses the ICI. USGS-NAWQA does not provide methods on the calculation of indices from its field data.

### 5.8.2 Sampling Locations

The method used to select sampling locations varies between programs. Programs frequently choose sites in order to assess a specific area such as previously studied target areas or point sources. However, the EMAP protocols use randomly chosen sites in order to make a regional assessment of stream quality. Also, there are differences in the habitat type in which benthic samples are

**Table 5-8.** Comparison of Benthic Macroinvertebrate Indices, Sampling Methods, Preferred Sampling Habitats, and Preferred Sampling Seasons

| | USEPA-EMAP-SW | USGS-NAWQA | USEPA-RBP | Ohio EPA | MDNR MBSS |
|---|---|---|---|---|---|
| **Indices** | | | | | |
| EPT | X | | X | | X |
| HBI | X | | X | | X |
| SBII | X | | | | |
| B-IBI | | | | | X |
| ICI | | | | X | |
| Shannon Diversity (H') | X | | | | |
| **Sampling Methods** | | | | | |
| D-Net | | | | | X |
| Dip Net | | X | X | X | |
| Kick Net | | | X | | |
| Modified Kick Net | X | | | | |
| Drift Net | X | | | | |
| Hester-Dendy | | | | X | |
| Slack Sampler | | X | | | |
| **Habitat Types** | | | | | |
| Riffle Areas | X | X | X | | X |
| Pool Areas | X | X | | | |
| Run Areas | | | X | X | |
| **Seasons** | | | | | |
| Spring Sampling | | | | | X |
| Summer Sampling | X | X | X | X | |

taken. MDNR samples only in riffle areas, Ohio EPA samples primarily in runs, USGS-NAWQA samples in riffles and pools, USEPA-RBP suggests sampling in riffle and run areas, and USEPA-EMAP-SW samples in riffles, runs, and pools.

### 5.8.3 Sampling Equipment

The USEPA-EMAP-SW uses a 595-µm modified kick net sampler and 595-µm drift nets, USEPA-RBP suggests using a 500-µm kick net and 500-µm dip net, USGS-NAWQA uses a 210-µm dip net for qualitative sampling and a 425-µm sieve for semi-quantitative sampling, MDNR uses a 600-µm mesh D net, and Ohio EPA uses a Hester-Dendy for quantitative sampling and 600-µm dip nets for qualitative sampling. The mesh size used for sampling is not consistent between programs and this may influence sample content. The various methods used to sample benthic macroinvertebrates from substrate result in characteristic sampling differences among the five programs. Ohio EPA uses both natural and artificial substrate samplers, while USGS-NAWQA, MDNR, USEPA-EMAP-SW, and USEPA-RBP use a natural substrate sampler. Using an artificial substrate sampler is a quantitative method that allows objective sampling to take place in areas that are difficult to reach. However, sampling with artificial substrate takes more time and personnel than does natural substrate sampling. Also, an artificial substrate sampler may selectively sample certain taxa and misrepresent the relative abundance of taxa in the natural substrates. Natural substrate sampling takes less time and personnel than does artificial substrate sampling, but it is less quantitative.

# Section 6
# Fish Assessment Methods

by
Joseph E. Flotemersch

The principal methods used by the five reviewed assessment programs to survey fish communities are electro-fishing or electro-fishing in conjunction with seines or nets. The differences between the programs lie in how sites are selected, the length of the sample reach, the amount of time spent sampling, how the seines or nets are implemented, and how the data are analyzed. The dissimilarities among the programs' methods are a result of the differences between the programs' regions as well as the differences between the programs' objectives.

## 6.1 USEPA-EMAP-SW Fish Data Collection Methods

Data collection occurs at randomly selected sites within a designated region (see section 1.1). Fish are sampled during a summer index period (July to September), which coincides with the low flow period of streams in the research areas. The elementary sampling unit used by USEPA-EMAP-SW program for assessment is the *sampling reach*. It has a length of 40 times the channel width with a minimum length of 150 m. No maximum length has been specified.

Currently, both wadeable and boatable streams are being sampled. However, the only methods that have been fully documented are those addressing wadeable systems (Lazorchak et al. 1998). Methods for boatable systems are currently being piloted. These methods will be discussed in this document, but they should be viewed as pilot methods.

### *6.1.1 Wadeable Streams*

The USEPA-EMAP-SW design utilizes a single-pass electro-fishing method covering the determined reach length. In wadeable streams, block nets are placed at the downstream and upstream limits of the sampling reach when the sample reach is a large continuous pool. An attempt is made to thoroughly fish the entire segment, sampling all available cover and habitat structures while a consistent effort is applied over the entire pass. Sampling is continued for at least 45 minutes and should not exceed three hours. Seining may be used in conjunction with electro-fishing to ensure sampling of those species which may otherwise be under represented by an electro-fishing survey alone (e.g., darters, sculpins, benthic cyprinids). Seines may also be used as block or kick nets to selectively isolate sections of the stream being electro-fished (e.g., snags, riffles, cut-banks), in sites where streams are too deep for electro-fishing to be conducted safely, or in turbid, simple, soft-bottomed streams where seining is more effective. Figure 6-1 shows a member of a field crew using backpack shocker to electro-fish a wadeable stream.

### 6.1.2 Boatable Streams

In boatable systems, the stream reach is fished with a boat-based electro-fishing unit (Figure 6-2). Electro-fishing begins at the furthest upstream section and proceeds downstream until the entire stream reach has been covered. If the width of a stream requires that sample reaches exceed 5 km, members of the pilot field crews have suggested that electro-fishing the entire reach may not be logistically wise. In these situations, options include truncating the reach or sampling every other transect.

### 6.1.3 Data Recorded

Captured fish are identified in the field, if possible, and counted. Sport fish and very large specimens are identified, measured and released (Figure 6-3). For other species, the maximum and minimum lengths are recorded. A voucher sub-sample of 25 individuals from each species is identified and preserved in approximately 20% formalin. Additional specimens (above the 25 voucher) are counted and released (McCormick and Hughes 1998).

## 6.2 USGS-NAWQA Fish Data Collection Methods

The objective of the USGS-NAWQA characterization of fish community structure

**Figure 6-1.** A field crew member uses a backpack electro-shocker to sample fish in a wadeable stream.

**Figure 6-2.** A member of a field crew samples fish using the boat-based electroshocking technique for boatable rivers.

**Figure 6-3.** Before being released, the fish are identified, measured and weighed and these data are recorded.

is to relate fish community traits to physical, chemical, and other biological factors as part of an integrated assessment of the nation's water-quality conditions. Protocols have been published for wadeable and boatable streams and both will be discussed in this document (Meador et al. 1993a).

Sampling sites (either *fixed* or *synoptic*) are chosen to represent the set of environmental conditions considered important for controlling water quality in the study unit. *Fixed sites* are located at or near USGS gaging stations where continuous discharge measurements are available. Three sampling reaches are used to represent environmental conditions associated with each fixed site. *Synoptic sites* are non-gaged sites where one-time samples of a limited number of physical and chemical characteristics are measured. Only one sampling reach is generally used to characterize a synoptic site. The purpose of a synoptic site is to answer questions regarding source, occurrence, or spatial distribution.

Sampling is conducted during low and stable-flow periods (usually mid-June to early October). These conditions increase the likelihood that samples throughout the study unit can be collected under similar flow conditions.

The primary determinant of sampling reach length is geomorphology. An attempt is made to include at least two types of geomorphic channel units in the sampling reach. Where this is not possible, reaches are chosen that include one meander wavelength, based on 20 times the distance of the channel width. The minimum and maximum lengths of sampling reaches in boatable streams are 500 m and 1,000 m, respectively. The minimum and maximum lengths of sampling reaches for fish sampling in wadeable streams is 150 m and 300 m, respectively. These parameters were set to ensure the efficient collection of representative fish samples.

## 6.2.1 Wadeable Streams

Wadeable streams are sampled with backpack (Figure 6-1) or towed electro-fishing gear and, in contrast to other programs, use a double-pass approach to sampling rather than a single-pass approach. Backpack electro-fishing is used in relatively small, shallow headwater streams, whereas towed electro-fishing is employed in relatively wide, wadeable streams with deep pools. Sampling is conducted in an upstream direction. All captured fish are placed immediately in either a holding box or live well for future processing. After the first pass is completed and all fish are processed, a second pass is conducted in the same manner, and usually in the same area, as the first pass. In order to avoid sampling the same individuals twice, no fish are released until the second pass is completed.

Following electro-fishing, seining is used to collect small-sized individuals, thereby allowing for a more representative sample to be taken. The seine configuration and method employed are dependent on the geomorphic channel units present and the degree of complexity of the habitat features within a sampling reach (Meador et. al. 1993a).

## 6.2.2 Boatable Streams

Boatable streams are sampled using electro-fishing boats (Figure 6-2). Sampling is conducted downstream, from the upstream boundary of the sampling reach along the shoreline. This is to allow the fish to swim into the approaching electrical field. The boat is operated at a speed equal to or slightly greater than water velocity. Sampling is conducted in two passes, one for each shoreline.

Boatable streams can also be sampled using the beach seine in wadeable shoreline areas. Three samples should be taken from accessible parts of the upper, lower, and middle sections of the boatable sampling reach. The fish from the three seine hauls are combined and processed before release.

### 6.2.3 Other Sampling Methods

Other sampling methods are used to obtain a representative sample of the fish community when electro-fishing and seining is not effective (e.g., in water with extremely-low conductivity). In situations where seining may be ineffective because a sampling reach contains a large number of woody snags, debris, or other obstructions, gill nets and hoop nets may be used to collect a representative sample of fish. Gill nets capture fish by entangling them in a fabric mesh that is not actively moved by man or machine. They require one trip for deployment, one trip for collection, and have the potential to be vandalized. Gill netting can kill fish, therefore, it must not be conducted in areas where endangered or threatened species may be present. The net should be set in the late afternoon and remain in the water for several hours, but no longer than 24 hours. The number of fish collected in the gill net is not linearly related to the duration of the set (Hubert 1983), so the exact duration of the set should depend on flow conditions and the presence of drifting debris.

Hoop nets capture fish by trapping them in an enclosed mesh trap. Unlike gill nets, fish caught by hoop netting can be released with little or no harm. The duration of the set should depend on the flow conditions and the presence of drifting debris. To harvest, the hoop net is raised at the cod end and the fish are removed. Two hoop nets are set within the sampling reach.

### 6.2.4 Data Recorded

Regardless of the sampling method, a representative sample is taken to provide information on the presence and relative abundance of the species which represent the fish community inhabiting the sampled stream. An attempt is made to identify all fish in the field to the species level. If there is uncertainty regarding the identification of specimens, representative samples are preserved in formaldehyde for later identification in the laboratory (Meador et al. 1993a).

## 6.3 USEPA-RBP Fish Data Collection Methods

The USEPA-RBP methods were designed to provide guidance on cost-effective approaches to problem identification and trend assessment of our nation's resources. The methods suggested by the USEPA-RBP for fish involves careful, standardized field collection, species identification and enumeration, and analyses using aggregated biological attributes. Data provided by the fish USEPA-RBP can serve to assess use attainment, develop biological criteria, prioritize sites for further evaluation, and assess status and trends of fish assemblage. The suggested fish collection procedure is a multi-habitat approach for wadeable streams, which allows the sampling of habitats in relative proportion to their local availability (Barbour et al. 1999).

The USEPA-RBP states that for assessment and monitoring, sites can either be *targeted*, i.e., relevant to special studies that focus on potential problems, or *random*, which provides information of the overall status or condition of the watershed, basin, or region. In a random or probabilistic sampling regime, stream characteristics may be highly dissimilar among the sites, but will provide a more accurate assessment of biological condition throughout the area than targeted design. Most studies conducted by state water quality agencies for identification of problems and sensitive waters are done with a targeted design.

The recommended sampling season is mid to late summer, when stream and river flows are moderate to low, and less variable than during other seasons. The USEPA-RBP suggest that the stream length to be sampled can be either a fixed or a proportional distance, with the selection based on the results of pilot studies.

The USEPA-RBP endorses electro-fishing as the most comprehensive and effective single method for collecting stream fishes. Protocols suggest that collection efforts begin at a shallow riffle, or other physical barrier at the downstream limit of the sample reach, and terminate at a similar barrier at the upstream end of the reach. Each sample should contain riffle, run, and pool habitats, when available. It is further suggested that if a reach contains a bridge or a road crossing, sufficient sampling be conducted upstream of the structure to minimize the hydrological effects on the overall quality of the habitat.

### 6.3.1 Wadeable Streams

The suggested sampling scheme for wadeable streams uses a two-person crew that electro-fishes in an upstream direction using a bank-to-bank sweeping technique that maximizes coverage area. All wadeable habitats within the reach should be sampled in a single pass which terminates at an upstream barrier. Fish are held in buckets for subsequent identification.

### 6.3.2 Boatable Streams

The USEPA-RBP state that a proportional-distance designation may be desirable in order to allow for variation in reach length based on stream width (e.g., 40 times wetted width). If a proportional distance approach is used in large streams, electro-fishing should be limited to a maximum distance of 500 m or a maximum time of three hours per sampling site (Klemm et al. 1993).

### 6.3.3 Data Recorded

Field identifications of collected fish are acceptable; however, voucher specimens preserved in a formalin solution must be retained for laboratory verification, particularly if there is any doubt about the correct identity of the specimens. Because the collection methods used are not consistently effective for young-of-the-year fish and because their inclusion may seasonally skew bio-assessment results, it is suggested that fish less than 20 mm in total length not be identified or included in standard samples (Barbour et al. 1999).

## 6.4 Ohio EPA Fish Data Collection Methods

The selection of fish sampling sites is based upon several factors including, but not limited to: 1) location of point source dischargers; 2) stream use designation evaluation issues; 3) location of physical habitat features; 4) location of non-point sources of pollution; 5) variations in macro-habitat; and 6) proximity to ecoregion boundaries. Ohio EPA methods for boatable and wadeable streams have been published (OEPA 1988) and both will be discussed in this document.

Fish sampling generally takes place between mid-June and mid-October. The total time a site is fished varies depending on the current, number of fish being collected, and amount and type of cover within a zone. However, an Ohio EPA review of electro-fishing samples suggest at least 1300-1500 seconds should be spent boat electro-fishing a 0.5 km stream segment (Ohio EPA 1989).

The principal method used by Ohio EPA to obtain fish relative abundance and distribution data is pulsed direct current electro-fishing. Boatable sites are electro-fished for 500 m and wadeable sites are electro-fished for 150-200 m. Each site is electro-fished two or three times during the sampling season (Ohio EPA 1988).

### *6.4.1 Wadeable Streams*

Wadeable streams are sampled with backpack (Figure 6-1), sportyak or longline electro-fishing methods developed by Ohio EPA.

### *6.4.2 Boatable Streams*

Boatable sites are sampled using electro-fishing methods based on the work of Gammon (1973, 1976) and the experience of the Ohio EPA.

### *6.4.3 Data Recorded*

Captured fish are identified in the field with laboratory vouchers required for any new locality records, new species, and those specimens that cannot be field identified. The collection techniques used are not consistently effective for fish less than 15-20 mm in length, therefore, identification and inclusion in the sample are not recommended.

## 6.5 MDNR-MBSS Fish Data Collection Methods

The Maryland Biological Stream Survey (MBSS) is a statewide monitoring survey to assess the status of biological resources in Maryland's non-tidal streams and determine the extent to which acidic deposition has affected or may be affecting critical biological resources in the state. The MDNR-MBSS targets streams of 3rd order and less. The Index of Biological Integrity (IBI) for fish that was derived and utilized by the state of Maryland compares the condition of biological assemblages to that of a regional reference representing conditions minimally influenced by anthropogenic disturbance.

Sample sites were selected in a probabilistic manner using a multi-stratification design. This geographic stratification facilitated the effective use of a limited number of crews. Two basins were randomly selected, without replacement, from each region for each sampling year. One randomly selected basin in each region was to be visited twice to quantify between-year variability in the response variables.

### *6.5.1 Wading Methods*

The MDNR-MBSS samples a fixed stream length of 75 m during the summer index period. Sites are sampled using a double-pass electro-fishing methodology. In general, a single electro-fishing unit is used when the segment width is less than ten meters and two or more units are used for greater widths. Block nets are placed at each end of the segment and direct current backpack electro-fishing units (Figure 6-1) are used to sample the entire segment. An attempt is made to throughly fish each segment, sampling all available cover and habitat structures throughout the segment. A consistent effort is applied over the two passes.

For each pass, all non-game species are weighed together for an aggregate biomass measurement and all game species are weighed in aggregate to the nearest 10 g. For each pass, up to 50 individuals of each gamefish species (i.e., trout, bass, walleye, pike, chain pickerel, or striped bass) are measured for total length (Figure 6-3). For both passes, up to 100 fish of each species are examined for visible external pathology or anomalies. This sampling approach allows for the computation of several metrics useful in calculating a biological index and in producing estimates of fish species abundance.

Also, supplemental electro-fishing is conducted at non-random sites in which only the presence of each fish species is recorded. This provides auxiliary qualitative information on fish distributions. For the supplemental samples, the sampling effort is based on a minimum of 600 seconds or double the elapsed time since the last new species was recorded.

### 6.5.2 Boating Methods

Because boatable streams do not fall within the framework of the program's objectives, the MDNR-MBSS does not provide methods for boatable streams.

### 6.5.3 Data Collected

Captured fish are identified to species, if possible, and counted. Any individuals which cannot be identified to species are retained for laboratory confirmation.

After the processing of the fish collection is completed in the field, voucher specimens are retained for each species not previously collected in the drainage basin and the remaining fish are released. All voucher specimens and fish retained for positive identification in the laboratory are examined and verified (Roth et al. 1997b).

## 6.6 Origin and Development of the IBI and Modified IWB

*The IBI* was first developed by Karr (1981) for use in small warm water streams in central Illinois and Indiana, and further refined by Karr et al. (1986). The original version had 12 metrics that reflected fish species richness and composition, number and abundance of indicator species, trophic organization and function, reproductive behavior, fish abundance, and condition of individual fish. Each metric received a score of five points if it had a value similar to that expected for a fish community characteristic of a system with little human influence, a score of one point if it had a value similar to that expected for a fish community that departs significantly from the reference condition, and a score of three points if it had an intermediate value.

The original version of the IBI quickly became popular. As it became more widely used, different versions were developed for different regions and different ecosystems. These new versions also had multi-metric structures, but differed from the original version in the number, identity, and scoring of metrics (Simon and Lyons 1995). Some versions developed for streams and rivers retain many of the original IBI metrics, with metrics usually being modified as a part of an effort to compensate for insensitivities to environmental degradation in a particular geographic area or type of stream. Similarly, the metrics used in versions of the IBI developed for other types of ecosystems, such as estuaries, impoundments, and natural lakes, usually bear a limited resemblance to those of the original version yet retain its overall structure (Simon and Lyons 1995).

The multi-metric indices currently used by USEPA-EMAP-SW, Ohio EPA, and MDNR-MBSS have all followed this same chain of development; all contain some metrics with origins in Karr et al.'s 1986 IBI. In general, selection of which metrics to drop, modify or add have been determined by first developing a list of candidate metrics (various attributes of the fish assemblages) and then statistically determining which formulations were effective in discriminating between reference sites and sites known to be degraded.

*The Index of Well-Being (Iwb)*, or composite index, was developed by Gammon (1976) to evaluate the response of riverine fish communities to environmental stress. This index was tested using data from the Wabash River in Indiana (Gammon 1976; Gammon et al. 1981) and subsequently from other rivers in Indiana, Ohio (Yoder et al. 1981; Gammon 1980), and Oregon (Hughes and Gammon 1987). Some investigators have modified the original Iwb for specific applications.

The Iwb incorporates four measures of fish communities that have traditionally been used separately; numbers of individuals, biomass, the Shannon diversity index (*H'*) based on numbers of fish, and the Shannon diversity index (*H'*) based on weights of fish (OEPA 1989). The computational formulas for the Iwb and Shannon index are provided in Appendix E.

## 6.7 Indices used by the Programs to Interpret Fish Data

There are two primary indices utilized by these assessment programs to interpret collected fish data. The USEPA-EMAP-SW, USEPA-RBP, Ohio EPA, and MDNR-MBSS programs endorse or have developed versions of the IBI (Karr 1981) for use in their respective waters. The IBI includes discrete measurements of assemblage attributes, or metrics based on species composition, trophic composition, abundance, and condition (Davis 1995). In addition to the IBI, Ohio EPA subjects data to a modified version of the Index of Well-Being (Iwb). The Iwb is based on structural attributes of the fish community whereas the IBI additionally incorporates functional characteristics. Their use in combination is suggested by Ohio EPA (1988) to provide a rigorous evaluation of overall fish community condition. The USGS-NAWQA program does collect information on aquatic vertebrates, but specific methods used to interpret data were not available as of the completion of this document. The USGS-NAWQA program does not rely on a single index approach such as the IBI; rather, a combination of multivariate and multimetric approaches to data analysis are used to examine factors affecting biological water-quality characteristics. Indices that have been locally or regionally calibrated to reference conditions are used at the study-unit level where required data are available.

### 6.7.1 USEPA-EMAP-SW Fish Data Interpretation

The goal of the USEPA-EMAP-SW program is to monitor the condition of the Nation's ecological resources, to evaluate the success of current policies and programs, and to identify emerging problems before they become widespread or irreversible (Gurtz and Muir 1994).

The USEPA-EMAP-SW program is in the process of developing an IBI for wadeable streams in the MAH region of the United

States. The USEPA-EMAP-SW MAH version of the IBI is being developed by examining the responses of fish community metrics to physical, chemical, habitat and landscape indicators of catchment disturbance. Univariate and multivariate analyses of relationships among fish community metrics, habitat integrity and anthropogenic disturbance are being used to develop this index. Table 6-1 lists the metrics proposed for inclusion in the index.

USEPA scientists developed their IBI by randomly selecting sampling sites in the designated study area, collecting field measurements, and then analyzing the resulting data, with respect to candidate metrics, in order to establish expectations for minimally degraded streams. Reference values were derived from sites scoring in the upper 15% of all sites sampled. Individual sites were therefore compared to this reference condition rather than upstream, or similar stream, individual "reference sites" selected as being minimally impacted, as is commonly practiced, by best professional judgement. This IBI is being developed for wadeable systems and its metrics are not adjusted for watershed size. This is probably a reflection of the size of the watersheds of the study area (most are less than 500 $km^2$), the fact that these were predominantly upland systems, and the historical biogeography of the fish fauna.

**Table 6-1.** Metrics in the Index of Biotic Integrity for the USEPA-EMAP-SW Program.

| Metric | Expected response to stress |
|---|---|
| Native species richness | Decrease |
| Native family richness | Decrease |
| Sensitive species richness | Decrease |
| Tolerant individuals | Increase |
| Benthic species richness | Decrease |
| Water column species richness | Decrease |
| Alien individuals | Increase |
| Number of trophic guilds | Decrease |
| Percent top carnivores | Decrease |
| Percent invertivore individuals | Decrease |
| Percent herbivores | Decrease |
| Percent omnivore individuals | Increase |
| Number of specialized reproductive strategies | Decrease |
| Proportion of gravel spawning species | Decrease |
| Proportion tolerant substrate spawners | Increase |
| Total abundance | Decrease |

The 16 metrics of the MAH IBI will be scored continuously from 0-10 and the resulting IBI scores converted from a range of 0-160 to a range of 0-100%. No information is currently available concerning the development of an IBI for boatable systems (McCormick and Hughes 1998).

The initial steps in deriving an IBI score for a wadeable location involves the collection and identification of samples and entering collected information into a database. Once this process is complete, species-specific information relevant to the metrics is determined. This information is obtained from a list that contains the taxa occurring in the waters of the study area as well as designations for use in IBI metrics (Appendix F). Parameters assigned to individual species include tolerance, trophic status, habitat preference, reproductive strategy, and watersheds to which the species is native. Totals are derived and metrics are scored and summed. Streams with an IBI value of >85% are used as the reference condition, scores between 70-85% are *acceptable*, streams with IBI values between 50-70% are *marginally impaired*, and IBI scores below 50% are *highly impaired*.

Protocols for the interpretation of fish data collected from boatable sites have yet to be developed (McCormick and Hughes 1998).

## 6.7.2 USGS-NAWQA Fish Data Interpretation

The methods used by the USGS-NAWQA program to interpret information collected on aquatic vertebrates program is not available as of the completion of this document (Meador et al. 1993a). The USGS-NAWQA program does not rely on a single index approach such as the IBI; rather, a combination of multivariate and multimetric approaches to data analysis are used to examine factors affecting biological water-quality characteristics. Indices that have been locally or regionally calibrated to reference conditions are used at the study-unit level where required data are available.

## 6.7.3 USEPA-RBP Fish Data Interpretation

The USEPA-RBP endorses the technical framework of the multi-metric Index of IBI developed by Karr (1981) for the assessment of fish assemblages. The 12 metrics included in Karr's (1981) original IBI are in Table 6-2.

**Table 6-2.** Metrics Recommended for Calculation by the USEPA-RBP

| Metric | Expected Response to stress |
|---|---|
| Total number of fish species | Decrease |
| Number and identity of darter species | Decrease |
| Number and identity of sunfish species | Decrease |
| Number and identity of sucker species | Decrease |
| Number and identity of intolerant species | Decrease |
| Proportion of individuals as green sunfish | Increase |
| Proportion of individuals as omnivores | Increase |
| Proportion of individuals as insectivorous cyprinids | Decrease |
| Proportion of individuals as top carnivores | Decrease |
| Number of individuals in sample | Decrease |
| Proportion of individuals as hybrids | Increase |
| Proportion of individuals with disease, tumors, fin damage, and skeletal anomalies | Increase |

Although the USEPA-RBP recommends the framework of Karr's (1981) IBI, they also recommend that some modifications may be needed to adjust for the regional differences between surveys. The protocols further state that the IBI "serves as an integrated analysis because individual metrics may differ in their relative sensitivity to various levels of biological condition" (Barbour et al. 1999). Calculation and interpretation of the IBI involves a sequence of activities including, fish sample collection, data tabulation, regional modification and calibration of metrics, and determination of expected values (Barbour et al. 1999). Once this process is complete, species-specific information relevant to the metrics can be assigned.

For each sampling location, metrics are developed and scores (1, 3, or 5) are assigned according to the thresholds established during the indicator development process. The final IBI score is the sum of all metric scores (Barbour et al. 1999).

### 6.7.4 Ohio EPA Fish Data Interpretation

The Ohio EPA assessment program was designed to support all state agency surface water programs. Ohio EPA has used measurable characteristics of instream fish since 1980. The principal measures of overall fish community health used by the Ohio EPA are the Iwb, developed by Gammon (1976) and modified by Ohio EPA, and the IBI developed by Karr (1981).

The IBI utilized by Ohio EPA contains 12 metrics specifically tailored to Ohio surface waters and Ohio EPA sampling methods. The IBI metrics used by the Ohio EPA to evaluate wading sites (Table 6-3; Appendix F) closely approximate those proposed by Karr (1981) and refined by Fausch et al. (1984) and Karr et al. (1986). Substantial modifications were necessary for the IBI metrics used for the boat sites and headwater sites. These changes were made in recognition of the different sampling efficiency and selectivity of the boat methods and the different faunal characters of larger streams and rivers and headwater areas. However, these modifications were made in keeping with the guidance given by Karr et al. (1986). Three basic divisions are made; wading sites, boat

**Table 6-3.** Metrics Employed by the Ohio EPA with Expected Response to Stress.

| Metric | Expected response to stress |
|---|---|
| Total number of species[1] (a,b,c) | Decrease |
| Number of darter species (a[2],b)/Percent round-bodied suckers[3] (c) | Decrease |
| Number of headwater species (a)/ Number of sunfish species (b,c) | Decrease |
| Number of minnow species (a)/ Number of sucker species (b,c) | Decrease |
| Number of sensitive species (a)/ Number of intolerant species (b,c) | Decrease |
| Percent tolerant species (a,b,c) | Increase |
| Percent omnivores (a,b,c) | Increase |
| Percent insectivorous species (a,b,c) | Decrease |
| Percent pioneering species (a)/ Percent top carnivores (b,c) | Decrease |
| Number of individuals[4] (a,b,c) | Decrease |
| Number of simple lithophilic species (a)/Percent simple lithophils (b,c) | Decrease |
| Percent DELT anomalies[5] (a,b,c) | Increase |

[a]Headwater sites, drainage areas less than 20 mi$^2$., sampled with wadeable methods.
[b]Wading sites, sites sampled with wadeable methods.
[c]Boat sites, these sites are sampled with boat methods.
[1]Excludes exotic species.
[2]Includes sculpins.
[3]Includes suckers in the genera *Hypentelium, Moxostoma, Minytrema,* and *Erimyzon*; excludes white sucker (*Catostomus commersoni*).
[4]Excludes species designated as tolerant, hybrids, and exotics.
[5]Includes deformities, eroded fins, lesions, and external tumors (DELT).

sites, and headwater sites. Generally, wading sites are those having a drainage area of less than 300 $mi^2$ but greater than 20 $mi^2$. Boat sites include streams and rivers that are too deep and large to sample effectively with wading methods. Boat sites generally exceed 100-300 $mi^2$ in drainage area. Headwaters sites are defined as sampling locations with drainage areas less than 20 $mi^2$.

The value of each metric is compared to the value expected at a reference site located in a similar geographic region where human influence has been minimal. Ratings of 5, 3, or 1 are assigned to each metric according to whether its value approximates (5), somewhat deviates from (3), or strongly deviates from (1) the value expected at a reference site. The maximum IBI score possible is 60 and the minimum is 12. Reference site scores are grouped by ecoregion (Omernik 1987) and used to statistically generate region specific use attainment criteria (OEPA 1988).

*The* Iwb used by the Ohio EPA is a modified version of that developed by Gammon (1976). The Iwb is based on structural attributes of the fish community. Four measures of fish communities that traditionally have been used separately are: numbers of individuals, biomass, and the Shannon diversity index (*H'*) based on numbers and weights of fish.

The modified Iwb retains the same computational formula as the conventional Iwb developed by Gammon (1976). The difference is that highly tolerant species, exotic species, and hybrids are eliminated from the numbers and biomass components of the Iwb. However, tolerant and exotic species are included in the two Shannon index calculations. This modification eliminates the *undesired* effect caused by the high abundance of tolerant species, but retains their *desired* influence on the Shannon indices. Computational formulas for the index of well being and the Shannon diversity index are in Appendix E.

### 6.7.5 MDNR-MBSS Fish Data Interpretation

Maryland scientists began their development of an IBI by first establishing expectations for minimally degraded streams and then comparing the ability of candidate metrics to discriminate between these reference sites and sites known to be degraded. The resulting IBI consists of eight metrics (Table 6-4), each of which quantitatively describe attributes of the biological community. Each of the metrics used has an expected direction of change in response to anthropogenic stress. For each sampling location, metrics are developed and scores (1, 3, or 5) assigned according to the thresholds established during the indicator development process. The final IBI score is the mean the metric scores. No IBI score is assigned to sites having watershed area less than 300 acres (Roth et al. 1997b).

The initial steps in deriving an IBI score for a location involves collecting, identifying, and entering collected information into a database. Once this process is complete, species specific information relevant to the metrics can be assigned. This information is obtained from a Maryland fish species list that contains designations for use in IBI metrics (Appendix F). Parameters assigned to individual species included tolerance, trophic status, native or non-native status by watershed, if the species was considered benthic, and if the species was a lithophilic spawner. Totals are derived and metrics scored as in Appendix E. The metrics used by the MDNR-MBSS for their IBI are given in Table 6-4 (Roth et al. 1997c; Stribling et al. 1998).

**Table 6-4.** Metrics Employed by MDNR-MBSS and Expected Response to Stress.

| Metric | Expected response to stress |
|---|---|
| Number of native species | Decrease |
| Number of benthic species | Decrease |
| Percent of tolerant individuals | Increase |
| Percent abundance of dominant species | Increase |
| Percent generalist, omnivores, and invertivores | Increase |
| Number of individuals per $m^2$ | Decrease |
| Biomass (g per $m^2$) | Decrease |
| Percent lithophilic spawners | Decrease |
| Percent insectivores | Decrease |

## 6.8 Comparison of the Fish Assessment Programs of the USEPA-EMAP-SW, USGS-NAWQA, USEPA-RBP, Ohio EPA, and MDNR-MBSS

*Site selection* - The method used to determine the location of the sampling sites varies among the five programs discussed in this document. For the USEPA-EMAP-SW and MDNR-MBSS, sampling sites are randomly selected. The USGS-NAWQA usually utilizes fixed sampling sites. Ohio EPA selects its sites based on site-specific and regional issues. The USEPA-RBP states that for assessment and monitoring, sites can either be “targeted”, i.e., relevant to special studies that focus on potential problems, or “random”, which provides information of the overall status or condition of the watershed, basin, or region.

*Sampling season/Index period* - All five programs reviewed either use or endorse the use of a summer index period. The general consensus for this is that this period coincides with the low and stable flow period; these conditions increasing the likelihood that samples throughout the study will be collected under similar flow conditions.

*Stream distance sampled/sampling reach* - The method used to determine the stream length to be sampled at a chosen site varies among the selected programs. The USEPA-EMAP-SW program uses a stream length that is 40 times the wetted width or 150 m, whichever is greater. The reach length sampled by the USGS-NAWQA program includes two types of geomorphic channel units or 20 times the channel width if repetitive geomorphic channel units are not present. Acceptable ranges for wadeable streams is 150 to 300 m where the acceptable range for boatable stream is 500 to 1000 m. Ohio EPA samples 150 to 200 m in wadeable streams and 500 m in boatable streams. MDNR-MBSS uses a fixed stream length of 75 m. The USEPA-RBP manual suggests that either a fixed-distance method or a proportional-distance method of determining reach length would be acceptable, but final decisions should be based on the goals of the study as well as results of pilot studies conducted in the study area.

*Sampling method* - All of the programs reviewed in this document use electro-fishing, either alone or in conjunction with other sampling gear, to assess fish populations. Ohio EPA uses electro-fishing exclusively in both wadeable and boatable streams. Each stream length is sampled in either 2 or 3 passes per sampling season with the electro-fishing gear. The USEPA-EMAP-SW and USGS-NAWQA use electro-fishing methods with the assistance of additional gear, principally seines. The two programs differ, however, in that the USEPA-EMAP-SW program electro-fishes one bank of the designated stream length in one pass whereas the USGS-NAWQA program uses a double-pass sampling scheme to sample both banks on the same day. The MDNR-MBSS also uses a double-pass electro-fishing method to sample both banks on the same day in addition to incorporating the use of block nets to delimit the reach if necessary. The use of seines to delimit a stream reach is also occasionally employed by the USEPA-EMAP-SW program. The USEPA-RBP endorses a single pass electro-fishing method supplemented with seining and further suggests the use of block nets to delimit the reach if necessary.

*Measure of fish community health* - Many of the metrics used in the regionally-developed IBIs overlap between the programs. Among the three programs that have published IBIs, the number of metrics employed varies. The USEPA-EMAP-SW IBI contains 16 metrics, the Ohio EPA IBI contains 12 metrics, and the MDNR-MBSS IBI contains 8 metrics. Within programs, some metrics vary depending upon the size of the stream sampled (Ohio EPA) or upon its location (MDNR-MBSS).

In addition to its own IBI, the Ohio EPA also uses a modified version of Gammon's (1976) Iwb. This index incorporates measurements concerning the structure of the fish community.

All sampled sites are scored against an established set of criteria. The USEPA-EMAP-SW program compares sampled sites to expectations for minimally degraded streams. Minimally impacted values were derived from sites scoring in the upper 15% of all sites sampled. Individual sites are therefore compared to a reference condition rather than values derived from minimally impacted reference sites. The USGS-NAWQA, USEPA-RBP, Ohio EPA, and MDNR-MBSS programs either use or suggest the use of reference sites. This involves comparing sampled sites to the value expected at a reference site located in a similar geographic region where human influence has been minimal.

## 6.9 Conclusions Regarding Potential Comparisons Of Fish Data

Different researchers and programs may have different reasons for conducting bioassessments and these differences do not necessarily require the same level or type of effort in sample collection, taxonomic identification, or data analysis (Gurtz and Muir 1994). However, different methods of sampling and analysis may yield comparable data for certain objectives despite differences in effort (Barbour et al. 1999). As an example, we can compare the conclusions drawn by different programs conducting research in the same areas. A pilot field study comparing some of the methods of three of the reviewed programs (USEPA-EMAP-SW, USGS-NAWQA, Ohio EPA) concurrently in large river systems was conducted in the summer of 1999. Such studies will yield useful information about methods employed, especially in reference to the effectiveness of compared methods in detecting differences when they exist and not detecting differences when they do not exist. Such comparisons would also be beneficial to cost and benefit analyses of methodologies.

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# Appendix A
# Descriptions Of The Habitat Assessment Parameters

## A.1 Description of USEPA-EMAP-SW Habitat Assessment Parameters

The habitat assessment index being developed by USEPA-EMAP-SW currently contains three distinct indices: 1) the Rapid Habitat Assessment (RHA) index; 2) the Physical Habitat Assessment (PHab) index; and 3) the Streams/Rivers Assessment (SRA) index. Short descriptions of the individual assessment metric comprising these indices are given below (Kaufmann and Robison 1998).

### *A.1.1. USEPA-EMAP-SW Rapid Habitat Assessment Index (RHA)*

The USEPA-EMAP-SW RHA index is very similar to both the MDNR-MBSS and USEPA-RBP indices. The 12 metrics used in the RHA index are described below. Each ranking category has a range of possible scores associated with it (i.e., Optimal 20 to 16, Sub-Optimal 15 to 11, Marginal 10 to 5, Poor 5 to 0) based on an assessment of the entire sample segment. A total maximum index score of 240 is possible. Unlike the QHEI, no negative metric scores are used and no habitat-ranking scheme has been produced.

1) *Instream Cover (Fish)* - Scores are based on the amount and diversity of useable fish cover types observed across the entire sampling segment. The highest scores are given to areas having more than a 50% mix of boulders, cobble submerged logs, undercut banks, or other stable habitat and judged to have adequate amount of habitat. The lowest scores are given to areas with less than 10% of these cover types and that obviously lack an adequate amount of habitat. Scored 0 to 20.

2) *Epifaunal Substrate* - Scores are based on assessing the entire sampling segment for the presence and size of riffles and the amount of cobble substrate present. The highest scores are given to areas that have well-developed riffles and runs and streams

with an abundance of cobble. The lowest scores are given to areas in which riffles and runs are almost non-existent and that lack cobble substrate. Scored 0 to 20.

3) *Velocity/Depth Regimes* - Scoring of this metric is based on the variety and velocity of velocity/depth regimes found within the stream sample segment. Streams with the four velocity regimes, *slow-deep*, *slow-shallow*, *fast-deep*, and *fast-shallow*, are scored the highest and those that are dominated by one velocity/depth regime (usually *slow-deep*) are scored the lowest. Scored 0 to 20.

4) *Frequency of Riffles* - Scores for this metric are based on the frequency and occurrence of riffles and the variety of habitat found within the stream sample segment. Streams with frequent riffles and diverse habitat are scored the highest. Streams with poor habitat and low frequency of well-developed riffles are scored the lowest. Scored 0 to 20.

5) *Channel Alteration* - Scoring of this metric is based on the type and amount of channel alteration and disruption found within the stream sample segment. Streams with no channelization or dredging present are scored the highest and those that are dominated (more than 80% of the reach) by channelization and disruption are scored the lowest. Scored 0 to 20.

6) *Bank Condition (Bank Erosion)* - Scores for this metric are based on evidence of bank stability and erosion. Streams with stable banks and showing little evidence of erosion or bank failure are scored the highest. Streams that have unstable banks, banks with many eroded areas, and banks showing 60 to 100% evidence of erosional scarring are scored the lowest. Scored 0 to 20.

7) *Embeddedness* - Scoring for this metric is based on the percentage of stream gravel, cobble, and boulder particle surface area that is surrounded by fine sediment or flocculent materials. High scores are given for areas with low embeddedness (0 to 25% surrounded) and low scores are given to areas with high embeddedness (more than 75% surrounded). Scored 0 to 20.

8) *Channel Flow Status* - Scores for this metric are based on the degree to which water fills the channel and the amount of exposed substrate that occurs within the channel. Streams in which the water reaches the base of both banks and a very small proportion of the channel substrate is exposed are scored the highest. Streams that have little water in the channel, most of which is in standing pools, are scored the lowest. Scored 0 to 20.

9) *Riparian Vegetation Zone Width (Least Buffered Side)* - Scores for this metric are based on the width of the riparian zone and the presence or absence of human disturbances. Streams with a riparian zone width of more than 18 m and no evidence of impacts from human activities are scored the highest. Streams with a riparian zone width of less than 6 m and evidence of human activities are scored the lowest. Scored 0 to 20.

10) *Sediment Deposition* - Scores for this metric are based on the degree of bar development and the extent that the stream channel is affected by sedimentation within the stream sample segment. Streams with little or no bar enlargement and those where less than 5% of the stream bottom is affected by sediment deposition are scored the highest. Streams with heavy deposits of fine sediment, increased bar development, and more than 50% of the bottom changing frequently due to sedimentation are scored the lowest. Scored 0 to 20.

11) *Bank Vegetative Protection* - Scores for this metric are based on the percentage of

the stream bank surfaces that are covered by vegetation. Streams that have more than 90% of their bank surfaces covered by vegetation are scored the highest. Streams that have less than 50% of their bank surfaces covered by vegetation are scored the lowest. Scored 0 to 20.

12) *Grazing or Other Disruptive Pressure* - Scores for this metric are based on the degree of vegetative disruption by mowing or grazing on the banks of the stream. Stream banks that are minimally disturbed are scored the highest. Streams with banks that have very disturbed vegetation (vegetation removed to an average of ≤ 2") are scored the lowest. Scored 0 to 20.

## A.1.2 USEPA-EMAP-SW Physical Habitat Assessment Index (PHab)

The PHab has four primary metrics, each of which is made up of a varying number of sub-metrics. Many of these sub-metrics are based on direct numerical measurements made in the field and are therefore quantitative rather than qualitative. Some of the PHab metrics are based on ranked categories of field measurements. The goal of the PHab sampling design is to assess habitat and other stream conditions over the sampling reach. No overall composite score is produced by this index.

1a-g) *Thalweg Profile* - The thalweg profile is a longitudinal survey of the sub-metrics: *Maximum Depth, Wetted Width, Bar Width, Soft/Small Sediment Presence, Channel or Pool Type, Pool Forming Element*, and *Side Channel Presence.* The thalweg measurements (except wetted width) are generally taken at 100 to 150 equally spaced points (10 to 15 intervals between each of 11 channel cross-section sampling stations) along the centerline of the stream between the two ends of the sample reach. Thalweg wetted width is measured at 21 equally spaced intervals (at each of 11 channel cross-section sampling stations and a station mid-way between cross-section sampling stations). Spacing of the thalweg measurements is based on the channel width. The samples are taken at 1 m, 1.5 m or 0.01 times reach length, for channel widths of less than 2.5 m, 2.5 to 3.5 m, and more than 3.5 m, respectively. Sampling is designed to resolve deep areas and habitat units that range from 1/3 to ½ the channel width. Sampling proceeds upstream along the middle of the channel. Data from the thalweg profile is intended to allow the calculation of indices of residual pool volume, stream size, channel complexity, and the relative proportions of habitat types such as riffles and pools.

1a) *Thalweg Profile, Maximum Depth* - The greatest depth in the channel is measured to the nearest cm, at each of the 100 increments of length upstream along the mid-channel line. The *thalweg maximum depth* is not necessarily the mid-channel line.

1b) *Thalweg Profile, Wetted Width* - The *thalweg wetted width* is the width between the left and right wetted boundaries (the point at which substrate particles are no longer surrounded by free water). It is measured across and over bars. Widths are measured to the nearest 0.1 m for widths up to 3 m and to the nearest 5% of the width if the width is greater than 3 m. They are usually only measured at 21 sample stations. However, if a higher resolution is needed, *thalweg wetted widths* can be taken at all 100 to 150 sample stations.

1c) *Thalweg Profile, Bar Width - Bars* are defined by PHab as channel features below the bankfull mark that are dry during baseflow conditions. Islands are features that are dry even during bankfull conditions. If a mid-channel feature is as high as the surrounding flood plain, it is treated as an island. When present, bar widths are determined at each thalweg.

1d) *Thalweg Profile, Soft/Small Sediment Presence* - When the rod or staff is used to make the thalweg depth measurement, it is also used to determine the presence or absence of small, loose, soft sediments at each of the thalweg sampling stations. Small/soft sediments are defined by PHab as fine gravel, sand, silt, clay, or muck.

1e) *Thalweg Profile, Channel or Pool Type* - A channel unit scale habitat classification is used to visually determine and classify channel or pool features into one of 12 possible categories at each of the thalweg sampling stations. These categories include: *glide, riffle, rapid, cascade, falls, dry channel*, or one of five pool types. The feature should be at least as long as the channel is wide if it is to be included.

1f) *Thalweg Profile, Pool Forming Element* - When present, pools are classified using seven categories, based on the element from which the pool is formed (e.g., boulder, large woody debris, etc.).

1g) *Thalweg Profile, Side Channel Presence* - The presence of side channels is noted at each of the thalweg sampling stations. Notes about their point of convergence and divergence with the main channel are taken.

*2) Woody Debris* - The large woody debris (LWD) measurement used by PHab is a simplified version of Robison and Beschta's (1990) method. It provides quantitative estimates of the number, size, total volume and distribution of wood in the stream reach. LWD is defined by PHab as woody material with a small end diameter of at least 10 cm and a length of at least 1.5 m. All pieces of LWD in (partially or fully) or spanning the active channel (flood channel up to bankfull) are tallied for the area between each sampling cross section. The tallies are assigned to separate categories based on: 1) location in the channel (above or in), 2) length (1.5 to 5 m, 5 to 15 m, or more than 15 m) and 3) large end diameter (more than 0.8 m, 0.8 to 0.6 m, 0.6 to 0.3 m, 0.3 to less than 0.1 m). When length is evaluated, only the part with a diameter more than 10 cm is included. Each piece of LWD is counted as one tally entry and the whole piece is included even if part of it is outside the bankfull channel. The LWD is assigned to the sampling cross section containing the large end.

3a-c) *Channel* and *Riparian Cross-Sections* - Three primary classes of measurements are performed at the 11 channel cross section stations: 1) quantitative measurements of channel cross-section dimensions, bank characteristics and stream channel gradient, sinuosity, and riparian cover; 2) visual estimates of substrate size class and embeddedness, areal cover class and type of riparian vegetation in canopy, mid-layer and ground cover, areal cover class of fish concealment features, aquatic macrophytes, and filamentous algae; and 3) recorded observations of human disturbances and their proximity to the channel.

3a) *Channel* and *Riparian Cross-Sections, Quantitative Measurements* - The cross-sectional dimensions, *bankfull width, wetted width* and *bar width* are measured as described above for the thalweg profile stations. The channel bankfull height is estimated as the height of the bankfull flow above the water level. The channel incised height is estimated as the height from the water surface to the first terrace of the flood plain (the area at or above the bankfull height). The slope or gradient, determined using a clinometer, and the bearing, determined using a compass, are measured between the cross section stations. Supplemental measurements are taken in situations where the direct line of sight between stations is obscured. Estimates of residual pool depth and volumes may be made possible, by applying methods described by Stack (1989) and Robison and Kaufmann (1994), to the slope and the thalweg depth and width measurements. Channel sinuosity can be computed using the bearing and distance measurements. Riparian canopy cover over the stream is quantified using a Convex Spherical Densiometer (Lemmon 1957). Four readings (one in each direction while standing in the center of the stream) are taken at each of the 11 cross section stations. Two bank side readings (one on each bank) are also taken at each site. These measurements are made with the observer's back to the stream.

3b) *Channel* and *Riparian Cross-Sections, Visual Estimates* - Substrate size class and embeddedness are evaluated at five equally spaced points centered between the wetted channel width boundaries, at each of the 11 channel cross section stations. Water depth and distance from the left bank is also determined at each sampling point. The substrate at each point is visually inspected and classified into one of 11 categories based on size or origin. For particles larger than sand, the average embeddedness in a 10 cm circle is estimated. Observations are made to estimate areal cover class and type of riparian vegetation in canopy (more than 5 m high), mid-layer or understory (0.5 to 5 m high), and ground cover (less than 5 m high). A portion of the riparian zone from the shoreline to a distance of 10 m on either side of the bank and 5 m up and down stream (10 m X 10 m area on each bank) is assessed at each of the 11 channel cross-section stations. For each 10 m X 10 m area, and for the canopy and understory cover categories, the percent total cover (expressed as one of four possible categories: 1 = Sparse, <1%; 2 = moderate, 10 to 40%; 3 = heavy, 40 to 75%, or 4 = very heavy, >75%) comprised by each of five broad vegetation types is noted. The percent total cover is also estimated for each bank area, using the same classification for big and small trees in the canopy, woody and non-woody vegetation in the understory; and woody, non-woody, and barren categories in the ground cover layer. Using the classification scheme outlined above, the percent total areal cover of seven kinds of fish concealment features (e.g., aquatic macrophytes, filamentous algae, woody debris, etc) is estimated for the area 5 m up and down stream at each of the 11 channel cross section stations.

3c) *Channel* and *Riparian Cross-Sections, Recorded Observations* - The presence and proximity of 11 categories of human influence in the riparian and stream areas 5 m up and down stream at each of the 11 channel cross section stations, is noted.

4) *Discharge* - Discharge is measured at one location in each sample segment by one of four methods: 1) velocity-area (Linsley et al. 1982), 2) portable weir, 3) calibrated bucket, or 4) time of movement of a neutrally buoyant object. The velocity-area method is preferred in streams large enough to use a water velocity meter. Using this approach, the water velocity at a depth of 0.6 of the total depth, at each of 15 to 20 points, equally spaced across the stream width, is measured. In smaller streams one of the other methods may need to be used. Discharge is measured at the point, where the water chemistry samples are taken.

## A.1.3 USEPA-EMAP-SW Streams/Rivers Assessment Index (SRA)

The USEPA-EMAP-SW SRA index is based on approximately 5 metrics (or components), depending on how they are grouped. Two of the components, *General Assessment* and *Local Anecdotal Information* are written descriptions. The remaining SRA metrics are based on ranked categories of field measurements and classified lists of field observations. No scores are assigned to any of the metrics. Like the measurements for the PHab, it is unclear how these measurements will be utilized in an analysis scheme and no overall index score for the SRA is available.

1) *Watershed Activities and Disturbances* - Watershed activities are broken into five major types: *residential*, *recreational*, *agricultural*, *industrial*, and *stream management*. Listed under each of these activity categories is are examples of typical disturbances associated with each activity. The presence or absence of each disturbance is noted and the intensity of each disturbance ranked into one of three categories, *low*, *moderate*, or *high*.

2a-c) *Reach Characteristics* - Three major categories: *vegetation cover type*, *land use*, and *water clarity* are used to describe and classify the character of the stream sampling reach.

2a) *Reach Characteristics, Vegetation Cover* - The vegetative cover observed at the sample reach is noted and classified into one of five possible categories: *forest*, *shrub*, *wetland*, *bare ground*, or *macrophytes*. During this process, each vegetation cover type is ranked, based on the percent of the reach it comprises (i.e., rare <5%, sparse 5 to 25%, moderate 25 to 75%, and extensive >75%).

2b) *Reach Characteristics, Land Use/Type* - The land use/type observed at the sample reach is noted and classified into one of four possible categories: *agriculture row crop*, *agriculture grazing*, *logging*, or *development*. During this process, land use/type is ranked, based on the percent of the reach it comprises (i.e., rare <5%, sparse 5 to 25%, moderate 25 to 75%, and extensive >75%).

2c) *Reach Characteristics, Water Clarity* - The type of water clarity observed at the site is ranked into one of four categories: *clear*, *murky*, *highly turbid*, or *storm influenced*.

3a-b) *Waterbody Character* - Two categories, *disturbance impact* and *aesthetic quality*, are used to assess the waterbody character at each sample reach.

3a) *Waterbody Character, Disturbance Impact* - The waterbody character at each sample reach is assessed for the degree of disturbance impact observed. This metric is ranked from 1 (highly disturbed) to 5 (pristine).

3b) *Waterbody Character, Aesthetic Quality* - The waterbody character at each sample reach is assessed for it's aesthetic quality. This metric is ranked from 1 (unappealing) to 5 (appealing).

4) *General Assessment* - A general assessment is conducted for stream reach by taking notes on the wildlife, vegetation diversity, and forest age class (0 to 25, 25 to 75, >75 yrs) observed at the site.

5) *Local Anecdotal Information* - Local anecdotal information for the study reach is described.

## A.2 USEPA-RBP

The USEPA-RBP index is very similar to both MDNR-MBSS and USEPA-EMAP-

SW RHA indices. A short description of each of the 13 metrics that comprise the USEPA-RBP habitat assessment index are listed below (Barbour et al. 1999). Three of the metrics, *embeddedness*, *frequency of riffles*, and *velocity/depth combinations*, are only used at high gradient sites, and three of the metrics, *pool substrate*, *pool variability*, and *channel sinuosity*, are only used at low gradient sites. As a result, only ten metrics total are used at any one site. Each ranking category has a range of possible scores associated with it (i.e., Optimal 20 to 16, Sub-Optimal 15 to 11, Marginal 10 to 5, Poor 5 to 0) based on an assessment of the entire sample segment. All of the metrics have a maximum score of 20 points. The metrics *bank stability*, *bank vegetation protection*, and *riparian vegetation zone width*, have maximum scores of 10 points for each bank (maximum 20 points total). A total maximum index score of 200 points is possible.

1) *Epifaunal Substrate and Available Cover* - Used to assess the relative quality of natural structures in the stream as sites for use as refugia, feeding, and reproduction. Scores are based on the amount and diversity of substrate for epifaunal colonization and fish cover observed across the entire sampling segment. The highest scores are given to areas having more than a 70% (in high gradient streams) or more than 50% (in low gradient streams) mix of favorable, stable, substrates and cover types such as submerged logs/snags, undercut banks, cobble, or other stable habitat and at a stage to allow full colonization. The lowest scores are given to areas with less than 20% (in high gradient streams) or less than 10% (in low gradient streams) of these cover types and that obviously lack an adequate or stable habitat. Scored 0 to 20.

2) *Velocity/Depth Combinations (High Gradient)* - This metric is only used for high-gradient streams. Scoring of this metric is based on the variety of velocity of velocity/depth regimes found within the stream sample segment. Streams with the four velocity regimes, *slow-deep*, s*low-shallow*, *fast-deep*, and *fast-shallow*, are scored the highest and those that are dominated by one velocity/depth regime (usually slow-deep) are scored the lowest. Scored 0 to 20.

3) *Pool Substrate Characterization (Low Gradient)* - This metric is only used for low-gradient streams. It is used to assess the type and condition of substrates found in pools. Scoring for this metric is based on the presence of particular substrate types, root mats, and submerged aquatic vegetation (SAV). Generally, an area with diverse substrates support a more diverse array of organisms as compared to areas with uniform substrates. Scores are high for areas exhibiting the presence of mixed substrates, gravel and firm sand, root mats, and SAV. Scores are low for areas with hard-pan clay or bedrock and no SAV. Scored 0 to 20.

4) *Pool Variability (Low Gradient)* - This metric is only used for low-gradient streams. It rates the overall mixture of pool types found in streams by size and depth. Scoring of this metric is based on the variety of basic pool types found within the stream sample segment. Streams that have all four pool types, *large-deep*, *large-shallow*, *small-deep*, and *small-shallow*, are scored the highest and those that are dominated by one pool type (usually small-shallow) or that lack pools, are scored the lowest. Scored 0 to 20.

5) *Frequency of Riffles or Bends (High gradient)* - This metric is only used for high-gradient streams. Scores for this metric are based on the frequency or occurrence of riffles and the variety of habitat found within the

stream sample segment. Streams with frequent riffles and diverse habitat are scored the highest. Streams with poor habitat and a low frequency of well-developed riffles are scored the lowest. Scored 0 to 20.

6) *Channel Alteration* - Is used to assess the impact of large scale changes on the shape of the stream channel. Scoring of this metric is based on the type and amount of channel alteration and disruption found within the stream sample segment. Streams with no channelization or dredging present are scored the highest and those that are dominated (>80% of the reach) by channelization and disruption are scored the lowest. Scored 0 to 20.

7) *Bank Stability (Condition of Banks)* - Scores for this metric are based on evidence of bank stability and erosion. Eroded banks indicate a problem of sediment movement and deposition, and suggest a scarcity of cover and increased organic input to streams. Streams with stable banks and showing little evidence of erosion or bank failure (<5% affected) are scored the highest. Streams that have unstable banks, banks with many eroded areas, and banks showing 60 to 100% evidence of erosional scarring, are scored the lowest. Scored 0 to 10 for each bank, 0 to 20 total.

8) *Embeddedness (High Gradient)* - This metric is only used for high-gradient streams. It is used to assess the extent to which stream substrates are buried by silt, sand or mud. Scoring for this metric is based on the percentage of stream gravel, cobble, and boulder particle surface area that is surrounded by fine sediment. Scores are high for areas of low embeddedness (0 to 25% surrounded) and low for areas with high embeddedness (>75). Scored 0 to 20.

9) *Channel Flow Status* - Scores for this metric are based on the degree to which water fills the channel and the amount of exposed substrate that occurs within the channel. Streams in which the water reaches the base of both banks and a very small proportion of the channel substrate is exposed are scored the highest. Streams that have little water in the channel, most of which are standing pools, are scored the lowest. Scored 0 to 20.

10) *Riparian Vegetation Zone Width (Least Buffered Side)* - Scores for this metric are based on the width of the riparian zone and the presence or absence of human disturbances. Streams with a riparian zone width of more than18 m and no evidence impacts from human activities are scored the highest. Streams with a riparian zone width of less than 6 m and evidence of human activities are scored the lowest. Scored 0 to 10 for each bank, 0 to 20 total.

11) *Sediment Deposition* - Is used to assess the impact of sedimentation on the stream bottom and pools. Scores for this metric are based on the degree of bar development and the extent that the stream channel is affected by sedimentation within the stream sample segment. Streams with little or no bar enlargement and those where less than 5% (for high-gradient streams) or less than 20% (for low-gradient streams) of the stream bottom is affected by sediment deposition are scored the highest. Streams with heavy deposits of fine sediment, increased bar development, and more than 50% (for high-gradient streams) or more than 80% (for low gradient streams) of the stream bottom changing frequently due to sedimentation, are scored the lowest. Scored 0 to 20.

12) *Bank Vegetative Protection* - This metric supplies information on the ability of the bank to resist erosion as well as some additional information on the potential for nu-

trient uptake by plants, the control of instream scouring, and stream shading. Scores for this metric are based on the percentage of the stream bank surfaces that are covered by vegetation. Streams that have more than 90% of the bank surfaces covered by vegetation, particularly native vegetation, with little evidence of grazing or mowing are scored the highest. Streams that have less than 50% of their bank surfaces covered by vegetation, disruption of streamside vegetation is very high, and vegetation has been removed to an average height of less than 5 cm, are scored the lowest. Scored 0 to 10 for each bank, 0 to 20 total.

13) *Channel Sinuosity (Low Gradient)* - This metric is only used for low-gradient streams. Scores for this metric are based on degree of meandering or sinuosity that occurs over the channel length. It is used for streams in which distinct riffles are uncommon. Streams in which the bends in the channel increases its length by three to four times are scored the highest. Streams with straight channels are scored the lowest. Channel braiding is considered normal in coastal plains and low-lying areas so this parameter is not easily ranked in these areas. Scored 0 to 20.

## A.3 Descriptions of Ohio EPA's (QHEI) Parameters

Listed below is a short description of each of the seven metrics that comprise the QHEI (Rankin, 1989). Six of the metrics are based on two or four scored sub-metrics. Each sub-metric is further divided into scored categories which are matched with field observations to produce the scores. The *Gradient* metric is the only metric that does not contain a sub-metric. To compute a final overall score for the QHEI, the scores of the sub-metrics are summed and then the scores of the composite metrics are summed. The maximum score for the composite metrics range from 8 to 20. The maximum total score of the QHEI index is 100.

1a-b) *Substrate (Type and Quality)* - Scores are based on evaluation of two submetrics, *substrate type* and *substrate quality*. The submetric *substrate type* includes identification and diversity of the substrate types present. The submetric *substrate quality* includes determining the origin of the benthic material (parent material), the extent of silt cover, and embeddedness at the sample site. Scored a maximum of 20.

1a) *Substrate, Type* - The type of substrate observed in the sample segment is selected from a list of ten scored categories. The scores range from 0 for artificial substrate to 10 for boulder/slabs. The two most common substrates at the sample site are identified from the list. A single category is selected twice if it predominates (more than 75-80% of the bottom area or clearly is the most functionally dominant type). The total number of substrate types (more than four = 2 points, or four or fewer = 0 points) is used to evaluate substrate diversity. Substrate types must comprise more than 5% of the sampling area to be included. Any substrate types observed but not included in the scored categories are recorded. Scored 0 to 21.

1b) *Substrate, Quality* - The type of parent material observed in the sample segment is selected from a list of seven scored categories. The scores range from -2 for coal fines to 1 for limestone or tills. All of the categories of parent materials observed at the sample site are identified from the list. The extent of silt cover observed at the sample segment is evaluated using four scored categories that range from silt heavy, (nearly all of the stream bottom covered with a deep layer of silt; -2 points) to silt free; (substrates exceptionally clean; 1 point). Silt cover is defined as a substrate being covered by more than one inch of silt. The extent of embeddedness observed at the sample segment is evaluated using four scored categories that range from extensive, more than 75% of the sample area (-2 points) to none (1 point). Substrates are considered embedded if more than 50% of the surface of the substrate is embedded in fine material and the substrate cannot be easily dislodged. Naturally sandy streams are not included, but streams embedded by sand as a result of human activities are included. Scored -5 to 3.

*2a-b) Instream Cover (Type and Amount)* - Scores are based on evaluation of two submetrics, *cover type* and *cover amount*. Scoring the submetric *instream cover type* entails identifying the cover types present. Scoring the submetric *instream cover amount* entails estimating the amount or extent of the useable cover at the sample site. (Limited to a maximum 20 points)

*2a) Instream Cover, Type* - All the cover types observed in the sample segment are selected from a list of nine scored categories. All of the categories are scored 1 point each except the deep pool category, which is scored 2 points. Cover types must comprise more than 5% of the sampling area to be included. Cover types in areas of the stream with insufficient depth (usually <25 cm) to make them useful are not scored. The undercut banks and rootwad categories are not selected unless undercut banks occur without rootwads are a major category. Scored 0 to 10.

2b) *Instream Cover, Amount* - The extent of the *instream cover* at the sample segment is estimated using four scored categories that range from extensive (more than 75% of the sample area, 11 points) to nearly absent (less than 5% of the sample area or when no large patch of cover exists any where in the sampling area, 1 point). If the estimated amount of cover falls between two categories, then both categories are chosen and the scores averaged. Scored 1 to 11.

3a-d) *Channel Morphology* - Scores are based on the evaluation of four submetrics, *channel sinuosity, development, channelization, and stability*. These submetrics were chosen to emphasize facets of the stream channel that are related to the creation and stability of stream habitat. Scoring *channel sinuosity* entails estimating the degree to which the channel meanders. Scoring *channel development* entails evaluating the presence and quality of riffle/pool habitat at the sample site. Scoring *channel channelization* entails evaluating the presence and status of man-made channel modifications at the sample site. Scoring *channel stability* entails estimating the degree channel bank stability. Scored a maximum of 20 points.

3a) *Channel Morphology, Sinuosity* - The degree of the *channel sinuosity* of the sample segment is estimated using four scored categories. Scoring of the categories is based on the number of outside bends, how well these bends are defined, and the development of deep outside areas and shallow inside areas. Scores for this submetric range from 4 points for two or three well-defined outside bends with deep outside areas and shallow inside areas, to 1 point for a straight channel. Scored 1 to 4.

3b) *Channel Morphology, Development* - The presence and quality of riffle/pool habitat at the sample site is evaluated using four categories, ranging in score from excellent (7 points) to poor (1 point), based on the definition and development of quality riffle/pool habitat. Higher scores are associated with areas that have distinct examples of deep pools that vary in depth, deep riffles and runs, and riffles with larger substrate (gravel, rubble or boulders). Lower scores are given to areas that are predominantly glides; that lack riffles, areas that have shallow riffles and pools, and that have riffles with sand and fine gravel substrates. Scored 1 to 7.

3c) *Channel Morphology, Channelization* - Evaluation of the presence and status of man-made channel modifications at the sample site is based on the presence and recovery status of man-made channel modifications. Sites are classified into four possible categories: none (6 points), recovered (4 points), recovering (3 points), or recent/no recovery (1 point). The specific modification is also classified into one of nine un-scored categories. Scored 1 to 6.

3d) *Channel Morphology, Stability* - The degree channel bank stability is classified into one of three categories, high (3 points), medium (2 points) or low (1 point), based on the quantity of bedload; signs bank erosion or effects of wide water level fluctuations; or the presence of false banks. Artificially stable (e.g., concrete) stream channels receive a high score, even though they generally have a negative impact on fish for reasons other than stability. More stable channels tend to have stable riffles and pools, little bedload, and banks with little or no erosion. Scored 1 to 3.

4a-c) *Riparian Zone* - Scores are based on evaluation of three submetrics, *(riparian zone width, quality and bank erosion*). These submetrics were chosen to emphasize the quality of the riparian zone buffer and the flood plain vegetation. Scoring for all three submetrics is accomplished by scoring both banks of the stream and then averaging the scores to get an overall score for the each sub-metric. For each sub-metric, only one category (for each bank) should be selected unless conditions are considered intermediate between two categories. In these instances the two categories are identified and the scores averaged. Scoring *riparian zone width* entails estimating the width of the stream side vegetation. Scoring *riparian zone quality* entails identifying the predominant type of floodplain land use or habitat along the banks of the site. Scoring *riparian zone bank erosion* entails evaluating the degree of bank alteration at the site. Scored a maximum of 10 points.

4a) *Riparian Zone, Width* - This sub-metric is defined as the width of the riparian vegetation. Width estimates are only made for forest, shrub, swamp and old field vegetation. Weedy urban and industrial lots are not included. Estimates are classified into five scored categories: *wide* (more than 50 m, 4 points), *moderate* (10-50 m, 3 points), *narrow* (5-10 m, 2 points), *very narrow* (5-10 m, 2 points), and *none* (0 points). Scores for both the left and right banks are averaged. Scored 0 to 4.

4b) *Riparian Zone, Quality* - The predominant type of land use or habitat observed along each bank of the site floodplain is selected is assigned to one of eight scored categories. The floodplain is the either the area immediately outside the riparian zone or greater than 100 ft from the stream (whichever is wider). Scores associated with the categories range from 0 points for open pasture/row crop, urban/industrial, and mining/construction, to 3 points for forest/swamp. The score for both banks are averaged to provide an overall estimate of riparian zone quality for the site. Scored 0 to 3.

4c) *Riparian Zone, Bank Erosion* - Riparian zone bank erosion is assessed using the Stream Bank Soil Alteration Ratings from Platts et al. (1983). Bank erosion is classified into one of three scored categories, none/little (3 points), moderate (2 points), or heavy/severe (1 point). The ranking categories are based on the percentage of the stream bank that is unstable, eroding, broken down or false (Platts et al. 1983). Both the left and right banks are scored and the scores averaged. Scored 1 to 3.

5a-c) *Pool/Glide Quality* - Scores are based on evaluation of three submetrics, *maximum depth, current type, and morphology*. These submetrics were chosen because they are related to the quality of pool/glide habitats. Scoring *maximum depth* entails estimating the maximum depth of the pool. Scoring *current type* entails evaluating the types and diversity of water current velocities found at the site. Scoring *morphology* entails assessing the ratio of pool width to riffle width observed at the sample site. Scored a maximum of 12 points.

5a) *Pool/Glide Quality: Maximum Depth* - The observed pool habitats are classified by maximum depth into five scored categories (>1 m, 6 points; 0.7-1 m, 4 points; 0.4-0.7 m, 2 points; <0.4 m, 1 points; and <0.2 m, 0 points). Pools and glides with maximum depths less than 20 cm are considered to have lost their function. Scored 0 to 6.

5b) *Pool/Glide Quality: Current Type* - Based on observed water flow patterns and other characteristics such as waves and water borne objects, the *Pool/glide current types* present at the site are classified into seven scored categories (Fast, Moderate, Slow and Eddies all are scored 1 point; Torrential and Interstitial, -1 point; and Intermittent, -2 points). All of the categories observed at a site are scored and then summed to provide an overall sub-metric score. Scored -2 to 4.

5c) *Pool/Glide Quality: Morphology* - Based on the ratio of pool width to riffle width observed at the sample site, the *pool/glide* morphology is classified into one of three scored categories: Wide, pool width>riffle width (2 points); Equal, pool width=riffle width (1 point); and Narrow, pool width<riffle width (0 points). If the entire area (including the areas outside the sampling zone) is pool then the pool = riffle category is used. Scored 0 to 2.

6a-c) *Riffle/Run Quality (Depth, Substrate Stability and Substrate Embeddedness)* - Scores are based on evaluation of three submetrics, *(depth, substrate stability and substrate embeddedness*). These submetrics were chosen because they are related to the quality of riffle/run habitats. Scoring the submetric *depth* entails estimating the depth of the riffle. Scoring the sub-metric *substrate stability* entails evaluating the type and stability of riffle habitats at the site. Scoring the submetric *substrate embeddedness* entails assessing the degree to which cobble, gravel and boulder substrates are surrounded or covered by fine material (sand, silt). Scored a maximum of 8 points.

6a) *Riffle/Run Quality, Depth* - The observed riffle habitats are classified by depth into one of four scored categories: *generally deeper than 10 cm with a maximum depth more than 50 cm* (4 points); *generally deeper than10 cm with a maximum depth less than 50 cm* (3 points), *generally 5-10 cm* (1 point), or *generally less than 5 cm* (0 points). Scored 0 to 4.

6b) *Riffle/Run Quality, Substrate Stabilit*y - Based on substrate type and stability, riffles are classified into three scored categories, *stable* (cobble, boulder, 2 points), *moderately stable* (pea gravel, 1 point), and *unstable* (gravel or sand, 0 points). Scored 0 to 2.

6c) *Riffle/Run Quality, Embeddedness* - The extent of embeddedness of the sample segment is evaluated using four scored categories that range from *extensive* (more than 75% of the sample area, -1 points) to *none* (2 points). Substrates are considered embedded if more than 50% of the surface of the substrate is embedded in fine material and the substrate can not be easily dislodged. Scored -1 to 2.

7) *Gradient* - Scores are assigned to the sites based on the local stream gradient calculated using a 7.5 topographic map. The gradient is calculated by measuring the stream length between first contour lines up and down stream of the sample site and dividing the distance by the contour interval. If the contour lines are too close together, a minimum distance of one mile should be used. Judgement may need to be exercised in areas containing features such as waterfalls and impoundments. Scores increase as the gradient increases to a maximum of 10 points for a gradient of 9.9 to 13.1 feet per mile, after which the scores decline with increasing gradient. The lowest score is assigned sites that have gradients in excess of 65.6 ft per mile (2 points). Scored a maximum of 10 points.

### *A.3.1 Ohio EPA QHEI Additional Miscellaneous Habitat Measurements*

*Miscellaneous Measurements Made* - Other measurements made in the course of completing an Ohio EPA QHEI include: 1) classification of channel morphology/modifications; 2) percent composition of pool, riffle and run features in the stream reach; 3) the gear distance, water clarity and water stage, during each of three electroshocking passes; 4) an aesthetic rating of the stream reach; 5) the percentage of canopy opening above the stream reach; 6) a ranking of the stream gradient (high, low, or moderate); 7) quantitative measurements of stream reach average width and average and maximum depth; 8) quantitative measurements of pool/glide/riffle/run length, width and depth; and 9) notes on the representativeness of the reach with regard to the stream and pollution impacts overall. These measurements/observations are not scored or used in the final QHEI scoring.

## A.4 Descriptions Of Maryland (MDNR-MBSS) Qualitative Habitat Assessment

Listed below is a short description of each of the 13 metrics that comprise the MDNR-MBSS QHA index (Roth et al. 1997b). Only 9 of the 13 metrics are scored. Each scored metric has a maximum score of 20 points. The index is still under development and no total index score been devised.

1) *Instream Habitat* - Scoring of this metric is based on the perceived value of the habitat to the fish community. Sites that display a variety of habitat types, particle sizes, and hypsographic complexity are assigned higher scores only where flows are sufficient for fish to utilize these habitats. Sites lacking these qualities are assigned low scores. The presence of ferric hydroxide does not cause a lower score unless precipitates have changed the gross physical nature of the substrate. Zero scores are assigned to segments where none of the habitat is usable by fish. Scored 0 to 20.

2) *Epifaunal Substrate* - The rating of this metric is based on the amount and variety of hard, stable substrates available for use by benthic invertebrates. The presence of features that inhibit colonization such as flocculent materials, fine sediments, and unstable substrates will reduce the scores assigned to segments. Scored 0 to 20.

3) *Velocity/Depth Diversity* - Scoring of this metric is based on the variety of velocity/depth regimes found within the stream segment. Low gradient streams are usually scored lower. Scored 0 to 20.

4) *Pool/Glide/Eddy/Quality* - Scoring of this metric is based on the variety and spatial complexity of slow or still water habitat within the sample segment. These habitats may include larger eddies in high gradient streams. Higher scores are assigned to segments that provide cover for fish (e.g., undercut banks or woody debris). Scored 0 to 20.

5) *Riffle/Run Quality* - Scores for this metric are based on the complexity and functional importance of riffle/run habitat. Higher scores are assigned to segments dominated by deep riffle/run areas, stable substrates and a variety of current velocities. Scored 0 to 20.

6) *Channel Alteration* - Scores for this metric are based on the degree and type of alteration of the stream channel. Some of the types alterations included are: concrete channels, artificial embankments, obvious straightening of the natural channel, rip-rap, or recent bar development. The type, placement and extent of bar development is used as an indicator of the degree of flow fluctuation and substrate stability. Greater bar development or a higher percentage of artificial armoring (e.g., rip-rap or concrete) of the steam bank results in lower scoring. Scored 0 to 20.

7) *Bank Stability* - Scoring of this metric is based on the presence of riparian vegetation or other bank stabilizing material. The scoring is explicitly based on a ranking of the bank stability, the degree of erosional scarring, the potential for erosion caused by flood conditions and the degree of bank sloping. The presence of steep slopes alone, does not result in the segment being scored low. Scored 0 to 20.

8) *Embeddedness* - Scoring for this metric is the percentage of stream gravel, cobble, and boulder particle surface area that is surrounded by fine sediment or flocculent materials.

9) *Channel Flow Status* - Scoring for this metric is the percentage of stream channel, minus exposed substrates and landforms, that has water.

10) *Riparian Buffer* - Scored as the minimum width of vegetated buffer (50 m maximum). Cultivated fields containing any bare soil are not considered riparian buffers. For segments which have variable buffer widths or receive direct delivery of storm runoff or sediments, the narrowest buffer in the segment is scored (e.g., 0 m if parking-lot runoff enters the stream directly), even though a portion of the segment may have a well developed buffer. If the riparian zone on one side slopes away from the stream and there is no direct runoff delivery point, the score should be based on the opposite bank. The dominant buffer zone is classified into one of five categories, *forest*, *old field*, *emergent vegetation*, *mowed lawn*, *tall grass*, or *logged area*, and the dominant adjacent land cover into one of 10 categories, *bare soil*, *railroad*, *paved road*, *parking-lot/industrial/commercial*, *gravel road*, *dirt road*, *pasture*, *orchard*, *cropland*, or *housing*.

11) *Shading* - Scoring for this metric is the percentage of segment that is shaded. Both the extent (total area) and the duration (day length) of shading is considered in scoring shading (e.g., full and dense shading all day in summer is 100% and full exposure all day in the summer is 0%).

12) *Aesthetic Rating* - Score is based on the visual appeal of the site, the presence of human refuse, and the degree of channelization and riparian vegetation disturbance. Segments in essentially a natural state, with no human refuse and that have a visually outstanding character are scored the highest. Scored 0 to 20.

13) *Remoteness* - Scoring is based on presence of detectable human activity and the difficulty in accessing the segment. The highest scores are given to streams that are difficult to access, are more than 0.25 miles from the nearest road, and that show little or no evidence of human activity. Segments which are immediately adjacent to roadside access or have an unnatural and/or unpleasant view, smell, or sound are noted, are scored the lowest. Scored 0 to 20.

## A.4.1 Additional Miscellaneous Habitat Measurements used by MDNR-MBSS

*Miscellaneous Measurements Made* - Other miscellaneous measurements made in the course of completing an MDNR-MBSS habitat assessment include: 1a, b) thalweg depth and velocity at 0, 25, 50 and 75 m along the sample segment; 2) wetted width; 3) maximum stream depth; 4) overbank flood height; 5) categorization of adjacent land use (11 categories); 6) categorization of stream character (26 categories); 7) number of woody debris; 8) number of rootwads; and 9) flow (Lat Loc, depth, velocity).

# Appendix B
# Periphyton Metrics Listed in the USEPA-RBP (1998).

## B.1 Diatom Metrics

### *B.1.1 Total Number of Diatom Taxa (TNDT)*

TNDT is an estimate of diatom species richness. High species richness is assumed for unimpacted sites and species richness is expected to decrease with increasing pollution. Slight levels of nutrient enrichment, however, may increase species richness in headwater or naturally unproductive, nutrient-poor streams (Bahls et al. 1992).

### *B.1.2 Shannon Diversity for Diatoms.*

The Shannon Index is affected by both the number of species in a sample and the distribution of individuals among those species (Klemm et al. 1990). Because species richness and evenness may vary independently, under certain conditions, Shannon diversity values can be misleading (e.g., when the total number of taxa is less than 10). Assessments for low-richness samples can be improved by comparing the assemblage Shannon Diversity value to the Maximum Shannon Diversity value (David Beeson; S.M. Stoller Corporation, personal communication). Species diversity, despite the controversy surrounding it, has historically been used with success as an indicator of organic (sewage) pollution (Wilhm and Dorris 1968, Weber 1973, Cooper and Wilhm 1975). Bahls et al. (1992) uses Shannon diversity because of its sensitivity to water quality changes, and Stevenson (1984) suggests that changes in species diversity, rather than the diversity value, may be useful indicators of changes in water quality.

### *B.1.3 Percent Community Similarity ($PS_c$) of Diatoms.*

The $PS_c$ index, discussed by Whittaker (1952), was used by Whittaker and Fairbanks (1958) to compare planktonic copepod communities. It was chosen for use in diatom bioassessments because it shows community similarities based on relative abundances, and therefore gives more weight to dominant taxa than to rare ones. $PS_c$ only applies to comparison to a control site, or to multivariate cluster analysis. If emphasis is comparison to regional reference condition (i.e., a composite of sites), $PS_c$ will not be useful. $PS_c$ values range from 0 (no similarity) to 100% (identical).

The formula for calculating $PS_c$ is:

$$PS_c = 100 - 0.5 \sum_{i=1}^{s} a_i - b_i$$

where $a_i$ = the percentage of species *i* in sample A and $b_i$ = the percentage of species *i* in sample B.

## B.1.4 Pollution Tolerance Index for Diatoms.

The pollution tolerance index (PTI) used by Kentucky DEP is most similar to that of Lange-Bertalot (1979) and resembles the Hilsenhoff biotic index for macroinvertebrates (Hilsenhoff 1987). Lange-Bertalot distinguished three categories of diatoms according to their tolerance to increased pollution, with species assigned a value of 1 for most tolerant taxa (e.g., *Nitzschia palea* or *Gomphonema parvulum*) to 3 for relatively sensitive species. For the PTI, Lange-Bertalot's list has been adapted to four categories to differentiate a large moderately tolerant group of species (similar to his splitting of category 2 diatoms into 2a and 2b); the Kentucky DEP diatom pollution tolerance values range from one (most tolerant) to four (most sensitive). Tolerance values have been generated from several sources, including Lowe (1974), Patrick and Reimer (1966, 1975), Patrick (1977), Lange-Bertalot (1979), Descy (1979), Sabater et al. (1988), Bahls et al. (1992), and Oklahoma Conservation Commission (1993).

The formula used to calculate PTI is:

$$PTI = \Sigma \frac{n_i t_i}{N}$$

where $n_i$ = number of cells counted for species i, $t_i$ = tolerance value of species *i* (1,2,or 3), and N = total number of cells counted.

## B.1.5 Percent Sensitive Diatoms.

The percent sensitive diatoms metric is the sum of the relative abundances of all intolerant species. This metric is especially important in smaller-order streams where primary productivity may be naturally low, causing the other metrics to underestimate water quality.

## B.1.6 Percent Motile Diatoms.

The percent motile diatoms is a siltation index, as the relative abundance of *Navicula* + *Nitzschia* + *Surriella*. This metric is especially important in smaller-order streams where primary productivity may be naturally low, causing the other metrics to underestimate water quality.

## B.1.7 Percent Achnanthes minutissima.

This species is a cosmopolitan diatom that has a very broad ecological amplitude. It is an attached diatom and often the first species to pioneer a recently scoured site, sometimes to the exclusion of all other algae. *A. minutissima* is also frequently dominant in streams subjected to acid mine drainage (e.g., Silver Bow Creek, Montana) and to other chemical insults. The percent abundance of *A. minutissuma* has been found to be directly proportional to the time that has elapsed since the last scouring flow or episode of toxic pollution. For use in bioassessment, the quartiles of this metric from a population of sites has been used to establish judgement criteria (e.g., 0-25% = no disturbance, 25-50% = minor disturbance, 50-75% = moderate disturbance, and 75-100% = severe disturbance). Least-impaired streams in Montana may contain up to 50% *A. minutissima* (Loren Bahls, retired phycologist and Chief of Nonpoint Section of the Montana Department of Environmental Quality, personal communication).

## B.2 Non-diatom Metrics

### B.2.1 Taxa Richness of Non-diatoms

In general, an inverse relationship exists between the number of soft algae present and impairment. Extremely low taxa richness of non-diatoms indicates the possible occurrence of a toxicity problem (e.g., acid mine drainage), while high taxa richness suggests clean water. However, extremely high taxa richness in low-order streams may indicate a minor degree of nutrient enrichment, while low taxa richness may be natural in low-order streams with low nutrient inputs.

### B.2.2 Indicator Non-diatom Taxa

Certain taxa are good indicators of pollution. Autecological information on these indicator taxa is available in published references (Palmer 1969, 1977; Prescott 1969; Lowe 1974; and Patrick and Reimer 1966, 1975). Indicator categories are provided in Table B-1. Presence and relative abundance of indicator taxa is recorded and used in conjunction with other data to determine water quality impairment.

**Table B-1.** Indicator Taxa (Taken From Kentucky DEP 1993).

| Taxa | Indicator Condition |
|---|---|
| Acidophilic taxa | Occur at a pH of 7 or below. |
| Alkaliphilic taxa | Occur at a pH of 7 or above. |
| Heterotrophic taxa | Have a growth requirement for organic nitrogen; often associated with wastewater treatment plant effluents. |
| Halophilic taxa | Tolerate elevated chloride concentrations (including brackish water forms). |
| Eutrophic taxa | Characteristic of water with high nutrient concentrations. |
| Aberrant diatoms | Morphological changes are an indication of physiological stress often found in association with toxic materials (e.g., metals). |
| Taste and odor taxa | All taxa that cause water to taste and/or smell noxious; taxa will be identified in streams used for domestic water supplies. |

## B.3 All Taxa (Diatoms and Non-diatoms)

### B.3.1 Relative Abundances of All Taxa

The relative abundances of all taxa can be calculated from counting a pre-determined number of cells or, relative abundance of each taxon (diatoms are combined under the heading Bacillariophyceae) can be estimated as follows:

| | |
|---|---|
| **Rare** | Present in <25% of the examined fields and only 1 unit per field |
| **Common** | Present in 25-75% of the examined fields and 2-10 units per field |
| **Abundant** | Present in >75% of the examined fields and > 10 units per field. |

## B.3.2 Number of Divisions Represented All Taxa

Representatives from several phyla of algae are common from sites with good water quality. The number of phyla represented is reported as an indicator of diversity.

## B.3.3 Chlorophyll a

Benthic chlorophyll *a* values are used as an estimate of algal biomass. Chlorophyll *a* values can be extremely variable because of the patchiness of periphyton distribution. Therefore, assessments are based on a mean of three or more replicate samples. These values are used to compare biomass accrual at the same station over time or between stations during the same sampling period. High chlorophyll *a* values may indicate nutrient enrichment, while low values may either indicate low nutrient availability, toxicity, or low-light availability because of shading, sedimentation, or high turbidity. Chlorophyll *a* values are used only in support of other analyses.

## B.3.4 Ash-free Dry-mass (AFDM)

Benthic AFDM values are used as an estimate of total organic material accumulated on the artificial substrate. This organic material includes all living organisms (algae, bacteria, fungi, protozoa, and macroinvertebrates) as well as non-living detritus. Ash-free dry-mass values have been used in conjunction with chlorophyll *a* as a means of determining the trophic status (autotrophic vs. heterotrophic) of streams. The Autotrophic Index (AI) is calculated as follows:

AI = AFDM (mg/m$^2$)/Chlorophyll *a* (mg/m$^2$).

High AI values (>200) indicate the community is dominated by heterotrophic organisms, and extremely high values indicate poor water quality (Weber 1973; Weitzel 1979; Matthews et al. 1980). This index should be used with discretion, as non-living organic detritus can artificially inflate the AFDW value.

The USEPA RBP (Barbour et al. 1999) recommends that the AI be modified as chl/AFDM. The index is then positively related to the autotrophic proportion of the assemblage and not the heterotrophic component. Also, the index will have better statistical properties as a proportion or percent (chl/AFDM is usually about 0.1% of the assemblage by mass) than in the original form as AFDM/chl.

# Appendix C
# Benthic-IBI Metrics

| | Scoring Scheme for the B-IBI | | |
|---|---|---|---|
| **Metrics Used in the Coastal Plain B-IBI** | **5** | **3** | **1** |
| Total number of taxa | >24 | 11-24 | <11 |
| Number of EPT taxa | >6 | 3-6 | <3 |
| Percent Ephemeroptera | >11.4 | 2.0-11.4 | <2.0 |
| Percent Tanytarsini of Chironomidae | >13.0 | >0.0-13.0 | 0.0 |
| Beck's Biotic Index | >12 | 4-12 | <4 |
| Number of scraper taxa | >4 | 1-4 | <1 |
| Percent clingers | >62.1 | 38.7-62.1 | <38.7 |
| **Metrics Used in the Non-Coastal Plain B-IBI** | **5** | **3** | **1** |
| Total number of taxa | >22 | 16-22 | <16 |
| Number of EPT taxa | >12 | 5-12 | <5 |
| Number of Ephemeroptera taxa | >4 | 2-4 | <2 |
| Number of Diptera taxa | >9 | 6-9 | <6 |
| Percent Ephemeroptera | >20.3 | 5.7-20.3 | <5.7 |
| Percent Tanytarsini | >4.8 | >0.0-4.8 | 0.0 |
| Number of intolerant taxa | >8 | 3-8 | <3 |
| Percent tolerant | <11.8 | 11.8-48 | >48 |
| Percent collectors | >31 | 13.5-31.0 | <13.5 |

## Coastal Plain

1. **Total number of taxa** - Measures the overall variety of the macroinvertebrate assemblage. *Expected to decrease with increasing perturbation.*

2. **Number of EPT taxa** - Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). *Expected to decrease with increasing perturbation.*

3. **Percent Ephemeroptera** - Percent mayfly nymphs in the sample. *Expected to decrease with increasing perturbation.*

4. **Percent Tanytarsini of Chironomidae** - Percent of chironomids in the tribe Tanytarsini. *Expected to decrease with increasing perturbation.*

5. **Beck's Biotic Index** - Weighted sum of intolerant taxa (= 2 x number of Class 1 taxa + number of Class 2 taxa; where Class 1 taxa have tolerance values of 0 and 1, Class 2

taxa have values from 2 to 4). *Expected to decrease with increasing perturbation.*

6. **Number of scraper taxa** - Number of taxa that scrape food from substrate. *Expected to decrease with increasing perturbation.*

7. **Percent clingers** - Percent of sample primarily adapted for inhabiting flowing water, as in riffles. *Expected to decrease with increasing perturbation.*

## Non-Coastal Plain

1. **Total number of taxa** - Measures the overall variety of the macroinvertebrate assemblage. *Expected to decrease with increasing perturbation.*

2. **Number of EPT taxa** - Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). *Expected to decrease with increasing perturbation.*

3. **Number of Ephemeroptera taxa** - Number of mayfly taxa. *Expected to decrease with increasing perturbation.*

4. **Number of Diptera taxa** - Number of "true" fly taxa (includes midges). *Expected to decrease with increasing perturbation.*

5. **Percent Ephemeroptera** - Percent mayfly nymphs in the sample. *Expected to decrease with increasing perturbation.*

6. **Percent Tanytarsini** - Percent of Tanytarsini midges to total fauna. *Expected to decrease with increasing perturbation.*

7. **Number of intolerant taxa** - Number of taxa considered to be sensitive to perturbation (Hilsenhoff values 0-3). *Expected to decrease with increasing perturbation.*

8. **Percent tolerant individuals** - Percent of sample considered tolerant of perturbation (Hilsenhoff values 7-10). *Expected to increase with increasing perturbation.*

9. **Percent collectors** - Percent of sample that feeds on detrital deposits or loose surface films. *Expected to decrease with increasing perturbation.*

# Appexdix D
# ICI Metrics

**1. Total Number of Taxa** - Taxa richness has historically been a key component in most all evaluations of macroinvertebrate integrity. Healthy, stable biological communities have high species richness and diversity. *Expected to decrease with increasing perturbation.*

**2. Total Number of Mayfly Taxa** - Mayflies are an important component of an undisturbed stream macroinvertebrate fauna. They are pollution sensitive and are often the first to disappear with the onset of perturbation. *Expected to decrease with increasing perturbation.*

**3. Total Number of Caddisfly Taxa** - Caddisflies are often a predominant component of the macroinvertebrate fauna in larger, relatively unimpacted Ohio streams and rivers. Though tending to be slightly more pollution tolerant than mayflies, they display a wide range of tolerances among types. Few can tolerate heavy pollution stress, and are therefore good indicators of environmental conditions. *Expected to decrease with increasing perturbation.*

**4. Total Number of Dipteran Taxa** - Of all major aquatic invertebrate groups, dipterans, especially midges of the family Chironomidae, have the greatest faunal diversity and display the greatest range of pollution tolerances. Under heavy pollution stress, they can often be the only insect collected. Larval taxonomy has improved greatly for the group and clear patterns of organism assemblages have become distinct under water quality conditions ranging from the pristine to the heavily organic and toxic. *Expected to decrease with increasing perturbation.*

**5. Percent Mayflies** - The percent abundance of mayflies in a sample can react strongly and rapidly to often minor environmental disturbances. Mayfly abundance is reduced considerably under slight impact and is essentially non-existent under severe impact. *Expected to decrease with increasing perturbation.*

**6. Percent Caddisflies** - Percent abundance of caddisflies is strongly related to stream size. Optimal habitat and availability of appropriate food type seem to be the main considerations for large populations of caddisflies. Because of their general position as an intermediately pollution-tolerant group between mayflies and dipterans, and because they disappear rapidly under environmental stress, zero scores are restricted to those sites draining areas less than 600 square miles where no caddisflies are collected. At sites draining areas greater than 600 square miles, appropriate habitat conditions are much more likely to exist, and caddisflies should be present in at least minimal numbers. *Expected to decrease with increasing perturbation.*

**7. Percent Tribe Tanytarsini Midges** - Tanytarsini midges are a tribe of the chironomid subfamily Chironomidae. The larvae are generally burrowers or clingers, and many species build cases out of sand, silt, and/or detritus. Many species feed on microorganisms and detritus through filtering and gathering though a few are scrapers. Eleven genera and up to 140 species occur in North America, though only 8 genera and 21 distinct taxa have been collected in Ohio. They appear to be relatively pollution sensitive and often disappear or decline under even minor pollution stress. *Expected to decrease with increasing perturbation.*

**8. Percent Other Dipterans and Non-insects** - Community percentage of all dipterans (excluding the midge tribe Tanytarsini) and other non-insect invertebrates, such as aquatic worms, flatworms, scuds, aquatic sow bugs, freshwater hydras, and snails. This metric is one of two negative metrics of the ICI. Taxa are those that generally tend to become predominant under adverse water quality conditions. *Expected to increase with increasing perturbation.*

**9. Percent Tolerant Organisms** - Those organisms that appear to be extremely pollution tolerant and tend to predominate in cases of severe perturbation. This is a negative metric. List of pollution-tolerant organisms used:

- Aquatic segmented worms: Oligochaeta
- Midges: *Psectrotanypus dyari, Cricotopus bicinctus, Cricotopus sylvestris, Nanocladius*
- distinctus, Chironomus, Dicrotendipes simpsoni, Glyptotendipes barbipes, Parachironomus
- hirtalus, Polypedilum fallax, Polypedilum illinoense
- Limpets: *Ferrissia*
- *Pond Snails*: Physella

Expected to increase with increasing perturbation.

**10. Total Number of Qualitative Ephemeroptera, Plecoptera, and Trichoptera Taxa** - Generated by the qualitative sample taken in conjunction with the artificial substrate sampling. Affected by the kinds of natural substances available in the sampling area, the metric is a measurement of habitat quality. *Expected to decrease with increasing perturbation.*

# Appendix E
# Modified Index Of Well-being (IWB)

$$Iwb = 0.5 \ln N + 0.5 \ln B + H(no.) + H(wt.)$$

where:

N = relative numbers of all species excluding species designated *highly tolerant*

B = relative weights of all species excluding species designated *highly tolerant*

$H$ (no.) = Shannon diversity index based on numbers.

$H$ (wt.) = Shannon diversity index based on weight.

## Shannon Diversity Index

$$H = - (n_i)/N \log_e (n_i)/N$$

where:

$n_i$ = relative numbers or weight of the $i^{th}$ species

N = total number or weight of the sample

Relative abundance (number and weight) data are derived from pulsed D.C. electro-fishing catches where sampling effort is based on a per kilometer basis for boat methods and on a 0.3 kilometer basis for wading methods (OEPA 1988).

# Appendix F
# Fish IBI Scoring

## Justification of Selected USEPA-EMAP-SW IBI Metrics

**1. Native species richness -** Modified from Karr's (1981) Species Richness. Native species richness is a classic measure of biodiversity with focus on natives. This is important where introductions are common.

**2. Native family richness -** Replaces Karr's (1981) Darter, Sunfish, and Sucker Richness. A measure of biodiversity at the family level of organization. Useful for assessing the degree to which the reach supports families typically represented by only a single species, and therefore whose losses mean the loss of entire families from the assemblage.

**3. Sensitive species richness -** Modified from Karr's (1981) Intolerant Species Richness. Species likely to be the first to disappear following anthropogenic disturbance and the last to recover following restoration. Most useful at discriminating among reaches with higher quality assemblages.

**4. Percent tolerant individuals -** Modified from Karr's (1981) percent Green Sunfish. Evaluates the tendency of one or more weedy species to dominate the assemblage. Typically highly disturbed sites are numerically dominated by tolerant species. In the Appalachians, the blacknose dace and creek chub are prime examples. However, these taxa may naturally dominate very small streams. Calculated as:

1-(proportion of tolerant individuals in excess of 10%).

**5. Benthic species richness** - Modified from Karr's (1981) Darter Species Richness. Measures quality of habitat (substrate) for small bottom dwelling species; includes darters, sculpins, benthic minnows (e.g., dace, lamprey).

**6. Water column species richness** - Modified from Karr's (1981) Sunfish Species Richness. Measures quality of water column (especially pools) for stronger swimming species that feed largely on drifting prey; includes sunfish, many minnows, salmonids.

**7. Percent alien individuals** - This is a measure of the degree to which the site is contaminated by biological pollution. Also, they represent a direct disturbance themselves as a result of predation and competition with species that are not adapted to coexisting with them; includes common carp, brown trout, rainbow trout, many sunfishes, and bass.

**8. Number of trophic guilds -** Measures niche diversity in streams.

**9. Percent top carnivore (invertivore-piscivore) individuals -** Modified from Karr's (1981) percent Carnivore; includes species that are piscivores or invertivore-piscivores as adults (bass, pike, several sunfishes, eel). Estimates the ability of the food chain to support fish that prey largely on other fish, vertebrates, or large macrobenthos. Calculated as:

> proportion of top carnivores/expected value of 10%.

**10. Invertivore individuals -** Measures the capacity of the food base to support the major trophic group of fishes in most streams. Prey includes both insects and other invertebrates. Calculated as:

> proportion of invertivores/expected value of 50%.

**11. Percent herbivores** - This metric includes herbivorous scrapers and phytoplanktivores. These species disappear when sediment decreases food quality. Calculated as:

> 1 - (proportion of herbivores in excess of 10%).

**12. Percent omnivore individuals -** A measure of the dominance of trophic guilds by individuals that can eat either plant or animal materials. These are trophic generalists with at least 25% of its diet as animals and at least 25% is plants. Ecomorphology (mouth gape, dentition, pharyngeal teeth, gut length) also suggest dietary niche. Calculated as:

> 1 - (proportion of omnivores in excess of 20%).

**13. Number of specialized reproductive strategies -** Replaces Karr's (1981) percent hybrids. The number of different reproductive strategies represented in the assemblage not to include generalist or broadcast spawners. A measure of niche diversity in streams, it evaluates the degree to which the reach supports a variety of reproductive strategies.

**14. Proportion of gravel spawning species -** Replaces percent Simple Lithophils metric of some authors. Comprised of some representatives of Balon's (1975) Lithophilic A.1, A.2, .1 and B.2 species.

**15. Proportion of tolerant substrate spawners -** They may spawn over gravel, vegetation, detritus, sand or silt or construct a nest, guard it against predation and maintain it, fanning or otherwise manipulating the eggs to remove silt or increase flow over the nest. Eggs are demersal and/or adhesive. Calculated as:

> 1 - (proportion of tolerant reproductive individuals in excess of 10%.

**16. Total abundance -** The number of individuals collected at the site. Low abundance may result from toxic or extremely oligotrophic waters. Calculated as:

> number of individuals/expected value of 500.

## Justification of Selected Ohio EPA IBI Metrics

**1. Total Number of Indigenous Fish Species -** This metric is used with all three versions of the IBI. Exotic species are not included. This metric is based on the well-documented observation that the number of indigenous fish species in a given size stream or river will decline with increasing environmental disturbance. (Karr 1981; Karr et al. 1986). Thus, the number of fish species metric is expected to give an indication of environmental quality throughout the range from exceptional to poor. Exotic (i.e., introduced) species present in a system through stocking or inadvertent releases do not provide an accurate assessment of overall integrity and their abundance may even indicate a loss of integrity (Karr et al. 1986).

**2. Number of Darter Species (Wading, Headwaters), Proportion of Round-bodied Catostomidae (Boat Method) -** The darter species metric is reflective of good water quality conditions (Karr et al. 1986). None of the species in this group have been found to thrive in degraded stream conditions. Eleven of the 22 Ohio species have been found to be highly intolerant of degraded conditions based on the Ohio EPA intolerance criteria. Life history data on this group show darters to be insectivorous, habitat specialists, and sensitive to physical and chemical environmental disturbances (Kuehne and Barbour 1983). These factors make darter species reliable indicators of good water quality and habitat conditions.

**3. Number of Sunfish Species (Wading, Boat), Proportion of Headwaters Species (Headwaters) -** This metric follows Karr (1981) and Karr et al. (1986) by including the number of sunfish species (Centrachidae) collected at a site, excluding the black basses (*Micropterus* spp.). The redear sunfish (*Lepomis microlophus*) is not included because, in Ohio, it is introduced and only locally distributed. Hybrid sunfish are also excluded from this metric.

**4. Number of Sucker Species (Wading, Boat), Number of Minnow Species (Headwaters) -** All species in the family Catostomidae are included in this metric. Suckers represent a major component of the Ohio fish fauna with their total biomass in many samples surpassing that of all other species combined. The general intolerance of most sucker species to habitat and water quality degradation (Karr 1981; Trautman 1981; Becker 1983; Karr et al. 1986) results in a metric with a sensitivity at the high end of environmental quality. In addition the relatively long life spans of many sucker species (10-20 years) (Becker 1983) provides a long-term assessment of past and prevailing environmental conditions. Of the 19 species still present in Ohio (one is extinct), seven are widely distributed throughout the state.

**5. Number of Intolerant Species (Wading, Boat), Number of Sensitive Species (Headwaters) -** The number of intolerant species metric is designed to distinguish streams of the highest quality. As a result, the sensitivity of this metric is at the highest end of biotic integrity. Designation of too many species as intolerant will prevent this metric from discrimination among the highest quality streams. Only species that are highly intolerant to a variety of disturbances were included in this metric so that it will respond to diverse types of perturbations; species intolerant to one type of disturbance, but not another were not included.

**6. Percent Abundance of Tolerant Species (Replacing Karr's % Green Sunfish) -** This metric is a modification of one of Karr's (1981) original IBI metrics, the percentage of the fish community comprised by green sunfish (*Lepomis cyanellus*). This metric was designed to detect a decline in stream quality from fair to poor. The green sunfish is a species that is often present in moderate numbers in many Midwest streams and can become a predominant component of the community in areas with degraded habitat and/or water quality. This ability to survive and reproduce in disturbed environments makes this species sensitive to changes in environmental quality in severely impacted areas. Although green sunfish are one of the most widely distributed and numerically abundant fish species found in the Midwest, they show a decided preference towards smaller sized and low gradient streams. This limits their utility in assessing impacts in larger streams and rivers. Karr et al. (1986) suggested that other species could be substituted for the green sunfish if they respond in a similar manner. Several species meeting this criterion were included to give this metric an improved sensitivity for the range of stream and river sizes encountered in Ohio. Because individual species have habitat requirements that are keyed to stream size, composition of the tolerant species metric shifts with drainage area and this metric remains useful among small, medium, and large streams and rivers.

**7. Percent Omnivores -** The Ohio EPA definition of an omnivorous species follows Karr (1981) and Karr et al. (1986) with two important distinctions added. Specialized filter-feeding species which technically are omnivorous are not included. Specialist filter feeders are represented in Ohio by the paddlefish (*Polyodon spathula*) and brook lamprey ammocoetes. These species are generally sensitive to environmental degradation. Since the omnivore metric is designed to measure increasing levels of environmental degradation due to a disruption of the food base it is not appropriate to include these sensitive, filter feeding species in this metric. This metric was further restricted to those species that did not show feeding specialization and were reported primarily as omnivores in all studies reviewed. This removes such species as channel catfish (*Ictalurus punctatus*) which may or may not feed as an omnivore under different environmental conditions.

**8. Proportion of Insectivores (All) -** This metric is designed to be sensitive over the middle range of biotic integrity. A low abundance of insectivorous species can reflect a degradation to the insect food base of a stream (Karr et al. 1986). As disturbance increases, the diversity of benthic insects decreases, production becomes more variable, and the community often becomes predominated by a few taxa (Jones et al. 1981). Thus, specialist feeders such as specialist insectivores will decrease and be replaced by generalist feeders such as omnivores. This represents a modification from Karr et al. (1986) using insectivorous Cyprinids alone.

**9. Top Carnivores (Wading, Boat), Proportion of Pioneering Species (Headwaters) -** Karr (1981) developed the top carnivore metric to measure community integrity in the upper functional levels of the fish community. And Karr (1981) and Karr et al. (1986) were followed in designating a species as a top carnivore. Species which feed primarily on other vertebrates or crayfish are included in this metric. As with the omnivore metric, species which display feeding plasticity are excluded (e.g., channel catfish).

**10. Number of Individuals in a Sample (All) -** This metric assesses population abundance as the number of individuals per unit of sampling effort. This metric is most sensitive at the low to middle end of biotic integrity when polluted sites yield fewer individuals (Karr et al. 1986). In such cases, the normal trophic relationships are disturbed enough to either have severe effects on fish production or directly reduce fish abundance through toxic effects. As integrity increases, total abundance increases and becomes more variable with natural factors such as ionic concentration, temperature, and amount of energy reaching the stream surface. However, certain perturbations, such as channelization with canopy removal, can lead to increases in the abundance of fishes, especially tolerant species, (e.g., bluntnose minnow). Thus, inclusion of these species may obscure negative environmental change. To decrease the variability in the scoring of this metric, it excludes species designated as tolerant.

**11. Proportion of Individuals as Simple Lithophilic Spawners -** This metric was designed as a replacement metric for the proportion of individuals as hybrids. In Ohio streams, the hybrid metric was not a consistent indication of water quality. Hybrids have been observed to occur in high quality Ohio streams (e.g., minnow hybrids), can arise from sensitive parent species (e.g., longear sunfish), are often times absent from headwaters streams and severely impacted streams, and they can be difficult to identify. Although the frequency of hybridization has often been associated with habitat degradation this did not appear consistently enough in the Ohio EPA data base to distinguish this type of impact.

**12. Proportion of Individuals with Deformities, Eroded Fins, Lesions, and Tumors (DELT) (replaces Karr's % diseased individuals) -** This metric keys in on the health of individual fish within a community using the percent occurrence of external anomalies and corresponds to the percentage of diseased fish in Karr's (1981) original IBI. Studies of wild fish populations have revealed that these and other anomalies are either absent or occur at very low rates at reference sites, but reach higher percentages at impacted sites (Mills et al. 1966; Berra and Au 1981; Baumann et al. 1987). Common causes of DELT anomalies are described in Allison et al. (1977), Post (1983) and Ohio EPA (1988) and include the effects of bacterial, viral, fungal, and parasitic infections, neoplastic diseases, and chemicals. An increase in the frequency of occurrence of these anomalies is generally an indication of stress and environmental degradation which may be caused by chemical pollutants, overcrowding, improper diet, excessive siltation, and other disturbances. Blackspot is not included because the presence and varying degrees of infection may be natural and not related to environmental degradation (Allison et al. 1977; Berra and Au 1981). Also, analysis of Ohio data has shown no clear relationship between black spot and stream degradation (Wittier et al. 1987). Other parasites are also excluded due to the lack of a consistent relationship with environmental degradation although their effects can resemble and lead to tumors, deformities, and lesions. Prior to using this metric, Ohio EPA (1987a) should be referred for consistent data-recording procedures and as a reference for specific anomalies included in each category.

## Justification of Selected MDNR-MBSS IBI Metrics

The metrics used in the IBI represent various attributes of the fish assemblage indicative of ecological quality, so that differences in metric values reflect important differences in stream conditions.

**1. Number of native species -** The concept of species richness has been used extensively to assess the quality of ecological systems. In most cases, the number of fish species supported by streams of a given size in a given region decreases with environmental degradation (Karr et al. 1986). The reduction in number of species may be as a result of reduced diversity of habitats, the loss of species that are sensitive to pollutants, or other human-induced impacts. Introduced species are not included in this metric because the presence of these species may result in a higher species number than would naturally be found in a given stream. In addition, the species richness value for a site in which species have been introduced would not reflect the lowered richness that may result from human disturbance at the site. Leidy and Fiedler (1985) found that species richness increased at sites with moderate human disturbance mostly due to the addition of introduced species. There are some potential exceptions to this rule. For example, minimally disturbed coldwater systems, dominated by salmonids and sculpin, tend to have low number of species.

**2. Number of benthic species -** Benthic fish species are sensitive to degradation of stream benthic habitats because of the their specific requirements for reproducing and feeding on the stream bottom (Page 1983). Benthic habitats are degraded by channelization, siltation, and reduction of dissolved oxygen and are often degraded in streams with watersheds that contain a great deal of impervious surface. Berkman and Rabeni (1987) documented reduced abundance of benthic insectivores in streams with increased amounts of silt in riffles. Benthic specialists included in this metric are darter, sculpin, madtom, and lamprey species.

**3. Percent tolerant individuals -** Intolerant species are among the first to be affected by perturbations (Jenkins and Burkhead 1993, Pflieger 1975, Smith 1979, Trautman 1981). As specific habitats required by habitat specialists are degraded, the relative abundance of tolerant, habitat generalists becomes greater.

**4. Percent abundance of the dominant species -** The contribution of the dominant (tolerant) taxa to the fish community is likely to increase as the amount and extent of degradation increases. As intolerant species become less abundant, tolerant species increase in relative abundance in degraded streams and may become the dominant taxa (Karr et al. 1986). This metric was calculated as the percent contribution of the single dominant fish species to the total number of individuals at a site.

**5. Percent of individuals as generalists, omnivores, or invertivores -** The dominance of generalist feeders increases as specific food sources become less reliable, i.e., when degraded conditions reduce the abundance of particular prey items. An opportunistic foraging strategy makes generalists more successful than specialized foragers because they are better suited to a shifting food base in the presence of degraded conditions than are more specialized feeders (Karr et al. 1986).

**6. Percent of individuals as insectivores -** This metric takes into account the response of fishes to impacts on lower trophic levels. Fewer insectivorous fishes are collected in degraded streams probably due to decreases in the supply of preferred insects, reflecting degraded chemical or habitat quality (Karr et al. 1986).

**7. Abundance (number of individuals) per square meter -** Degraded streams are generally expected to yield fewer individuals than less severely impacted streams. Streams of similar size with greater heterogeneity of habitat generally contain larger numbers of individuals than streams with homogeneous habitat as a result of anthropogenic impact on the stream. In addition, streams with degraded chemical or habitat tend to support only tolerant species of fishes are likely to have depressed overall numbers of fishes. One notable exception is elevated abundance in the presence of excess nutrients, particularly of tolerant species.

**8. Biomass per square meter -** The biomass that a stream can accommodate is a function of the quantity and quality of available stream habitat. As with abundance, the biomass in a stream is expected to be lower in degraded streams compared to higher quality streams. In general, more and larger fishes are expected in higher quality streams. Larger individuals of a species may be indicative of longevity of the individuals. Long lived individuals indicate that the streams may have a history of good stream quality.

**9. Percent of individuals as lithophilic spawners -** Lithophilic spawners (Balon 1975) utilize rocks, rubble, or gravel substrates for egg deposition. Because they require clean spawning substrates and may use interstitial spaces, lithophils are particularly susceptible to siltation. Since silt is likely the most common stream pollutant in the state of Maryland, this metric may be useful in identifying streams that are degraded with substantial silt loads.

**MDNR-MBSS Method for Deriving IBI Scores for the State Data Sets**

| **Coastal Plain Metrics** | **1** | **3** | **5** |
|---|---|---|---|
| Number of native species | Criteria vary with stream size* | | |
| Number of benthic species | Criteria vary with stream size* | | |
| Percent tolerant individuals | More than 80 | 80 to 31 | Less than 31 |
| Percent abundance of dominant species | More than 78 | 78 to 31 | Less than 31 |
| Percent generalists, omnivores, and invertivores | More than 99 | 99 to 88 | Less than 88 |
| Number of individuals per square meter | Less than 0.47 | 0.47 to 0.62 | More than 0.62 |
| Biomass (g per $m^2$) | Less than 5.1 | 5.1 to 9.6 | More than 9.6 |
| Percent lithophilic spawners | 0 | 0 to 0.6 | More than 0.6 |
| **Non-Coastal Plain Metrics** | | | |
| Number of native species | Criteria vary with stream size* | | |
| Number of benthic species | Criteria vary with stream size* | | |
| Percent tolerant individuals | More than 82 | 82 to 50 | Less than 50 |
| Percent abundance of dominant species | More than 78 | 78 to 51 | Less than 51 |
| Percent generalists, omnivores, and invertivores | More than 95 | 95 to 59 | Less than59 |
| Number insectivores | Less than 5 | 5 to 33 | More than 33 |
| Number of individuals per $m^2$ | Less than 0.22 | 0.22 to 0.63 | More than 0.63 |
| Percent lithophilic spawners | Less than 6 | 6 to 32 | More than 32 |

*Metrics were adjusted for watershed area as follows: adjusted value = observed value/expected value, where expected value = m x log (watershed area in acres)+b. Values of m and b are:

| | Coastal Plain | | Non-Coastal Plain | |
|---|---|---|---|---|
| | Slope (m) | Intercept (b) | Slope (m) | Intercept (b) |
| Number of native species | 5.2142 | -7.7258 | 6.3258 | -12.7351 |
| Number of benthic species | 1.4478 | -2.5532 | 0.9016 | -1.2345 |

| Scoring Criteria For Adjusted Metrics | | | |
|---|---|---|---|
| **Coastal Plain** | **1** | **3** | **5** |
| Number of native species-adjusted value | More than 0.74 | 0.74 to 1.05 | More than 1.05 |
| Number of benthic species-adjusted value | Less than 0.70 | 0.70 to 0.99 | Less than 0.70 |
| **Non-Coastal Plain** | | | |
| Number of native species-adjusted value | Less than 0.47 | 0.47 to 0.77 | More than 0.77 |
| Number of benthic species-adjusted value | Less than 0.44 | 0.44 to 0.82 | More than 0.82 |

# Appendix G
# Fish IBI Metrics used by USEPA-EMAP-SW, Ohio EPA and the MDNR-MBSS Programs. Metrics Are Grouped By Association Or Similarity

| | Alternative IBI Metrics | USEPA-EMAP-SW | Ohio EPA | Ohio EPA Headwater | MDNR-MBSS Coastal | MDNR-MBSS Non-Tidal Plains |
|---|---|---|---|---|---|---|
| 1 | # Species | | | X | X | |
| | # Native fish species | X | X | X | | |
| 2 | # Native families | X | | | | |
| 3 | # Darter species | | X | | | |
| | # Darter and sculpin species | | | X | | |
| | # Benthic species | X | | | X | X |
| 4 | # Sunfish species | | X | | | |
| | # Headwater species | | | X | | |
| | % Headwater species | | | X | | |
| 5 | # Sucker species | | X | | | |
| | # Minnow species | | | X | | |
| 6 | # Intolerant species | | X | | X | X |
| | # Sensitive species | X | | X | | |
| 7 | % Tolerant species | X | X | X | X | X |
| 8 | % Omnivores | X | X | X | | X |
| | % Generalists, omnivores, invertivores | | | | | X |
| 9 | % Insectivores | | | | X | X |
| | % Insectivorous species | X | X | X | | |

(continued)

**Appendix G (continued)**

| | Alternative IBI Metrics | USEPA-EMAP-SW | Ohio EPA | Ohio EPA Headwater | MDNR-MBSS Coastal | MDNR-MBSS Non-Tidal Plains |
|---|---|---|---|---|---|---|
| 10 | % Top carnivores | X | X | | | |
| | % Pioneering species | | | X | X | |
| 11 | # Individuals | X | X | X | X | |
| | Density of individuals | | | | | X |
| | % Abundance of dominant species | | | | | X |
| | Biomass | | | | X | |
| 12 | % Simple Lithophils | | X | | | X |
| | # Simple Lithophilic species | | | X | | |
| | % Silt-intolerant spawners | | | | X | |
| | Proportion of gravel spawning species | X | | | | |
| | Proportion of tolerant substrate spawners | X | | | | |
| 13 | % Diseased individuals | | X | X | X | X |
| 14 | # Alien Individuals | X | | | | |
| 15 | # Trophic Guilds | X | | | | |
| 16 | % Herbivores | X | | | | |
| 17 | # Specialized Reproductive strategies | X | | | | |