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This manual contains procedures for collecting samples and measurement data from various biotic and abiotic components of non-wadeable streams and rivers in the Mid Atlantic and Pacific Northwest. These procedures were developed and used between 1997 and 1998 in research studies of the U.S. Environmental Protection Agency’s (EPA) Environmental Monitoring and Assessment Program (EMAP). The purposes of this manual are to: (1) Document the procedures used in the collection of field data and various types of samples for the various research studies; and (2) provide these procedures for use by other groups implementing river monitoring programs. These procedures are designed for use during a one-day visit by a crew of four or five persons to sampling sites located on larger, non-wadeable streams and rivers (generally stream order 4 or greater in the Mid Atlantic and Northwestern U.S.).

1.1 Overview of EMAP-Surface Waters

The U.S. EPA has designated EMAP to develop the necessary monitoring tools to 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 nation's ecological resources are a national heritage, as essential to the country now and in the future as they have been in the past. Data indicate that regional and international environmental problems may be endangering these essential resources. The potential threats include acid rain, ozone depletion, point and nonpoint sources of pollution, and climate change.

The tools being developed by EMAP include appropriate indicators of ecological
condition, and statistical sampling designs to determine the status and extent of condition, and to detect regional-scale trends in condition. When fully implemented in a national monitoring framework, such as that being developed by the White House Committee on Environment and Natural Resources (CENR; Committee on Environment and Natural Resources, 1997), these tools will provide environmental decision makers with statistically valid interpretive reports describing the health of our nation's ecosystems (Whittier and Paulsen, 1992). Knowledge of the health of our ecosystems will give decision makers and resource managers the ability to make informed decisions, set rational priorities, and make known to the public costs, benefits, and risks of proceeding or refraining from implementing specific environmental regulatory actions. Ecological status and trend data will allow decision makers to objectively assess whether or not the nation's ecological resources are responding positively, negatively, or not at all, to existing or future regulatory programs.

The following three objectives guide EMAP research activities (U.S. EPA, 1998):

- Estimate the current status, extent, changes and trends in indicators of the condition of the nation's ecological resources on a regional basis with known confidence.

- Monitor indicators of pollutant exposure and habitat condition and seek associations between human-induced stresses and ecological condition.

- Provide periodic statistical summaries and interpretive reports on ecological status and trends to resource managers and the public.

The EMAP Surface Waters Resource Group (EMAP-SW) is charged with developing the appropriate tools to assess the health of lakes, streams, rivers, and wetlands in the United States. The first phase of the program started with a study of northeastern lakes between 1991 and 1996 (Larsen and Christie, 1993; Baker et al., 1997). In 1992 and 1993, a pilot study of wetland ecosystems was conducted in the Prairie Pothole region of the northern plains region of the U.S. (Peterson et al., 1997). In 1993 - 1994 the U.S. EPA Office of Research and Development and Region 3 Office, with assistance from the U.S. Fish and Wildlife Service and States in the eastern United States (WV, NY, PA, VA and MD) conducted the first EMAP wadeable stream pilot which was called the Mid-Atlantic Highlands Assessment (MAHA). In 1997 - 1998 the pilot was expanded to additional states (DE, NJ, and NC) and ecoregions and both wadeable and non-wadeable streams were sampled. The 1997 - 1998 study was called the Mid-Atlantic Integrated Assessment (MAIA). Protocols that were used in wadeable streams in the MAHA and MAIA studies are contained in the manual "Environmental Monitoring and Assessment Program - Surface Waters. Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams. EPA/620/R-94/004F." (Lazorchak, et al., 1998). Many of the protocols used on non-wadeable streams in 1997-1998 in the eastern and western United states were adapted or modified from the 1997 manual. The specific indicators dealing with non-wadeable streams and rivers are described in more detail in the following section.

1.2 Summary of Ecological Indicators

The following sections describe the rationale for each of the ecological indicators.
Currently, EMAP considers two principal types of indicators, condition and stressor (U.S. EPA, 1998). Condition indicators are biotic or abiotic characteristics of an ecosystem that can provide an estimate of the condition of an ecological resource with respect to some environmental value, such as biotic integrity. Stressor indicators are characteristics that are expected to change the condition of a resource if the intensity or magnitude is altered.

1.2.1 Water Chemistry

Data are collected from each river for a variety of physical and chemical constituents. Information from these analyses is used to evaluate river condition with respect to stressors such as acidic deposition, nutrient enrichment, and other inorganic contaminants. In addition, rivers can be classified with respect to water chemistry type, water clarity, mass balance budgets of constituents, temperature regime, and presence of anoxic conditions.

1.2.2 Physical Habitat

Naturally occurring differences among surface waters in physical habitat structure and associated hydraulic characteristics contribute to much of the observed variation in species composition and abundance within a zoogeographic province. The structural complexity of aquatic habitats provides the variety of physical and chemical conditions to support diverse biotic assemblages and maintain long-term stability. Anthropogenic alterations of riparian areas and river channels, wetland drainage, grazing and agricultural practices, and river bank modifications such as revetments or development, generally act to reduce the complexity of aquatic habitat and result in a loss of species and ecosystem degradation.

Stressor indicators derived from data collected about physical habitat quality will be used to help explain or diagnose river condition relative to various condition indicators. Important attributes of physical habitat in rivers 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). Overall objectives for this indicator are to develop quantitative and reproducible indices, using both multivariate and multimetric approaches, to classify rivers and to monitor biologically relevant changes in habitat quality and intensity of disturbance. Kaufmann et al. (1999) discuss procedures for reducing EMAP field habitat measurements and observations to metrics that describe channel and riparian habitat at the reach scale.

1.2.3 Periphyton Assemblage

Periphyton are the algae, fungi, bacteria, and protozoa associated with substrates in aquatic habitats. These organisms exhibit high diversity and are a major component in energy flow and nutrient cycling in aquatic ecosystems. Many characteristics of periphyton community structure and function can be used to develop indicators of ecological conditions in streams. Periphyton are sensitive to many environmental conditions, which can be detected by changes
in species composition, cell density, ash free dry mass (AFDM), chlorophyll, and enzyme activity (e.g., alkaline and acid phosphatase). Each of these characteristics may be used, singly or in concert, to assess condition with respect to societal values such as biological integrity and trophic condition.

A hierarchical framework was used in the development of the periphyton indices of river condition. The framework involved the calculation of composite indices for biotic integrity, ecological sustainability, and trophic condition. The composite indices were calculated from measured or derived first-order and second-order indices. The first-order indices included species composition (richness, diversity), cell density, AFDM, chlorophyll, and enzyme activity (e.g., Saylor et al., 1979), which individually are indicators of ecological condition in streams. Second-order indices were calculated from periphyton characteristics, such as the autotrophic index (Weber, 1973), community similarity compared to reference sites, and autecological indices (e.g., Lowe, 1974; Lange-Bertalot, 1979; Charles, 1985; Dixit et al, 1992).

### 1.2.4 Sediment Community Metabolism

Ecosystems are complex, self-regulating, functional units defined by rates and processes, such as energy flow or material cycling. These processes are mediated by the trophic structure of the ecosystem, and integrate the functioning of the entire community. Energy flow and material cycling are important components of two major concepts in stream ecology: The river continuum concept and resource spiraling. Heterotrophic microorganisms (bacteria and fungi) are responsible for oxygen sags in streams and for much of the decomposition of organic matter deposited in them. Measuring the rate of oxygen consumption within the soft sediments of a river provides a functional indicator of energy flow and material transformation within the ecosystem.

### 1.2.5 Benthic Macroinvertebrate Assemblage

Benthic macroinvertebrates inhabit the sediment or live on the bottom substrates of rivers. The macroinvertebrate assemblages in rivers reflect overall biological integrity of the benthic community and monitoring these assemblages is useful in assessing the status of the water body and discerning trends. Benthic communities respond differently to a wide array of stressors. As a result of this, it is often possible to determine the type of stress that has affected a benthic macroinvertebrate community (Plafkin et al., 1989; Klemm et al., 1990). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, macroinvertebrate community structure is a function of past conditions.

Two different approaches are currently being evaluated to developing ecological indicators based on benthic invertebrate assemblages. The first is a multimetric approach, where different structural and functional attributes of the assemblage are characterized as "metrics". Individual metrics that respond to different types of stressors are scored against expectations under conditions of minimal human disturbance. The individual metric scores are then summed into an overall index value that is used to judge the overall level of impairment of an individual river reach. Examples of multimetric indices based on benthic invertebrate assemblages include

The second approach being investigated is to develop indicators of condition based on multivariate analysis of benthic assemblages and associated abiotic variables. Examples of this type of approach as applied to benthic invertebrate assemblages include RIVPACS (Wright, 1995), and BEAST (Reynoldson et al., 1995). Rosenberg and Resh (1993) present various approaches to biological monitoring using benthic invertebrates, and Norris (1995) briefly summarizes and discusses approaches to analyzing benthic macroinvertebrate community data.

1.2.6 Aquatic Vertebrate Assemblages

Aquatic vertebrate assemblages of interest to EMAP include fish and amphibians (more so in the western U.S. where fish taxa richness is less). The fish assemblage represents a critical component of biological integrity from both an ecosystem function and a public interest perspective. Historically, fish assemblages have been used for biological monitoring in streams more often than in lakes (e.g., Plafkin et al., 1989; Karr, 1991). Fish assemblages can serve as good indicators of ecological conditions because fish are long-lived and mobile, forage at different trophic levels, integrate effects of lower trophic levels, and are reasonably easy to identify in the field (Plafkin et al., 1989). Amphibians comprise a substantial portion of vertebrate biomass in streams of many areas of the U.S. (Hairston, 1987; Bury et al., 1991). Reports of dramatic declines in amphibian biodiversity (e.g., Blaustein and Wake, 1990; Phillips, 1990) has increased the level of interest in monitoring these assemblages. Amphibians may also provide more information about ecosystem condition in headwater or intermittent streams in certain areas of the country than other biological response indicators (Hughes, 1993). The objective of field sampling is to collect a representative sample of the aquatic vertebrate assemblage by methods designed to 1) collect all except very rare species in the assemblage and 2) provide a measure of the abundance of species in the assemblages (McCormick, 1993). Information collected for EMAP that is related to vertebrate assemblages in rivers includes assemblage attributes (e.g., species composition and relative abundance) and the incidence of external pathological conditions.

Indicators based on vertebrate assemblages are being developed primarily using the multimetric approach described in Section 1.3.5 for benthic macroinvertebrates, and originally conceived by Karr and others (Karr et al., 1986). Simon and Lyons (1995) provide a recent review of multimetric indicators as applied to stream fish assemblages.

1.2.7 Fish Tissue Contaminants

Indicators of fish tissue contaminants attempt to provide measures of bioaccumulation of toxic chemicals in fish. When coupled with study designs such as those being developed by EMAP, these indicators can be used to estimate regional risks of consumption to predators of fish (either wildlife or human), and to track how this risk changes with time in a region. It is also meant to be used in conjunction with the other stressor indicators (physical habitat, water chemistry, land use, population density, other records of relevant anthropogenic stresses) and condition indicators (fish, macroinvertebrates, periphyton) to help diagnose whether the probable
cause of river degradation, when it is shown by the condition indicators to occur, is water quality, physical habitat, or both.

The various studies that have been done on fish tissue contaminants have focused on different parts of the fish: whole fish, fillets, livers. For EMAP-SW, the focus is on whole fish because of the emphasis on the ecological health of the whole river (as opposed to a focus on human health concerns). Whole fish are a better indicator of risk to piscivorous wildlife than fillets. We also should be able to address potential risks to human health by analyzing whole fish. Whole fish also present fewer logistical problems for field crews (no gutting required in the field) and the analytical lab (no filleting necessary).

Samples are prepared for two major categories of fish species. One sample is prepared using a species whose adults are small (e.g., sculpins and small minnows). The second sample is prepared using a species whose adults are of larger size (e.g., squawfish, trout, suckers, and sunfish). In addition to being more ubiquitous than the larger fish (and therefore more likely to be present in sufficient numbers to composite), small fish have other advantages over large fish. Most importantly, it may be possible to get a more representative sample of the contaminant load in that river segment (although it could be at a lower level of bioaccumulation) by creating a composite sample from a larger number of small individuals than by compositing a few individuals of larger species. Small fish may be a more appropriate indicator for assessing ecological risk, as they might be expected to be prey for a larger number of fish-eating animals (the majority of which will be piscivorous birds and small mammals). The major advantage that larger fish could potentially offer, whether predators (piscivores) or bottom feeders, is a higher level of bioaccumulation and thus greater sensitivity to detect contaminants. The relative bioaccumulation of contaminants by large and small river fish is not known, thus the reason for preparing two samples in this study.

1.3 Objectives and Scope of the Field Operations and Methods Manual

Only field-related sampling and data collection activities are presented in this manual. Laboratory procedures and methods (including sample processing and analytical methods) associated with each ecological indicator are summarized in Chaloud and Peck, 1994 and Lazorchak et al. 1998); detailed procedures will be published as a separate document.

This manual is organized to follow the sequence of field activities during the 1-day site visit. Section 2 presents a general overview of all field activities. Section 3 presents those procedures that are conducted at a "base" location before and after a river site visit. Section 4 presents the procedures for verifying the site location and defining a reach of the river where subsequent sampling and data collection activities are conducted. Sections 5 through 12 describe the procedures for collecting samples and field measurement data for various condition and stressor indicators. Specific procedures associated with each indicator are presented in stand alone tables that can be copied, laminated, and taken into the field for quick reference. Section 13 describes the final activities that are conducted before leaving a river site. Appendix A contains a list of all equipment and supplies required by a crew to complete all field activities at a river. Appendix B presents a set of brief
summaries of field procedures and activities that can be laminated, collated into a 3-ring binder, and taken into the field along with the procedure tables. This waterproof handbook can serve as the primary field reference for field teams after they complete an intensive training program. Appendix C contains a list of vertebrate species names and corresponding species codes developed for use in the eastern U.S. and Oregon studies.

Depending on the specific project and approach to information management, field teams may also be provided with an information management handbook that contains instructions for tracking samples and generating sampling status reports as well as using the computers and associated hardware and software. Field teams are also required to keep the field operations and methods manual available in the field for reference and to address questions pertaining to protocols that might arise.

### 1.4 Quality Assurance

Large-scale and/or long-term monitoring programs such as those envisioned for EMAP require a rigorous quality assurance (QA) program that can be implemented consistently by all participants throughout the duration of the monitoring period. Quality assurance is a required element of all EPA-sponsored studies that involve the collection of environmental data (Stanley and Verner, 1986). Field teams should be provided a copy of the QA project plan (e.g., Chaloud and Peck, 1994) for EMAP-SW activities. The QA plan contains more detailed information regarding QA/QC activities and procedures associated with general field operations, sample collection, measurement data collection for specific indicators, and data reporting activities.

Quality control (QC) activities associated with field operations are integrated into the field procedures. Important QA activities associated with field operations include a comprehensive training program that includes practice sampling visits, and the use of a qualified museum facility or laboratory to confirm any field identifications of biological specimens. The overall sampling design for EMAP-SW related studies usually includes a subset of sites (10 to 15 percent) that are revisited within a single sampling period and/or across years (e.g., Larsen, 1997; Urquhart et al., 1998). Information from these repeat visits is used in part to describe overall sampling and measurement precision for the various ecological indicators.

### 1.5 Literature Cited


Klemm, D.J., P.A. Lewis, F. Fulk, J.M. Lazorchak. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. U.S. Envi-


