REPORT OF THE INTERAGENCY BIOLOGICAL METHODS WORKSHOP

Edited by Martin E. Gurtz and Thomas A. Muir

U.S. GEOLOGICAL SURVEY

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OHIO ENVIRONMENTAL PROTECTION AGENCY
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FOREWORD

The Interagency Biological Methods Workshop and this report are part of an ongoing intergovernmental effort to improve water-quality monitoring nationwide. Beginning in 1992 under the leadership of the U.S. Geological Survey (USGS) and the U.S. Environmental Protection Agency, a partnership of Federal, State, and Tribal organizations initiated the Intergovernmental Task Force on Monitoring Water Quality (ITFM). Operating under the sponsorship of the Federal Water Information Coordination Program that is led by the USGS, the ITFM is evaluating water-quality monitoring and is developing recommendations to improve water-quality information at all levels of government and in the private sector. In 1995, the ITFM will submit its final report to the Office of Management and Budget, Congress, States, and others. Based on the recommendations of the ITFM, agencies will implement the proposed strategy to improve water-quality monitoring.

From its beginning, the ITFM has recognized the importance of biological monitoring that supports efforts to protect human health, to preserve and enhance ecological conditions, and to sustain a stable economy. The ITFM has identified as a priority the need to develop comparable biological and microbiological methods for interagency use. In response to this priority, the USGS National Water-Quality Assessment (NAWQA) Program hosted the Interagency Biological Methods Workshop to explore the development of comparable biological methods. Many ITFM member organizations participated in the workshop.

This report documents the results of these initial interagency deliberations. The participants identified similarities and differences in objectives, program designs, and methods among agencies. The workshop and this resulting report set the stage for future collaboration to foster the development and adoption of comparable methods wherever appropriate and consistent with agency missions and program objectives.

Interagency collaboration and the use of comparable biological methods can reduce costs, expand the base of biological information useful for decision making, and enhance scientific understanding of biological processes in relationship to physical and chemical processes in ecosystems. The ITFM appreciates the important step that the NAWQA Program and the workshop participants from other agencies have made toward improving water-quality monitoring generally and biological monitoring specifically.

Elizabeth Fellows

Elizabeth Fellows
U.S. Environmental Protection Agency
Chairperson, ITFM

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FIGURES

Multiply	By	To obtain
	Length	
micron (μm)	0.00003937	inch
millimeter (mm)	0.03937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
kilometer (km)	0.6214	mile
	Area	
square centimeter (cm^2)	0.001076	square foot
square meter (m^2)	10.76	square foot
	Mass	
gram (g)	0.03527	ounce, avoirdupois
	Volume	
liter (L)	0.264	gallon
milliliter (mL)	0.000264	gallon

Temperature: Water temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = 1.8\ (°C) + 32$$

Following is a list of abbreviations of agency and program names used throughout this report:

BEST	**Biomonitoring of Environmental Status and Trends (NBS; part of USFWS at the time of the workshop)**
BLM	**Bureau of Land Management (DOI)**
BOR	**Bureau of Reclamation (DOI)**
DOI	**U.S. Department of the Interior**
EMAP	**Environmental Monitoring and Assessment Program (USEPA)**
FDA	**Food and Drug Administration (USDA)**
ITFM	**Intergovernmental Task Force on Monitoring Water Quality**
NAWQA	**National Water-Quality Assessment Program (USGS)**
NBS	**National Biological Survey (DOI)**
NCBP	**National Contaminant Biomonitoring Program (NBS; part of USFWS at the time of the workshop)**
NC-DEHNR	**North Carolina Department of Environment, Health, and Natural Resources**
NOAA	**National Oceanic and Atmospheric Administration (U.S. Department of Commerce)**
NPS	**National Park Service (DOI)**
NS&T	**National Status and Trends Program (NOAA)**
Ohio EPA	**Ohio Environmental Protection Agency**
ORSANCO	**Ohio River Valley Water Sanitation Commission**
RAT	**River Action Team (TVA)**
RBP's	**Rapid Bioassessment Protocols (State Programs)**
TVA	**Tennessee Valley Authority**
USDA	**U.S. Department of Agriculture**
USEPA	**U.S. Environmental Protection Agency**
USFS	**U.S. Forest Service (USDA)**
USFWS	**U.S. Fish and Wildlife Service (DOI)**
USGS	**U.S. Geological Survey (DOI)**

The following abbreviations are used in figures 1-7 of this report:

AHH	Aryl hydrocarbon hydroxylase
ANOVA	Analysis of Variance
BI	Biotic index
CDF	Cumulative Distribution Function
CERCLA	Comprehensive Environmental Response, Compensation and Liabilities Act
DC	Direct current
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribose nucleic acid
DTH	Depositional-targeted habitat (NAWQA)
FY	Fiscal year
GC/MS	Gas chromatography/mass spectrometry
GIS	Geographic Information System
GPS	Global Positioning System
IBI	Index of Biotic Integrity
ICI	Invertebrate Community Index
MIWb	Modified Index of Well-being
NASQAN	National Stream Quality Accounting Network (USGS)
NATT	National Target Taxa (NAWQA)
NAWQMN	National Ambient Water Quality Monitoring Network
NODC	National Oceanographic Data Center
NPDES	National Pollutant Discharge Elimination System
NPMP	National Pollutant Monitoring Program
PCB's	Polychlorinated biphenyls
PVC	Polyvinyl chloride
QA/QC	Quality assurance/quality control
QCTV	Qualitative community tolerance value
QHEI	Qualitative Habitat Evaluation Index
QMH	Qualitative multihabitat (NAWQA)
RCRA	Resource Conservation and Recovery Act
RF3	River Reach File, Version 3
RTH	Richest-targeted habitat (NAWQA)
SV	Screening Value
TMDL	Total maximum daily load
VDC	Volts DC (direct current)
WLA	Waste load allocation
WWTP	Wastewater treatment plant

Report of the Interagency Biological Methods Workshop

Edited by Martin E. Gurtz *and* Thomas A. Muir

ABSTRACT

The U.S. Geological Survey hosted the Interagency Biological Methods Workshop in Reston, Virginia, during June 22-23, 1993. The purposes of the workshop were to (1) promote better communication among Federal agencies that are using or developing biological methods in water-quality assessment programs for streams and rivers, and (2) facilitate the sharing of data and interagency collaboration. The workshop was attended by 45 biologists representing numerous Federal agencies and programs, and a few regional and State programs that were selected to provide additional perspectives. The focus of the workshop was community assessment methods for fish, invertebrates, and algae; physical habitat characterization; and chemical analyses of biological tissues. Charts comparing program objectives, design features, and sampling methods were compiled from materials that were provided by participating agencies prior to the workshop and formed the basis for small workgroup discussions. Participants noted that differences in methods among programs were often necessitated by differences in program objectives. However, participants agreed that where programs have identified similar data needs, the use of common methods is beneficial. Opportunities discussed for improving data compatibility and information sharing included (1) modifying existing methods, (2) adding parameters, (3) improving access to data through shared databases (potentially with common database structures), and (4) future collaborative efforts that range from research on selected protocol questions to followup meetings and continued discussions.

INTRODUCTION

Development of water-quality assessment programs having regional or national scope requires the use of consistent methods wherever possible to facilitate comparative analyses of spatial and temporal patterns. Although biological methods have been used in water-quality assessments for many years, only recently has there been an effort made to develop and implement consistent methods on a national scale in Federal programs. The U.S. Geological Survey (USGS) has developed a series of biological sampling protocols for use in its National Water-Quality Assessment (NAWQA) Program. These protocols are designed to provide national consistency in collecting samples of biological communities, characterizing physical habitat conditions at several spatial scales (basin, segment, and reach), and collecting samples of biological tissues for chemical analyses of organic compounds and trace elements. In an effort to promote better communication among Federal agencies that are using or developing related methods for use in other programs of regional or national scope, and to facilitate the sharing of data and interagency collaboration, the USGS hosted the Interagency Biological Methods Workshop in Reston, Virginia, during June 22-23, 1993. This workshop also supported activities of the Intergovernmental Task Force on Monitoring Water Quality (ITFM) and the developing needs of the National Biological Survey (NBS). The workshop was attended by 45 biologists representing numerous Federal agencies and programs, as well as several regional and State programs selected to provide some perspectives from non-Federal biological monitoring programs (Appendix I).

The objectives of the workshop were to

1. Discuss protocols for collecting and processing biological samples as part of water-quality or other resource-assessment activities (active or planned) having regional or national focus,
2. Compare sampling methods in the context of individual program objectives in order to highlight similarities and differences among programs, and
3. Increase the potential for data compatibility and interagency collaboration.

The scope of the workshop included biological water-quality assessment methods for streams and rivers, with emphasis on methods being used by NAWQA and corresponding methods of other programs. Thus, the focus was on community assessment methods for fish, invertebrates, and algae; physical habitat characterization; and chemical analyses of biological tissues. Other biological methods (such as toxicity studies or biomarkers) and other types of water bodies (such as lakes or estuaries) were generally considered to be beyond the scope of the workshop. However, such topics were included in the discussions where appropriate--for example, in comparisons of programs for tissue studies. Participants were, for the most part, scientists knowledgeable about the technical details of the protocols used by their respective agencies.

The workshop was structured around a series of workgroup discussions that focused on methods in each of the principal categories--fish, invertebrates, algae, habitat, and tissues (Appendix II). Following an overview of the NAWQA Program and its biological components, plenary talks were given on the role of biological monitoring in State programs and the activities of the biological indicators group within ITFM. The remainder of the 2-day meeting was spent predominantly in small-group discussions (Appendix III), focusing on each category of protocol, narrative summaries of which are included in this document.

Prior to the workshop, participating agencies were provided a series of protocol charts that presented details of the objectives, design, and methods used in the NAWQA Program, with blank space provided for entering comparable information for other programs. Each agency or program was requested to return the completed charts, and any appropriate protocols, or to bring them to the workshop. Copies of charts and protocols were distributed to participants at the beginning of the workshop and formed the basis for much of the discussion. Comparison charts (figs. 1-7 located at the back of this report) were compiled from materials provided by the participating agencies. To minimize duplication of information for programs represented by more than one component, general program objectives and design features are presented for several programs in figure 1. The NBS's Biomonitoring of Environmental Status and Trends (BEST) Program is included only in figure 1 because specific protocols are still in the development stage. (This program was part of the U.S. Fish and Wildlife Service (USFWS) at the time of the workshop.) Comparison charts for fish (fig. 2), invertebrates (fig. 3), algae (fig. 4), habitat (figs. 5 and 6), and tissues (fig. 7) include programs that provided the required information.

The primary focus of the workshop was on national-scale Federal programs because of the particular challenges associated with comparability of methods across such a large spatial scale; most, but not all, major agencies and programs using related methods were represented in the workshop. Because many regional, State, and local organizations have long histories of conducting biological assessments, representatives of several regional and State programs were invited to participate in the workshop. An overview of rapid bioassessment protocols (RBP's) was provided as applicable to the majority of States. Completed protocol charts also were provided by the Ohio Environmental Protection Agency (Ohio EPA) for fish, invertebrates, and habitat, and by the Ohio River Valley Water Sanitation Commission (ORSANCO) for tissues; this information is included in the comparison charts. Several other agencies, such as the Tennessee Valley Authority (TVA) and the North Carolina Department of Environment, Health, and Natural Resources (NC-DEHNR), were represented in one or more workgroups and provided materials for discussion, but are not represented in the comparison charts.

Purpose and Scope

The purpose of this report is to summarize discussions that took place at the Interagency

Biological Methods Workshop and facilitate communication and collaboration among Federal agencies using biological methods in water-quality assessments. A series of charts is provided that compares protocols in use or under development by the participating agencies. It is expected that this report will be a springboard for further discussion and future interagency collaborative efforts.

Acknowledgments

The success of this workshop was due in large part to the high degree of interest and support provided by all of the participating agencies. The editors particularly thank Elizabeth Fellows of the U.S. Environmental Protection Agency (USEPA) for encouraging the leaders of the NAWQA Program to take the initiative in hosting the workshop. Members of the interagency committee who met April 29, 1993, to plan the workshop included the following: Bill Breed of the U.S. Department of Energy, Wade Bryant of the USFWS, Tony Cappelucci of the Bureau of Reclamation (BOR), Chris Faulkner of the USEPA Office of Water, Marty Gurtz of the USGS, Ron Huntsinger of the Bureau of Land Management (BLM), Ron Preston of the USEPA Region III, Gary Rosenlieb of the National Park Service (NPS), Steve Sorenson of the USGS, and Rick Swanson of the BLM. Tom Muir also participated in the planning session, representing both the USFWS and the Implementation Committee of the NBS. The editors also thank Chris Yoder of the Ohio EPA, who gave a presentation at the workshop on the activities of ITFM related to biological indicators; Mike Barbour of Tetra Tech, Inc., who gave a presentation on biological monitoring activities of State agency programs; and Dallas Peck (then Director) of the USGS, who made the opening remarks at the workshop.

The protocol-comparison charts represent considerable effort on the parts of the participating agencies to provide summaries of program features and sampling methods in a consistent format. Contributors to those parts of this report include:

Component	Program (Agency)	Contributor
Objectives/ Design Features	NAWQA (USGS)	Marty Gurtz
	EMAP (USEPA)	Steve Paulsen
	RBP's (State Programs)	Mike Barbour
	BEST (NBS)	Chris Schmitt
	Ohio EPA	Chris Yoder
Fish	NAWQA (USGS)	Mike Meador
	EMAP (USEPA)	Frank McCormick
	RBP's (State Programs)	Mike Barbour
	Ohio EPA	Chris Yoder
Invertebrates	NAWQA (USGS)	Tom Cuffney
	EMAP (USEPA)	Brian Hill
	BLM/USFS	Mark Vinson
	RBP's (State Programs)	Mike Barbour
	Ohio EPA	Chris Yoder
Algae	NAWQA (USGS)	Stephen Porter
	EMAP (USEPA)	Brian Hill
Habitat	NAWQA (USGS)	Cliff Hupp, Mike Meador
	EMAP (USEPA)	Phil Kaufmann
	RBP's (State Programs)	Mike Barbour
	Ohio EPA	Chris Yoder

Component	Program (Agency)	Contributor
Tissues	NAWQA (USGS)	Kent Crawford
	EMAP (USEPA)	Roger Yeardly
	Fish Contamination Program (USEPA)	Skip Houseknecht
	National Study of Chemical Residues in Fish (USEPA)	Ryan Childs
	National Contaminant Biomonitoring Program (NBS)	Chris Schmitt
	National Status and Trends Program (NOAA)	Adriana Cantillo
	ORSANCO	Jerry Schulte

These contributors--except for Steve Paulsen, Phil Kaufmann, Roger Yeardly, and Jerry Schulte--were participants in the workshop.

REPORT OF FISH WORKGROUP

by Michael R. Meador

The fish workgroup was attended by Mike Meador (facilitator), USGS; Frank McCormick, (recorder/reporter), USEPA; Ron Preston, USEPA; Mike Rexrode, USEPA; Charlie Saylor, TVA; Terry Short, USGS; Chris Yoder, Ohio EPA; and Steve Zylstra, representing the BEST program of the USFWS (now NBS). Written protocols for sampling activities as part of NAWQA, EMAP, and Ohio EPA were contributed. A general discussion of sampling procedures conducted by TVA also was presented.

Discussion of the expectations of the workgroup revealed that the participants hoped to achieve a better understanding of how different programs conduct their respective sampling activities and the "why" behind decision-making. There was unanimous agreement that tremendous potential for cooperation and collaboration exists among the programs represented, and that these need to occur at the technical level. The goals of the workgroup were to (1) compare and contrast current protocols for sampling fish community structure, (2) complete a comparison chart of the protocol methods represented, (3) establish a working relationship for continued interagency cooperation, and (4) discuss opportunities for future research and collaboration. In order to accomplish goals (1) and (2), the similarities and differences among agency protocols (fig. 2) were examined.

Similarities

The USGS, USEPA, Ohio EPA, and TVA are currently involved in programs that describe fish community structure based on a representative sample of the fish community. In these programs, fish community structure is assessed by sampling all habitats within the selected stream reach, which is identified based on the geomorphology of the stream. Stream reach lengths range from a minimum of 150 meters (m) to a maximum of 500 m. Sampling seasons vary but depend on specific sampling objectives and are related to ideal flow conditions; high flows and abnormal turbidity are avoided. Fish samples are collected in all programs by conducting at least one pass using a pulsed-DC electrofishing unit during daylight hours. The Ohio EPA and NAWQA Program recognize that night sampling of some rivers may be more effective than day sampling, especially in larger rivers; Ohio EPA conducts night sampling in the Ohio River. The Ohio EPA and NAWQA protocols recommend a minimum reach length of 500 m for nonwadeable sites. During electrofishing, wadeable sites are generally fished in an upstream direction, whereas nonwadeable sites are generally fished in a downstream direction.

All fish are identified to species in the field when possible, and examined for anomalies; some or all fish are measured. It was agreed that only ichthyologists should identify fish collected in the programs; however, there are no accepted criteria for establishing the credentials of an ichthyologist to make taxonomic identifications. This difficulty seems common to all programs.

Specimens are retained for laboratory identification and (or) vouchers by fixing them in 10-percent buffered formalin for later storage in alcohol. At minimum, data analysis consists of providing species richness and relative abundance information. In all programs, consideration is being given to the use of multimetric indices and multivariate analytical procedures depending upon objectives.

Differences

The only programs represented that include sampling of fish communities in nonwadeable streams were the Ohio EPA and NAWQA programs. The Environmental Monitoring and Assessment Program (EMAP) determines reach lengths in wadeable streams based on about 40 times the channel width. However, because EMAP protocols are designed for wadeable streams only, this channel width determination results in reach lengths that are within the 150-m to 500-m range for wadeable streams that is common to all programs. The Ohio EPA's method consists of two passes through a sampling reach, using electrofishing gear. NAWQA, EMAP, and TVA conduct multiple-gear sampling using a combination of electrofishing and seining. However, NAWQA conducts electrofishing using two passes through a sampling reach; EMAP conducts electrofishing using one pass through a reach; and the number of passes conducted by TVA varies depending upon the number of fish and habitat types.

The participants generally felt that the differences in fish sample collection methods among programs were relatively minor and would not affect the ability to compare data among programs. None of the programs is designed to determine estimates of populations. Therefore, no program is designed to sample all individuals, and some rare and possibly some "canary" species (for example, highly sensitive species, or species that are indicative of a specific environmental condition) can be missed.

There was considerable discussion regarding the types of ancillary data that can contribute to the understanding of fish community structure as it relates to water quality. Ancillary data include information on length, weight, and external anomalies. The group agreed that such data are generally important, particularly in situations where species richness is naturally low.

Opportunities for Collaboration and Research

The participants emphasized the need to work closely with the USFWS (and State biologists) on endangered species issues as they relate to sampling efforts. Collaborative sampling efforts could help to reduce concerns regarding the effects of sampling activities on endangered species. Some discussion also focused on animal rights issues and fish sampling activities; all protocols are being examined regarding these issues.

Some discussion was generated regarding a common electronic database; however, there was not sufficient time to pursue this topic in detail. All agreed that efforts should be made to make the data broadly available on electronic media.

The use of relative abundance data for fish in relation to environmental conditions has grown in acceptance among the scientific community. All agreed that data from the programs must be reported not only internally, but through peer-reviewed scientific journals as well. However, there is still an indication that managers and policy makers lack an understanding of the use of such data analyses. It is hoped that the efforts of all programs will help advance the acceptance of such data analyses by the non-scientific community.

Opportunities for collaboration include joint coordination of workshops or symposia to address issues such as the need to identify qualified ichthyologists, field and laboratory quality assurance/quality control, and the use of museums in archiving material from water-quality assessment programs. Pilot projects also provide opportunities to evaluate field approaches of the participating programs, as well as opportunities to examine data collected by the various agencies.

REPORT OF INVERTEBRATES WORKGROUP

by Thomas F. Cuffney

The invertebrates workgroup consisted of Tom Cuffney (facilitator/reporter), USGS; Marc Sylvester (recorder), USGS; Steve Ahlstedt, TVA; Ted Angradi, U.S. Forest Service (USFS); Marjorie Coombs, USEPA; Wayne Davis, USEPA; Chris Faulkner, USEPA; Brian Hill, USEPA; Roy Irwin, NPS;

Dave Lenat, NC-DEHNR; Mark Nelson, BOR; and Mark Vinson, BLM.

Printed materials distributed to workgroup participants included descriptions of the following biomonitoring programs or activities of Federal agencies: NAWQA (USGS), EMAP and Rapid Bioassessment Protocols (RBP's) (USEPA), National Aquatic Monitoring Center (BLM/USFS), Biotic Condition Index protocol (USFS), and TVA's River Action Team (RAT) reconnaissance methods. Other written materials describing State programs of Ohio EPA, NC-DEHNR, and Kansas Department of Wildlife and Parks, and several volunteer stream-monitoring programs (including Streamside Bioassessment--Izaak Walton League of America, Intensive Stream Bioassessment--Maryland Save Our Streams, and the River Watch Network) also were distributed. Protocol charts provided by NAWQA, EMAP, BLM/USFS, State programs (RBP's), and Ohio EPA are included in the comparison chart (fig. 3).

The session began with participants providing brief overviews of their biomonitoring programs, including NAWQA, EMAP, RBP's, RAT, North Carolina's stream-monitoring program, and the BLM/USFS stream-biomonitoring programs. Dave Lenat presented some preliminary results from a collaborative study that investigated the comparability of benthic invertebrate-based water-quality assessment techniques used by several State and Federal agencies. Discussions then centered on similarities and differences among the programs, issues of common interest, and data comparability among programs. Principal discussion points included issues related to program objectives, reference sites, gear type (including mesh size), sampling designs, metrics, taxonomic resolution, sampling season, appropriate effort for objectives, and subsampling (100- and 300-organism counts). Because of time constraints, discussions emphasized mesh size, identification of midges (Chironomidae), reference sites, and subsampling.

Similarities

All invertebrate biomonitoring programs discussed by the workgroup have a similar objective--to provide an accurate assessment of the structure of the benthic invertebrate community that has relevance to water-quality conditions. Programs are oriented toward documenting the status of invertebrate communities, determining trends (spatial and temporal) in community condition, and establishing cause-and-effect relations. Other major areas of similarity include the following:

1. All programs integrate physical, chemical, and biological approaches to some extent.
2. Sampling is generally focused within a defined length of stream (sampling reach) associated with a sampling site. The size of the sampling reach and the criteria for establishing the sampling reach vary among programs.
3. Sampling within the sampling reach typically involves single-habitat sampling (usually a riffle) supplemented with a composite sample collected from multiple habitats within the reach.
4. All programs use multimetric approaches to data analysis, although some also use multivariate techniques.
5. Reference sites are important for data comparisons and interpretations in all programs, although the dependence on reference sites varies among programs.

Differences

The various biomonitoring programs differ in the size of mesh used in sampler netting. USEPA uses 595-micron (µm) mesh for EMAP and recommends it for use in RBP's, though specific mesh requirements are not included in the RBP's. Mesh sizes of 425 µm and 210 µm are used by NAWQA; the BLM/USFS program uses 280-µm mesh, and the TVA programs use 900- to 1,000-µm mesh. The consensus of the group was that any mesh size from 210 to 1,000 µm could be (and has been) successfully used for water-quality assessment. In theory, the smaller the mesh size the more complete the representation of the community. However, this theoretical consideration must be balanced with the drawbacks associated with smaller mesh size: sampler backwash, large sample volumes, increased sample-processing costs, and increased numbers of partially identified small organisms. A mesh size of 300 to 600 µm was offered as a good operational range for community

assessments. However, the choice of mesh size needs to be determined by the objectives of the individual program and its budget and should include considerations of available historical data and the taxonomic groups deemed important to the interpretation of water-quality conditions. For example, if the program needs to represent smaller invertebrate groups, such as the chironomids, then a smaller mesh size is appropriate. Programs that focus on "EPT" taxa (Ephemeroptera, Plecoptera, Trichoptera) may prefer a larger mesh size.

Most programs select sampling locations on the basis of anticipated effects (point source or desired combination of environmental conditions). EMAP is unique in that it randomly selects sampling sites according to a national sampling grid. Differences in selection of sampling sites reflect the differences in objectives of each monitoring program. For example, some monitoring programs (RBP's and RAT) focus on rapidly identifying sites with degraded water-quality conditions and emphasize sampling large numbers of sites, quickly and inexpensively, to identify relatively large changes in water quality. Other programs focus on relating physical and chemical water-quality changes to biological changes (NAWQA), or to assessing water-quality conditions over broad geographic areas (EMAP), and require different site-selection procedures, sampling equipment, sample-processing strategies, and data-analysis procedures to support their objectives.

Programs differ in the degree of sample processing (field picking and subsampling) used to determine community structure. EMAP and NAWQA process samples with the intent of achieving a high level of taxonomic resolution (usually identification to lowest practicable taxon) and processing at least one-fourth of the entire sample. RBP's, TVA, and BLM/USFS typically base processing on the removal of a minimum specified number of organisms (100, 200, or 300) from the sample. Identifications for RBP's may be at the lowest practicable taxon or at a higher level depending upon the level of RBP's used. TVA and RBP's procedures also can involve field picking of samples, whereas EMAP, NAWQA, and BLM/USFS depend upon picking of invertebrates in the laboratory. It was apparent from some of the experiences of the workshop participants that employing 100- or 300-count field picks expedited sample processing considerably but missed substantial numbers and varieties of small invertebrates, particularly small chironomids and oligochaetes. Preliminary analyses of NAWQA Program pilot-study samples also indicated that 100- or 300-count subsamples of invertebrates would have lead to significant underestimation of benthic invertebrate community composition and richness. However, 100- and 300-count subsamples have been used with great success in the RBP's and other State and Federal programs. Thus, decisions on sample-processing techniques should be based on the objectives of the various programs.

Programs also differ in whether taxonomic identifications include a detailed (genus or species) assessment of the chironomid community. For example, most current bioassessment metrics focus on EPT taxa and have relatively little emphasis on chironomid identifications made beyond family level. The major problems are the increased sampling effort and costs associated with collecting representative samples of midges, and identifying and quantifying midges. These problems must be balanced with the realization that in many instances the family Chironomidae constitutes more than half of the species at a site. Further, chironomid taxonomy has improved to the point that most individuals can be readily identified at least to genus, and chironomids are becoming increasingly diagnostic (increase information and differentiation) in bioassessment programs that include identifications at the genus or species level. The inclusion of chironomid identifications beyond family is largely dependent upon the program's objectives. Identification to family level is probably adequate for screening or impact assessment studies. However, for diagnostic studies of source, cause and effect, and trends, identification of chironomids to genus or species is probably preferable.

Opportunities for Collaboration and Research

The group discussed the possibility of establishing a single approach to benthic-invertebrate biomonitoring that could be used for all agencies and programs, but the majority of the group felt that each program must use sampling methods and design that support its objectives and fit within budget constraints. It is apparent that the programs represented here constitute a continuum in effort that ranges from quick screenings intended to rapidly and inexpensively identify significant water-quality problems (the "scratch and sniff" approach as described by Steve

Ahlstedt) to detailed cause-and-effect studies that require integrated physical, chemical, and biological assessments. Consequently, it would be very difficult to develop a unified set of methods that could be readily adapted to the multiple objectives of all existing State and Federal water-quality assessment programs.

Discussions on data comparability focused on two issues: (1) integrating data sets and (2) comparing assessment results. Because of the different objectives and techniques employed by the various State and Federal programs, the group agreed that combining data sets should be done very cautiously. This is particularly true when combining quantitative information (densities or relative abundances). However, qualitative data, particularly the presence of a taxon at a site, presented fewer problems provided that there was sufficient confidence (quality assurance) in the identification. Unfortunately, data on the absence of a taxon are much more sensitive to differences in sampling design and need to be interpreted cautiously. There was basic agreement that the water-quality assessments produced by different programs could be fairly easily used among the various programs, particularly as a guide to the selection of sites. Additional interagency efforts to compare and contrast assessment results, such as one conducted by NC-DEHNR in January 1993, are needed to fully define data comparability issues.

A potential area for collaboration is in establishing reference sites and determining reference conditions. All programs recognized the value of reference sites and their use in ranking biological conditions among sites. Key issues related to the use of reference sites are (1) the availability of reference sites for certain types of streams (particularly, larger rivers), (2) criteria for selecting reference sites and matching them with assessment sites, and (3) the evaluation of the natural temporal (seasonal and annual) variability within, and spatial variability among, reference sites.

The efforts of the various State and Federal programs can and should be highly complementary, despite their apparent differences. Mutual benefits will accrue from continued communication and collaboration on methods, site selection, quality-assurance and quality-control techniques, databases, assessment metrics, taxonomy, reference site selection, and assessment of variability related to sampling method, season, year, site, and metric. Interagency efforts, such as the development of an interagency taxonomic database (National Oceanic and Atmospheric Administration (NOAA), USEPA, and USGS) and the comparison of field methods, are important steps to fostering the exchange of consistent high-quality data among State and Federal agencies.

REPORT OF ALGAE WORKGROUP

by Stephen D. Porter

The algae workgroup was attended by Stephen Porter (facilitator/reporter), USGS; Brian Hill (recorder), USEPA; Steve Sorenson, USGS; and Marty Gurtz, USGS. Other informal discussions held with Chris Faulkner (USEPA), Ted Angradi (USFS), Mark Nelson (BOR), and Chris Yoder (Ohio EPA) during the course of the workshop contributed additional information concerning existing or proposed uses of algae for environmental assessments. The objectives of the workgroup meeting were to (1) compare and discuss current protocols and methods for using benthic algae (periphyton) for regional and national assessments of water quality, (2) compare and contrast program objectives and approaches relative to site selection, sampling strategies, methods of laboratory analyses, and data interpretation, (3) determine compatible uses of interagency algal data, (4) establish a working relationship for continued interagency cooperation and information exchange, and (5) discuss opportunities for future research and collaboration. Regional- or national-scale programs presently involved in sampling algal communities for water-quality assessments include only NAWQA and EMAP (fig. 4).

Similarities

The NAWQA and EMAP programs use similar approaches to collecting and processing algal samples, and analyzing data. Following are the similarities in algal protocols between these two programs:

1. Benthic algal samples are collected from natural substrates in conjunction with the collection of invertebrate samples.
2. Samples are collected from erosional and depositional habitats using similar (or compatible) methods; however, the habitats are targeted in the NAWQA program and randomized in the EMAP program.

3. Areas of benthic substrate sampled according to each protocol are similar, although the composite area of each habitat sampled by EMAP varies with the relative abundance of erosional and depositional areas in the sampling reach.
4. In both programs, quantitative periphyton samples are analyzed for species composition and abundance, chlorophyll a, and ash-free dry mass (although these analyses are a study-unit option for NAWQA, rather than a protocol requirement).
5. The same sample preservatives are used by both programs.
6. Both EMAP and NAWQA target sampling activities to be conducted during stable, low-flow hydrologic conditions, and similar (at least qualitatively) ancillary information is collected by both programs.

Brian Hill indicated that EMAP will consider adding qualitative multihabitat sampling of periphyton to its algal protocol, as well as adopting protocols similar to NAWQA for sampling epipsammic and epipelic microhabitats. If EMAP personnel record the predominant microhabitat sampled in erosional and depositional habitats, certain algal data (quantitative epilithic, epipsammic, and epipelic samples) should be compatible with NAWQA data. Qualitative multi-habitat periphyton samples collected by certain State agencies (for example, Kentucky and Montana) appear to be compatible with NAWQA protocols for the purpose of documenting a list of algal taxa and estimating taxa richness. Other State agencies (for example, Ohio and North Carolina) are in the process of establishing procedures for sampling benthic algae and have expressed interest in the NAWQA algal protocol.

Differences

With the exception of NAWQA, EMAP, and State monitoring programs, most agencies have not incorporated algal sampling into environmental assessment programs except for the purposes of (1) monitoring phytoplankton chlorophyll a concentrations to evaluate eutrophication processes or taste-and-odor problems; (2) using periphyton (including aquatic mosses) to evaluate contaminant sources (for example, bioconcentration of metals (BOR) and acidification (USFS) from mine drainage, and numerous studies concerning effects of point-source discharges (State agencies)); and (3) reconstruction of historical changes in the pH or trophic state of lakes by examining diatom-frustule assemblages from sediment cores (EMAP-Great Lakes).

The primary differences between NAWQA and EMAP algal protocols include differences in the selection of instream sampling habitats, methods of sample compositing, and relative emphasis on obtaining structural or process measures of the algal community to assess spatial variation. These issues correspond to differences in the statistical design criteria and approaches of NAWQA and EMAP. Other differences include sampling frequency (NAWQA samples once per year for 3 successive years, with multiple-reach replication, at intensive ecological assessment sites; EMAP samples once every 4 years, with 10-percent annual resampling of sites). The EMAP protocol calls for routine determinations of chlorophyll a, ash-free dry mass, alkaline/acid phosphatase, and benthic metabolism, whereas the NAWQA protocol lists determinations of chlorophyll and ash-free dry mass as study-unit options. Further, the EMAP protocol presently does not provide for qualitative sampling of periphyton, quantitative sampling of phytoplankton, or alternative methods for sampling periphyton when the prescribed sampling gear cannot be used effectively (for example, methods for sampling epiphytic and epidendric microhabitats and certain epilithic microhabitats such as gravel substrates).

Quantitative algal protocols for NAWQA and EMAP differ considerably from those used by other agencies. Although the use of artificial substrates by State agencies meets their objectives for water-quality monitoring purposes, algal data obtained by this method are not comparable with NAWQA or EMAP data from natural substrates. However, artificial substrates are listed as a study-unit option in the NAWQA protocol (for example, for synoptic or case studies); study units could benefit from State-agency data and collaboration in cases where the type of artificial substrate and length of exposure period can be standardized. Phytoplankton chlorophyll samples are routinely collected by a number of agencies to assess

trophic conditions in lakes and reservoirs and to evaluate the effects of nutrient enrichment in large rivers. Quantitative phytoplankton samples (for analyses of chlorophyll and algal community structure) are listed as a study-unit option in the NAWQA algal protocol. However, the NAWQA data may not be directly comparable with data reported by other agencies because of differences in sample-collection methodology (width- and depth-integrated samples collected by the NAWQA program compared with subsurface or euphotic-zone samples collected by other agencies); these differences apply equally to water-chemistry data.

Opportunities for Collaboration and Research

Opportunities for future collaboration and research include (1) increased standardization of collection and analysis protocols within the constraints of specific program objectives and approaches, (2) field testing the comparability of sampling procedures, (3) interagency cooperation concerning the development and enhancement of taxonomic and autecological databases, (4) development of protocols for assessments of large rivers, and (5) sharing of compatible data to supplement interpretation and synthesis of factors that contribute to observed environmental conditions. Interagency collaboration can ensure that protocols are continually refined to maximize information content for evaluating environmental differences and change, and to enhance the transferability of data among agency programs with different objectives or approaches. The costs of compiling and maintaining large taxonomic databases (including associated physical and chemical data) could be shared among environmental-assessment agencies. Similar cost savings can accrue by developing a common mechanism for taxonomic verifications, voucher repositories, quality-assurance procedures (for example, evaluation of sample variance associated with specific field and laboratory procedures), and integrated databases.

Workgroup members agreed that all programs would benefit from identifying key scientists and other individuals involved with regional-scale biological assessments of water quality and establishing informal interagency networks and coordination mechanisms. This process could be enhanced by developing interagency training programs, conducting periodic workshops, and circulating protocols and experimental results. Improved standardization (or understanding of compatibility among protocol procedures) should increase the transferability of biological and water-chemistry data among programs. This understanding could potentially allow each program to interpret greater spatial resolution of factors that control water chemistry, habitat, and biological communities. For example, NAWQA could benefit from greater spatial characterization of reference reaches (particularly lower-order streams), and EMAP could benefit from information obtained from larger streams that integrate one or more human factors within distinct ecoregions. Collaboration with State agencies could enhance both programs and possibly lead to the development of opportunities for basic research and cooperative studies.

REPORT OF HABITAT WORKGROUP

by Cliff R. Hupp

This workgroup was composed of Cliff Hupp (facilitator), USGS; Mike Barbour (recorder/reporter), Tetra Tech, Inc.; George Gibson (reporter), USEPA; Susan Jackson, USEPA; Kerry Overton, USFS; Rick Swanson, BLM; Ron Parker, USEPA (one day); Stephen Porter, USGS (one day); and Dick Smythe, USFS (one day). The EMAP program was represented by the written comments provided by Phil Kaufmann (Oregon State University) prior to the meeting. The group established four goals for this meeting: (1) determine compatible uses of interagency habitat data, (2) discuss reasons for various elements in each protocol, (3) relate purpose/objective for conducting assessments to the methods used, and (4) establish a foundation for continued interagency cooperation and information exchange. For the most part all of these goals were attained, although there was insufficient time for detailed discussions of advantages and disadvantages of particular approaches. Participants now have a much clearer idea of the habitat programs in other agencies, which is a substantial achievement in itself.

The group listed several justifications for habitat descriptions that were common to all of the programs represented:

1. Habitat description provides the foundation for assessing and

interpreting the status of the biological communities.

2. Habitat description is necessary for the characterization of fluvial geomorphic and vegetative conditions, which are highly important parameters regardless of instream biota.
3. Habitat description is necessary to document habitat or environmental impairment or degradation.
4. Habitat description is necessary to distinguish between habitat alteration and other forms of environmental degradation--for example toxins, nutrients, and sediment--that may be of interest where biota are used as environmental barometers.

In order to facilitate mutual understanding, a distinction in general objectives of the programs was made early in the discussions. A habitat characterization was defined as an attempt to objectively describe the composition and structure of the physical and vegetative environment. A habitat assessment further included judgments of the quality of the habitat for support of the biotic feature of interest. No program objective was strictly one or the other, but some clearly leaned toward assessment or characterization. The NAWQA Program is oriented strongly toward characterization, whereas State programs are focused toward assessment. EMAP, USFS, BLM, and BEST have a nearly equal split in general objectives between characterization and assessment. Thus, most agencies used habitat description to relate habitat to biota, to characterize the composition and structure of the local environment, to determine impaired habitats, and to distinguish habitat alterations from other perturbations that can affect instream biotic communities.

Other major points that were raised during the workgroup's discussion of program objectives included the following:

1. Reference sites were the cornerstones of most programs;
2. Objectives and questions drive the scale of the programs;
3. What constitutes "good" habitat can be defined; and
4. As objectives tend to converge, the various protocols should become more similar.

Topics 1, 2, and 4 require further interagency discussion if the programs are to minimize duplication of effort and improve opportunities for cooperation and sharing of mutually useful information and data. All felt it was imperative to continue such discussions, especially considering current executive and departmental objectives.

Similarities

The protocol-comparison chart (fig. 5) compares approaches for four programs that completed the matrix prior to the workshop. Discussions also included BEST, as well as monitoring activities in the USFS and BLM. Although no two programs were identical, NAWQA, BEST, and EMAP are reach-based and share, in a general way, many approaches. USFS and BLM are very similar to each other but are both non-reach based systems. Nearly half of the discussion focused on delineating individual elements of the various protocols, including aspects of the riparian zone (fig. 6). The similarities substantially exceeded the differences in both parameters and measurement tools.

Differences

Several significant differences among programs were identified by the workgroup. Future discussions and probable protocol alteration will be necessary for data to be fully compatible among agencies for the following protocol elements:

1. Light measurement--All agencies would like to see improvement on quantification of this parameter. Some agencies use a canopy densiometer or direct light measurement at specific times or light conditions; nothing has been standardized.
2. Measurement of canopy cover/density--As with light measurement, the group felt that using a canopy densiometer would help quantify the light parameter beyond canopy opening. All felt the solar pathfinder has some advantages but is not yet standard.
3. Bank angle determination--Some agencies measure bank angle as degrees from vertical, others as degrees from horizontal.

4. Velocity and discharge measurement--The various agencies noted a wide range of effort in obtaining data for these parameters. Most programs incorporate some measurements of current velocity, although they vary in the methods used and in the procedures for determining locations for the measurements. Only the USGS routinely measures stream discharge continuously at fixed sites; this approach was recognized as being desirable, but participants doubted whether continuous discharge records were achievable for all important sites for other agencies nationwide.
5. Reach definition/transect location--No two agencies have exactly the same definition of reach or approach to delineation and sub-reach sampling. This is an area that could use more interagency priority attention. However, the group found no insurmountable differences among the reach-based assessments that would seriously limit data exchange, although neither USFS nor BLM has a reach-based approach.

Approaches for describing the extent and character of the riparian zone also differed among agencies and programs. The group unanimously viewed the riparian zone as one of the most important aspects of stream assessments and noted that this zone has only recently received the attention deserved. The level of effort made to describe the extent and character of the riparian zone varies widely among agencies. The USFS and BLM have the most complete protocols for characterizing the riparian zone, spending as much effort there as on instream assessment. The USFS uses a longitudinal transect for visual estimates and a "greenline" riparian survey for quantitative data. The BLM also conducts visual estimates for the entire reach, with transects that yield quantitative information; additionally, BLM conducts intensive quantitative riparian surveys. NAWQA uses a plotless technique (point-quarter method) for quantifying riparian woody vegetation at all fixed sites, and permanent riparian plots at a subset of sites. EMAP uses both visual observations and quantitative transects of riparian vegetation. State programs typically do not quantify characteristics of riparian zones, but rely solely on visual estimation techniques.

Opportunities for Collaboration and Research

Opportunities and needs for future interagency collaboration include

1. Identifying key individuals, sharing staff directories, and regional coordination among programs, surveys, and field work;
2. Ensuring that all protocols are evolving documents so that programs remain flexible to converge in methods where appropriate;
3. Seeking standardization of parameters and sampling methods where possible;
4. Demonstrating common use of data among agencies;
5. Documenting and sharing exact methodology;
6. Identifying a minimum data set that is common to all programs at regional and national scales;
7. Developing a comparison of riparian classification systems among agencies; and
8. Developing interagency training, workshops, and information-transfer networks.

The group noted that the most immediate steps should include exchange of staff directories, resolution of methodology differences, identification of common reference sites, and evaluation of other habitat methods.

REPORT OF TISSUES WORKGROUP

by Steven Goodbred and J. Kent Crawford

Members of this workgroup included Kent Crawford (facilitator), USGS; Chris Schmitt (reporter), USFWS; Steve Goodbred (recorder), USFWS; Sarah Gerould, USGS; Ryan Childs, USEPA; Skip Houseknecht, USEPA; and Jim Lazorchak, USEPA. Adriana Cantillo, NOAA, and Candy Brassard, USEPA, each participated for one day only; Steve Sorenson, USGS, and Roy Irwin, NPS, also participated in a portion of the discussions. The workgroup defined the basic objective of the workshop

as completion of the protocol-comparison chart (fig. 7). Presentations were made describing objectives, design, target organisms, analyte list, analytical methods, and quality assurance for the following programs: NAWQA, EMAP, BEST, National Study of Chemical Residues in Fish (USEPA), Fish Contamination Program (USEPA), National Status and Trends Program (NOAA), and National Contaminant Biomonitoring Program (USFWS; now NBS). These presentations continued into the second day of the workshop, and were followed by a general discussion.

Similarities

The degree of similarity among programs was highly dependent on program objectives, which generally could be categorized as follows: (1) human health, (2) water quality, and (3) ecological health. Programs that focus on human health issues emphasize the analysis of edible portions of biota (for example, fish fillets or soft tissues of mussels). Water-quality programs use biological tissues as a tool to assess the occurrence and distribution of contaminants that are bioavailable. Other programs assess ecological health through indicator species and an understanding of trophic transfer. Greatest similarities occurred among programs with similar objectives. Fish were included among target taxa for all programs, and there was substantial similarity among lists of target analytes.

Differences

Each tissue analysis program represented at the workshop specifies a list of target taxa for collection and analysis. Each program includes fish as a target group, but all three USEPA programs and ORSANCO target only fish. The NAWQA Program lists the Asiatic clam as the preferred target taxon for both trace elements and organic compounds; in addition to fish, aquatic insects and aquatic plants also are targeted for trace elements in NAWQA. NOAA's National Status and Trends Program targets mussels and oysters in the Mussel Watch Project, and fish in the Benthic Surveillance Project.

Selection of target analytes commonly followed the reasoning used by USEPA's National Study of Chemical Residues in Fish, in which three criteria were applied for selecting a contaminant:

1. How much of a contaminant is in the environment and what portion will bioaccumulate in biota and the food chain?
2. How persistent is this contaminant and what are its sources-- past, present, and future?
3. How toxic is it?

A notable difference among analyte lists of different programs is polychlorinated biphenyls (PCB's). Some agencies are analyzing only total PCB's; some are analyzing specific congeners (not always the same ones); and some are summing congener-specific PCB's to get a total concentration. Even with congener-specific PCB data, which are very expensive to obtain, the toxicologically significant congeners--the aryl hydrocarbon hydroxylase (AHH) inducers--are usually present in very small quantities that are masked in gas chromatography by the less toxic and more common PCB's. A unique tiered approach is being proposed by BEST, using total PCB concentrations as a screen and then doing a followup immunoassay analysis (using H4IIE, a continuous line of laboratory rat cells, which are exposed to contaminants and monitored for mutations or other responses) for samples with high total PCB concentrations to determine if toxicologically significant PCB congeners are present. This test is apparently cost effective and may be relevant for many tissue programs.

Opportunities for Collaboration and Research

The most important recommendation for future collaboration among agencies is that national, regional, and State laboratories become involved in NOAA's Quality Assurance (QA) Project for tissue. This Project consists of a series of interlaboratory comparison exercises wherein each laboratory analyzes a variety of tissue matrices (currently both inorganic and organic); however, the number of participating laboratories may be limited by the amount of tissue sample material available. Results from these "round-robin" exercises will enable comparison of tissue data among programs and agencies. At this point, the National Contaminant Biomonitoring Program (NCBP), BEST, and NAWQA Program will be participating in NOAA's QA Project.

Compatibility and availability of tissue databases also were discussed. For example, USEPA's STORET database has been valuable in many ways but is still difficult to use. The BEST program is exploring the possibility of using USGS's National Water Information System database.

The workgroup concluded that improving compatibility of tissue data among programs requires answers to several critical research questions, including the following:

1. How are edible portions related to whole fish body burdens? What ancillary variables should be measured? If conversion factors can be developed, what degree of confidence do they have?
2. How important are interspecific differences in contaminant body burdens? Are there ecologically homologous species that are similar in their ability to accumulate and transfer contaminants? Are there differences in trophic transfers to humans as opposed to wildlife?
3. Can we identify levels of concern for contaminants that bioaccumulate in fish? The group felt that there is a substantial need for a comprehensive and thorough update of national wildlife contaminant criteria; the only published criteria were developed by the National Academy of Sciences/ National Academy of Engineering Committee on Water Quality Criteria more than 20 years ago (in 1972).
4. How can we optimize sampling effectiveness (maximize information return per dollar invested in sampling and analysis)? This can be achieved by optimizing compositing strategy, species selection, time of sampling, and ancillary variables.

The meeting ended with several participants discussing the possibility of a followup meeting to work on target analyte lists to make the various tissue programs more compatible. The session was successful in the exchange of protocols, compilation of information for the comparison chart, establishment of a dialogue, and identification of directions for future collaboration and research.

SUMMARY

by Martin E. Gurtz and Thomas A. Muir

The Interagency Biological Methods Workshop succeeded in its principal goal of promoting better communication among governmental organizations that use biological methods in water-resources programs. Participants freely exchanged information about existing and planned program activities and sampling protocols. Improving data comparability among programs can be accomplished only after first assessing differences and similarities and discussing the reasons for methods chosen by different programs. This workshop accomplished that important first step. This meeting and associated efforts are part of a larger process to consolidate protocols among Federal, State, local, and private agencies and organizations.

A consistent theme throughout the workshop was that differences in methods among programs largely occur because of differences in objectives of the individual programs. For example, programs represented in this workshop use different approaches to conducting tissue-analysis studies; depending on whether the objectives are driven by considerations for human health, water-quality assessment, or ecological health, the lists of target taxa (or target tissues within selected taxa) and target analytes vary substantially. Participants also noted some important similarities; EMAP and the NAWQA Program, for example, have converged on many aspects of sampling methods, including a stream-reach based approach for both biological sampling and habitat characterization. However, key differences in how reaches are selected and in how the sampling effort is distributed throughout the reach (for example, random transects as opposed to targeted habitats), are driven by basic design considerations of the programs and may influence the comparability of the data.

The consensus of the workshop participants was that the differences in objectives and sampling designs were appropriate and necessary, and that the programs represented were complementary rather than duplicative because the different approaches allow different questions to be addressed. Where programs have identified similar data needs, however, the use of common methods is mutually beneficial. Each workgroup was able to identify several opportunities for improving data compatibility and information sharing among programs. These opportunities could be

categorized as (1) modifying existing methods, (2) adding parameters, (3) improving access to data through shared databases (potentially with common database structures), and (4) encouraging future collaborative efforts that range from research on selected protocol questions to followup meetings and continued discussions.

Most programs incorporate "reference," or "minimally impaired," conditions into the sampling design in some way, but programs differ in how those reference conditions are defined and whether reference comparisons need to be regional or site specific. Some participants felt that defining reference conditions might be an appropriate role for State agencies; such information is essential for the development of biological criteria by States and would be valuable information in support of numerous regional and national programs. A continuing dialogue among Federal and State programs would help to prioritize regional characteristics for which reference conditions need to be defined. Future partnerships among government agencies could lead to identification of types of data, and methods to be used, that represent a consistent common denominator among State and Federal programs--for example, in the characterization of reference conditions.

Compatibility of databases was another consistent theme among workgroup discussions. Ongoing efforts through the Intergovernmental Task-Force on Monitoring Water Quality have begun the process of exchanging information about database and communication systems currently in use or being designed by Federal agencies. Participants in the workshop seemed unanimous in their view that these efforts deserve high priority.

Interagency discussions occur routinely among government biologists, through coincidental or planned exchanges at professional society meetings, participation on advisory panels, and collaboration involving informal comparative studies or formal interagency memoranda. Nevertheless, the Interagency Biological Methods Workshop provided a unique opportunity for biologists from a broad spectrum of agencies, with diverse missions and program objectives, to meet and exchange information about the programs they represent and the methods presently in use or under consideration. Participants agreed that the workshop was a successful forum for communication and improved understanding. The protocol charts in this report are expected to stimulate additional discussions among the workshop participants and other scientists who were not present at the workshop. We hope that this workshop report will provide a valuable framework for developing future interagency collaborative efforts.

COMPARISON CHARTS

Following are figures 1-7, which provide comparison charts of program objectives of the various participating agencies in the Interagency Biological Methods Workshop, as well as protocol comparisons for fish, invertebrates, algae, habitat, and tissues. (Lists of abbreviations and units of measure used in figures 1-7 are on page VI and VII at the front of this report.)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
PROGRAM OBJECTIVES	**Describe status and trends in the quality of the Nation's surface- and ground-water resources and provide an improved understanding of the natural and human factors that affect the quality of these resources. Program uses an integrated physical, chemical, and biological approach to assess water quality.** A. Retrospective Analysis. Interpret existing water-quality data to develop a conceptual model of factors affecting spatial and temporal patterns of water quality within the study unit and guide the design of NAWQA data-collection activities. B. Occurrence and Distribution Assessment. Characterize geographic and seasonal distributions of water-quality conditions in relation to major contaminant sources and background conditions. C. Trend and Change Assessment. Assess long-term (decadal) trends and changes in physical, chemical, and biological water-quality conditions in relation to changes in land use, point sources, and other factors related to changes in contaminant sources. D. Case Studies of Sources, Transport, Fate, and Effects. Investigate causes and governing processes of water-quality conditions and trends directed at the highest priority water-quality issues for individual study units and the Nation.	**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. This will be achieved via four specific objectives:** A. Estimate the current status, trends, and changes in selected indicators of the condition of the Nation's ecological resources on a regional basis with known confidence. B. Estimate the geographic coverage and extent of the Nation's ecological resources with known confidence. C. Seek associations between selected indicators of natural and anthropogenic stresses and indicators of the condition of ecological resources. D. Provide annual statistical summaries and periodic assessment of the Nation's ecological resources. The Nation's ecological resources are divided into 7 resource categories: agroecosystems, arid lands, estuaries, forests, Great Lakes, surface waters (lakes and streams), and wetlands. There are additional landscape ecology and landscape characterization components to provide the spatial context for the resource group information.	**Identify and document water-resource quality and provide an understanding of effects from human activities. Programs use an integrated physical, chemical, and biological approach to assess the quality of the water resource.** A. Aquatic Life Use Designation. Assess the aquatic life use attainment for the State's streams and rivers. The incorporation of bioassessment into this process is the basis for biological criteria. B. Sensitive Waters Identification. Characterize high quality waters for protection, which become part of the reference database. Assess in relation to spatial and temporal changes. C. Diagnostics. Determine sources of impairment and potential stressors. Use biological response signatures in conjunction with chemical, toxicological, and physical data to identify cause of impairment. D. Program Evaluation. Monitor effectiveness of pollution abatement programs, including wastewater treatment, watershed restoration, and other water-resource quality improvement programs. Use biosurveys to assess recovery of community structure and function.	**Determine the status and trends of contaminants and their effects on Trust resources. These resources include Service Lands, migratory birds, endangered species, anadromous and interjurisdictional fishes, and certain marine mammals. Four sampling networks will be used:** A. Problem Identification -- Service Lands. Identify existing or potential contaminant-related problems on Service Lands. B. Status and Trends of Service Lands. Provide statistically sound assessments of contaminant condition and changes in this condition over time. C. Exposure and Response of Trust Species. Provide measures of contaminant exposure and effect on populations. D. Status and Trends -- Trust Species Habitat. Provide statistically sound assessments of contaminant condition and changes in this condition of important habitats of Trust species.	**Support all agency surface-water programs including:** A. Water-Quality Standards. Use classifications to determine which water-quality and biological criteria apply to specific water bodies. Biocriteria and habitat assessment provide the basis for assigning use designations and for ground-truthing chemical and toxicological criteria. B. NPDES Permits. In addition to above ambient chemical and biological information, provide a characterization of the effect of discharges. Response signatures assist in distinguishing impacts in complex areas. C. Status and Trends (both site-specific and Statewide). Biocriteria provide the basis for determining extent and severity of use impairments reported via specific basin and sub-basin reports and the biennial water-resource inventory (305b report). Same information provides basis for Nonpoint Source Management. D. 404/401 Dredge and Fill. Biocriteria and habitat assessment provide a legal means to regulate habitat-impacting activities. Other programs supported include: TMDL/WLA, Clean Lakes, Great Lakes Initiative, CERCLA Site Characterization, WWTP Construction Issues.

Figure 1.--Comparison chart of program objectives and design features. (page 1 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
PROGRAM DESIGN FEATURES	**A.** Study-unit investigations in 60 major hydrologic basins (study units) that cover a large part of the United States, encompass the majority of national water use, and include diverse hydrologic systems that differ widely in the natural and human factors that affect water quality, thus ensuring that the most important national water-quality issues can be addressed by comparative studies. **B.** Intensive assessment activities for 4-5 years in each study unit (including a 3-year intensive data-collection period), followed by 5 years of low-level assessment activities, then repeating the cycle. **C.** Study-unit intensive assessment activities are conducted on a rotational basis, with approximately one-third studied intensively at a given time. **D.** First set of 20 study units began in FY 91; intensive data-collection began in FY 93. **E.** Liaison committees are established for each study unit to provide additional technical expertise and management perspectives in the design and interpretation of study-unit investigations. -- continued	**A.** EMAP-Surface Waters will address both lakes (and reservoirs) and streams. Several features are key to the approach: • Biological Indicators of Condition -- primarily used to describe status and trends. Will focus on a variety of biological assemblages in both lakes and streams (fish, invertebrates, algae, tissue residue). Chemical and physical habitat measures will be made simultaneously to evaluate expected biological conditions and also to draw associations to anthropogenic stresses. • Indexing -- Because all systems cannot be measured at all times, systems will be sampled during a 2-month window and conditions during that period tracked over years. • Sample Survey -- A survey approach will be used, i.e., study many different systems rather than a few very intensively. • Probability Sample Selection -- Because findings are to be extrapolated to all lakes and streams with confidence, study sites are selected using a probability design so the conditions of all systems can be inferred from the sample with rigorous statistical procedures. -- continued	**A.** Study units are non-randomly selected stations throughout States on all targeted water bodies. For streams and rivers, biological stations are established as reference sites and as assessment sites selected in relation to a suspected stressor. Upstream (site-specific) control stations or paired watersheds are used by a few States for reference sites. **B.** Regionalization, usually as ecoregions or subregions, is used to classify sites and stream types to structure assessments and site comparisons. **C.** Representative aquatic assemblages are selected. The majority of States survey benthic macroinvertebrates in stream systems. Approximately 40 percent survey fish. A small number of States (perhaps 4-8) survey periphyton. **D.** An index period is established for most States. A few States conduct seasonal collections. Response to catastrophic events may fall outside index period, in which upstream-downstream or paired watershed approaches are employed. -- continued	**A.** Four categories of methods: • Analytical Chemistry • Bioassays/Toxicity Tests • Biomarkers • Indicators of Population and Community Health **B.** Methods are divided into two tiers: • Tier 1 -- screening • Tier 2 -- specific **C.** Will focus on contaminant presence, organism exposure, and effects at various ecosystem levels. **D.** Site selection and methods depend on sampling network: • Problem Identification -- Service Lands: Site selection based on known sources, pathways, and endpoints. Methods selection driven by contaminant. • Status and Trends of Service Lands: Service lands are categorized into eco-geographical groups. Habitats within each group are selected for monitoring and appropriate "Tier 1" methods are universally applied. Results from Tier 1 drive selection of more specific Tier 2 methods. • Exposure and Response of Trust Species: Sampling plans are designed for each species of interest. • Status and Trends -- Trust Species Habitat: Methods and site selection are determined by the habitat.	**A.** A Five-Year Basin Approach to NPDES Permitting and Monitoring determines where ambient monitoring will be conducted. The State is divided into 25 sets of basin/sub-basin aggregations; survey areas are selected within 5 of these during each year. Priority issues to be addressed are based on input from all surface-water interests. With existing resources the highest priority areas are surveyed every 5-10 years. This process was formally initiated in 1990, but survey work has been ongoing since 1980. **B.** NAWQMN Network -- a network of fixed stations that have been monitored since 1974. Approximately 60 macroinvertebrate sites are sampled every 3 years. **C.** Special Studies -- numerous special studies have been accomplished, ranging from 1- to 2-day surveys to long-term surveys of special problem areas and larger water bodies. **D.** The basic design of the basin/sub-basin surveys of the Five-Year Basin Approach involves a synoptic arrangement of sampling sites in combination with the more traditional upstream/downstream approach. -- continued

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 2 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
PROGRAM DESIGN FEATURES (continued)	F. National synthesis combines key findings of study-unit investigations with existing information to produce regional and national-scale assessments for priority water-quality issues. G. Surface-water quality assessments utilize integrated physical, chemical and biological approaches. All biological components (fish, invertebrates, algae, habitat, and tissues) for the Occurrence and Distribution Assessment are conducted at Basic Fixed Sites in each study unit (8-12 sites selected to represent important environmental settings), where physical and chemical characteristics are also assessed. All community-level assessments, and habitat characterizations, are based on a common sampling reach at each site; specimens for tissue analyses are also typically collected in the same sampling reach (but may extend beyond reach boundaries in some cases). Multiple-reach and multiple-year sampling of biological communities are conducted at a subset of the Basic Fixed Sites (Intensive Ecological Assessment sites). Synoptic studies to address specific study-unit objectives may include any or all of the biological components sampled at the Basic Fixed Sites.	• Interagency Planning and Management -- The program covers many resource types and issues of interest and a mandate to other agencies besides USEPA. Thus program design, implementation, analysis, and management are best accomplished as an interagency effort. B. Approximately 3,600 lakes and 3,600 stream sites will be sampled over a 4-year period (approximately 800 per year) across the United States. Regional and national estimates will be available on an annual basis. The density of samples regionally will reflect the density of lakes or streams within the region. Sample size will be driven by inherent variability of measures and desire to detect trends of specified magnitude. C. Regional and national assessments will be conducted. Because of the probability nature of the design, results can be aggregated by ecoregions, hydrologic units, or political units for regional and national assessments.	E. Cost effective procedures are employed by the majority of States to (1) maximize limited resources, (2) maximize the number of sites assessed in a field season, and (3) minimize "turn-around" time of data analysis and judgment of condition. F. A multimetric approach is the primary design feature of most States, whereby measures of community elements and processes are integrated to assess the status of biological condition.		Sampling site density can range from 6-12 sites in relatively simple areas to more than 80-100 sites in major basins. An integrated assessment includes chemical measures (water column, effluent, sediments, tissues), physical measures (hydrological/morphological, habitat), and biological measures (fish and invertebrates). Standardized protocols for site selection and location, sampling methods, laboratory analysis, and data processing are followed.

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 3 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
SITE SELECTION STRATEGY	In each study unit, biological-community sampling and habitat evaluations are conducted at: **A.** Basic Fixed Sites -- (approximately 8-12) selected to represent important environmental settings (e.g., relatively homogeneous land use, physiography, and reference conditions) in each study unit, or that integrate water-quality conditions at key locations in the drainage basin. ***All biological components (fish, invertebrates, algae, habitat, and tissues) for the Occurrence and Distribution Assessment are conducted at all (or most) Basic Fixed Sites (minimum: one reach, one year--typically during the first year of the 3-year intensive data-collection period).*** **B.** Intensive Ecological Assessment Sites -- a subset of Basic Fixed Sites, selected to represent the range of conditions affecting variability within the study unit (typically including the Intensive Fixed Sites where frequent pesticide sampling is conducted). ***Multiple-reach and multiple-year sampling (fish, invertebrate, and algal communities, and selected habitat parameters) are conducted at these sites.*** -- continued	Physical habitat and biological measurements and observations will be made at all EMAP sample reaches; design targets specify approximately 3,200 sample reaches over the U.S., one-fourth of which would be sampled each year: **A.** EMAP Probability Sample Sites: chosen as a randomized, spatially-balanced systematic sample from several size strata of streams, to ensure that sampling effort is spread somewhat evenly across stream sizes, rather than being concentrated on small streams. One-fourth of the sample sites in each region will be sampled each year, so that all sites in the sample are visited at least once over a period of 4 years. A subset of about 10 sites in each region will be resampled each year to contribute to an assessment of interannual variability and improve initial trend detection capability. At least for the first sampling cycle, approximately 20 sites in each region will be sampled twice within the sampling season to measure index period variation. -- continued	Biological assessments are done at non-randomly selected fixed stations in State Programs. Sites are selected for four basic purposes: **A.** Fixed Sites. Fixed sites are selected to (1) represent natural environmental conditions (e.g., relatively homogeneous land use and physiographic conditions) that will become the reference database; (2) establish baseline, whereby monitoring of program effectiveness can be conducted on a periodic basis. **B.** Intensive Bioassessment. Intensive assessments are done for a specific purpose and generally incorporate more stations and parameters in the ecological assessment. States generally use intensive bioassessments to conduct a basin-level survey and may incorporate chemical, toxicological, and physical measurements. **C.** Site-Specific Surveys. These synoptic surveys are geared to a specific pollution source, and may be designed as an upstream/downstream or paired watershed survey. Site-specific studies are done by States that do not have an established reference database. -- continued	*No information provided*	Macroinvertebrates and fish are collected, and habitat assessments conducted, using standardized protocols at each sampling site in the following: **A.** Five-Year Basin Approach: Sampling site density ranges from 6-8 to 100 sites depending on study area extent and complexity. Sites are selected relative to both site-specific and regional issues. **B.** Regional Reference Sites: More than 360 reference sites were used to establish ecoregional biocriteria. In 1990 an effort to resample these sites at a rate of 10 percent per year was initiated. This effort is approximately 40 percent complete as of 1994. **C.** Special Studies: Level of intensity varies with complexity of the study (e.g., may collect only qualitative invertebrate samples in lieu of artificial substrates in simple, small stream situations. Special studies also include exploratory research on the Ohio River and Lake Erie estuaries. In addition, macroinvertebrates are collected at the following: **D.** NAWQMN Fixed Stations: Approximately 60 sites are sampled on a rotating basis (once every 3 years).

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 4 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
SITE SELECTION (continued)	**C.** Ecological Synoptic Studies are designed to meet specific study-unit objectives within national priorities, for example, to evaluate the spatial distribution of selected ecological characteristics in relation to causative factors, such as land uses or contaminant sources and instream habitat conditions. ***These studies may include any or all of the biological components sampled at the Basic Fixed Sites.*** (*Also see* fig. 7.)	**B.** EMAP Reference Sites: A sufficient number of hand-picked reference sites will be chosen in each region to describe the biological, chemical, and physical characteristics of relatively undisturbed streams and also those subject to gradients of important anthropogenic stressors. Reference sites may also be located in reaches where other, more intensive data monitoring or other related research is taking place.	**D.** Emergency Response. Following a catastrophic event (e.g., chemical spill, fish kill, etc.), selected stations within a watershed would be surveyed to evaluate magnitude of impact. Emergency response sampling is sometimes conducted under a different program or agency.		
UNIT OF SAMPLING	Sampling Reach. Length is determined by a repetitive geomorphic sequence (e.g., 2 riffles and 2 pools), or 20 channel widths if repetitive geomorphic channel units are not present. Acceptable range for wadeable streams is 150 m to 500 m. Range for nonwadeable streams is 500 m to 1,000 m (longer reach may be necessary in some cases for habitat assessment). If multiple reaches are sampled, they are located so as to ensure minimal fish movement between reaches (e.g., separated by natural or man-made barriers or a minimum distance of 150 m). Tissues may be collected from areas upstream or downstream from the reach if needed to obtain an adequate sample, as long as there are no intervening contaminant sources.	Sampling Reach. For wadeable streams, reach length is determined as 40 times the wetted width or 150 m, whichever is greater. To determine the width that defines the reach, several representative measurements are taken within a distance of about 10 channel widths from the mapped point that defines the midpoint of the reach (see below). The reach is then "laid out" 20 channel-widths' distance upstream and 20 channel-widths' distance downstream. The 2 reach ends may be adjusted plus or minus 4 channel-widths' distance so that they coincide with breaks in channel habitat.	Sampling unit is determined by individual State programs and is dependent on parameters. For example, sampling for fish is usually conducted within a 100- to 500-m reach. Sampling for macroinvertebrates is from specified habitat types within a specific location or reach. Instream habitat parameters focus on sampling reach; channel morphological features and watershed parameters require a larger sampling unit.	*No information provided*	Specific sampling reach (zone) distances for sampling the fish community (and evaluating habitat) were established via special studies. For wading methods, each sampling reach is 150-200 m. For boat methods, sampling reach length is 500 m. Qualitative Habitat Evaluation Index (QHEI) sampling reaches correspond to fish sites. For invertebrates, artificial substrates are positioned in a run habitat where flow will be continuous over the 6-week colonization period. For natural substrates, sample sites consist of a riffle, run, margin, and pool sampling for a minimum of 30 minutes and thereafter until no new taxa are observed based on gross examination.

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 5 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
LOCATION OF SAMPLING UNIT AT SITE	Sampling reach is located such that instream and riparian habitat conditions are representative of the local area and in close proximity to the location (Basic Fixed Site) where hydrologic and chemistry (water, sediment, tissues) data are collected. There should be no major discontinuities -- relative to geomorphology or other major natural or human influences likely to affect water quality -- between the sampling reach and the Basic Fixed Site.	Sampling reach midpoint latitude/longitude coordinates are predetermined during the site selection procedure. Crews enter the field with an "X" drawn on a 1:24,000 map showing the reach midpoint. They locate that spot in the field by orienteering, locating landmarks, and using hand-held GPS. Sites are relocated in the same way upon subsequent visits (after 1 to 4 years), with the aid of semipermanent site markings, photos, and descriptions.	Sampling reach is located so that instream and riparian habitat conditions are representative of the local area and in close proximity to the location (fixed site) where hydrologic and chemistry data are collected (water, sediment, tissue). There should be no major discontinuities -- relative to geomorphology or other major natural or human influences on water quality -- between the sampling reach and the fixed site.	*No information provided*	Sampling sites (zones) are selected to be in proximity to the source(s) being evaluated and with respect to site comparability. A synoptic watershed design is used in combination with the upstream/downstream and longitudinal design. Sites for fish, invertebrates, chemistry, and physical sampling are co-located or immediately adjacent to each other.
REPLICATION	Multiple reaches (at least 3) and multiple years (at least 3) are sampled at Intensive Ecological Assessment Sites (subset of Basic Fixed Sites) for fish, invertebrate, and algal community sampling, and selected habitat parameters. (*See* fig. 7 for replication recommended for tissue samples.)	Approximately 20 reaches per region are resampled within the same sampling season to estimate protocol, crew, and within-season temporal variance. Approximately 10 reaches per region are sampled on an annual basis; the remainder are resampled every 4 years.	Replication is generally not done except as temporal replication (i.e., 2 or 3 passes in a sampling season for fish-community collections). Some States take individual samples, particularly with artificial substrates; however, samples are usually composited and not replicated.	*No information provided*	Fish-community samples are collected at each site 1 to 3 times within an index period, depending on the complexity of the study area. Ten percent of all macroinvertebrate artificial substrate samples are replicated.
SAMPLING FREQUENCY	A. Basic Fixed Sites: Once per 3-year intensive period (cycle repeated every 9 years). B. Intensive Ecological Assessment Sites: Once per year for 3-year intensive period. C. Ecological Synoptic Survey Sites: At least once per 3-year intensive period [study-unit option to do more than once; may include seasonal sampling]. (*See* fig. 7 for frequency recommended for tissue samples.)	One-fourth of the total number of reaches in each region are sampled on a rotating basis once every 4 years, so that over a 4-year period, all sites are visited.	Most programs base sampling on index period. Some States sample 2 or more seasons per year.	*No information provided*	Samples are usually collected once during each survey year (6-week duration for macroinvertebrate artificial substrate samples, with qualitative sample collected at the time of retrieval). For the Five-Year Basin Approach, sites are resampled on a 5- to 10-year return basis. Regional Reference Sites are revisited once every 10 years.

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 6 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
SAMPLING SEASON (and other temporal considerations)	Factors to consider (in order of priority): **1.** Sample invertebrates at time of year when the majority of insect species are at or near maturity and few species are in early instars or resting stages (late fall to early spring in most temperate streams). Sample algae concurrently with collections of invertebrates unless local (study-unit) conditions or objectives require separate collection activities. **2.** Flow regime: avoid high-flow season, when access to stream may be restricted and invertebrate communities may be recovering from flood disturbance. Sample during season of minimum flow variability, if possible. Adjust field schedules to minimize influence of antecedent high flows, if possible. Avoid abnormally low-flow periods. **3.** Avoid spawning and migration periods of fish. **4.** Consider seasonal land-use activities (e.g., agriculture) and other human or natural factors that can influence water quality. **5.** Consider stream access problems due to seasonally adverse road conditions (mud, snow). -- continued	To keep costs down, EMAP is attempting to make most or all routine monitoring measurements during a 1- to 2-day period at each site. Many of the sampling considerations hinge upon the optimal or desirable times for biological sampling. Preferred sampling season is when robust measurements can be made in a practical manner, and when such measurements yield useful information concerning ecological responses of the stream to important anthropogenic stresses. The exact sampling periods have not yet been fixed for EMAP in all parts of the U.S., but summer baseflow sampling is probably the best alternative for most regions. However, in arid and acid-stressed regions, EMAP is piloting the spring season.	Selection of index period is based on three factors -- (1) minimize between-year variability due to natural events, (2) maximize gear efficiency, and (3) maximize target assemblage accessibility. Minimal between-year variability is partially addressed by sampling within the same index period as the previous year's sampling. By applying this temporal consideration in sampling, correction for natural variability due to seasonal cycles is accomplished. Water quantity and quality and climatic conditions should be such that sampling gear is at its maximum efficiency. If certain flow conditions are necessary for gear performance, sampling should be targeted to coincide with those conditions. Finally, sampling should occur when there is maximum accessibility to the targeted assemblage. The index period varies with the geographical area, but is generally in mid to late summer, which is after the spring high flows and insect emergence and is before the drought conditions and autumn temperature declines.	*No information provided*	For fish sampling, the following considerations apply: **1.** Flow. Acceptable conditions are prescribed by standard protocols (Ohio EPA, 1989). High flows and abnormal turbidity are avoided. **2.** Index Period. All fish sampling is conducted during June 15 - October 15. This is a time of relative stability in terms of community dynamics, pollution stresses are high, and flow is low. **3.** Sites sampled 3 times are spaced at a minimum of 3 weeks. Sites sampled 2 times are spaced 6 weeks apart. One-time samples are delayed until late August, or September if possible. For invertebrates: **1.** Sampling is conducted during the summer months; the index period is June 15-September 30. This is a time of relative stability in terms of community dynamics, pollution stresses are high, and flow is low. **2.** High-flow periods are avoided due to sampler setting/retrieval impracticability and qualitative sampling considerations.

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 7 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
SAMPLING SEASON (and other temporal considerations) (continued)	All sites sampled for a similar objective (e.g., fixed sites, synoptics) should be sampled within a 3- to 4-week period. Sites for multiple-year comparisons should be sampled during similar conditions of season and flow to the extent possible. Field habitat (reach-level) assessments are typically conducted as close to base-flow conditions as possible. Some elements may be done whenever it is convenient (e.g., surveys of monumented cross sections may be easiest after leaf-fall). Tissue sampling is typically conducted in late summer or early fall (base-flow conditions preferred; avoid spawning periods of target taxa).				

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 8 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
PROGRAM DESIGN DOCUMENTS	1. **Gurtz, M.E.**, 1994, Design of biological components of the National Water-Quality Assessment Program, Chapter 15, *in* Loeb, S.L., and Spacie, A., eds., Biological monitoring of aquatic systems: Boca Raton, Fla., Lewis Publishers, p. 323-354. 2. **Leahy, P.P.**, Rosenshein, J.S., and Knopman, D.S., 1990, Implementation plan for the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 90-174, 10 p.	1. **Klemm, D.J.**, and Lazorchak, J.M., eds., 1993, Environmental Monitoring and Assessment Program-Surface Waters and Region 3 Regional-EMAP 1993 Pilot Field Operations and Methods Manual--Streams: Cincinnati, Ohio, U.S. Environmental Protection Agency. 2. **Larsen, D.P.**, and Christie, S.J., eds., 1993, Surface Waters 1991 Pilot Report: EPA/620/R-93/003. 3. **Larsen, D.P.**, Stevens, D.L., Selle, A.R., and Paulsen, S.G., 1991, Environmental Monitoring and Assessment Program, EMAP-Surface Waters, A Northeast Lakes Pilot: Lake and Reservoir Management, v. 7, p. 1-11. 4. **Merritt, G.D.**, and others, 1992, Environmental Monitoring and Assessment Program: Surface Waters 1992 Northeast Lakes Pilot Survey--Field Operations and Training Manual Vol. I and II.	1. **Stribling, J.B.**, Gerritsen, J., Barbour, M.T., and Karr, J.R. (In review) Biological Criteria: Technical Guidance for Streams and Small Rivers, EPA/822-B-94-001: Washington, D.C., USEPA, Office of Science and Technology. 2. **Karr, J.R.**, Fausch, K.D., Angermeier, P.L., Yant, P.R., and Schlosser, I.J., 1986, Assessing biological integrity in running waters: A method and its rationale, Special Publication 5: Urbana, Ill., Illinois Natural History Survey, 28 p. 3. **Klemm, D.J.**, Lewis, P.A., Fulk, F., and Lazorchak, J.M., 1990, Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters, EPA/600/4-90-030: Washington, D.C., USEPA, Office of Research and Development.	*No information provided*	1. **Ohio Environmental Protection Agency**, 1987-1989, Biological Criteria for the Protection of Aquatic Life, Volumes I-III: Division of Water Quality Planning and Assessment. 2. **Ohio Environmental Protection Agency**,1990, The use of biocriteria in the Ohio EPA Surface Water Monitoring and Assessment Program: Division of Water Quality Planning and Assessment. 3. **Rankin, E.T.**,1989, The Qualitative Habitat Evaluation Index: Rationale, Methods, and Application: Division of Water Quality Planning and Assessment.

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 9 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	BEST (NBS)	Ohio EPA
PROGRAM DESIGN DOCUMENTS (continued)		**5. Paulsen, S.G.**, and Linthurst, R.A., 1994, Biological monitoring in the Environmental Monitoring and Assessment Program, Chapter 14, *in* Loeb, S.L., and Spacie, A., eds., Biological monitoring of aquatic systems: Boca Raton, Fla., Lewis Publishers, p. 297-322. **6. Paulsen, S.G.**, Larsen, D.P., Kaufman, P.R., Whittier, T.R., and others, 1991, EMAP-Surface Waters Monitoring and Research Strategy--Fiscal Year 1991, EPA/600/3-91/022: Corvallis, Oreg., U.S. Environmental Protection Agency, Office of Research and Development, 183 p. **7. Whittier, T.R.**, and Paulsen, S.G., 1992, The surface water component of the Environmental Monitoring and Assessment Program (EMAP): An overview: Journal of Aquatic Ecosystem Health, v. 1, p. 13-20.	**4. Plafkin, J.L.**, Barbour, M.T., Porter, K.D., Gross, S.K., and Hughes, R.M., 1989, Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish, EPA/440/4-89-001: Washington, D.C., USEPA, Office of Water. **5. U.S. Environmental Protection Agency**, 1990, Biological criteria: National program guidance for surface waters, EPA-440/5-90-004: Washington, D.C., Office of Water.		

Figure 1.--Comparison chart of program objectives and design features.--Continued (page 10 of 10)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
COMPONENT OBJECTIVE (Fish) *[Note: Also see fig. 1 for Program Objectives and Design Features.]*	(Occurrence and Distribution Assessment): Evaluate the structure (species composition and relative abundance) of fish communities within selected sampling reaches. *[Note: This chart is most appropriate for the Occurrence and Distribution Assessment; modifications may be made to meet other Program objectives (e.g., trends and cause/effect case studies).]*	Evaluate fish community from representative samples.	Evaluate the structure of fish assemblages (communities) within targeted habitats, develop taxonomic lists, and assess the functioning of the assemblage based on knowledge of ecological processes.	Distribution and relative abundance of fishes within selected sampling reaches. Evaluate structural and functional condition.
SAMPLE TYPES	Representative sample of fish from the sampling reach based on 2 sampling methods. *[Relative Abundance Information]*	Representative sample of fish from the sampling reach.	A multiple-habitat sampling is emphasized for fish and incorporates all habitats within a sampling reach.	Representative sample of the fish community using generator-powered pulsed-DC electrofishing as a single method.
SAMPLING STRATEGY (General approach, including compositing)	The most common sample consists of 2 passes of an electrofishing unit (backpack, barge, or boat). This is followed by seine collections that are most appropriate for collecting fish in that reach (three 50-m seine hauls and (or) or kick seine sampling in riffles at wadeable sites; 3 shoreline seine hauls in 3 representative sections of the reach at nonwadeable sites). If electrofishing and (or) seining cannot be conducted because of reach conditions (for example, extreme conductivity and (or) a debris load that prohibits seining), then other methods (gill netting, hoop netting, snorkeling, etc.) are conducted in consultation with local fish ecologists.	Single pass with backpack electrofishing unit, supplemented with seining for selected pool habitats and riffles.	Usually a single pass constitutes the sample. Some states conduct several passes of a given reach. Block nets are sometimes used, and depletion population estimates conducted.	**A.** Electrofishing samples are collected at each site 1 to 3 times within the index sampling period (June 15 - October 15). **B.** All sampling is conducted during daylight hours, except in the Ohio River where night sampling was determined to be more effective.

Figure 2.--Comparison chart of fish protocols. (page 1 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
SAMPLE GEAR	**A.** Battery- (24-volt deep cycle) or generator-powered (115-volt gasoline) backpack electrofishing unit **B.** Barge-operated electrofishing unit **C.** Boat-operated electrofishing unit *[Note: All electrofishing units should be pulsed-DC.]* **D.** Bag seine*: 7.6 - or 9.1-m by 1.2-m seine attached to 2 wooden poles 31.8 mm in diameter; usually 6.4-mm mesh size **E.** Kick seine*: "common sense" seine, 3-m by 1.2-m seine with wooden poles 31.8 mm in diameter; 6.4-mm mesh size **F.** Beach seine*: 30.5- to 61-m by 2.8-m seine with wooden poles (usually 51 mm by 51 mm); 9.5-mm mesh size **G.** Experimental gill net*: 1.8 m deep with 6 sections of monofilament mesh, 7.6 m long each, using 13-, 25-, 38-, 51-, 76-, and 102-mm mesh size (bar measure) **H.** Hoop net*: fiberglass hoops 0.6 m in diameter, 3.7 m long; untreated 38-mm mesh (bar measure); throats on 2nd and 4th of the 7 hoops **Note: Net sizes may be tailored to local conditions; mesh sizes are the maximum acceptable.*	**A.** Electrofishing: 360-watt pulsed-DC electrofishing unit; dip nets with 7-mm mesh **B.** Seining. Kick/beach seines	**A.** Electrofishing. Pram-operated units or shore-based units are the dominant form of gear. Backpack electrofishing units are used in small streams, particularly headwaters. **B.** Seining. Kick seines are sometimes used in riffles, and bag seines are used in pools. Usually this gear is employed only when electrofishing is not possible.	All methods employ pulsed-DC electrofishing using gasoline generators. Minimum wattages are specified below. **A.** Wading Methods: A T&J 1736 VDC electrofisher/ generator combination is used (minimum 1,750 watts output). This is either towed in a small, plastic towboat (Sport Yak) or a 100-m heavy-duty extension line is used as a bank-set. This is used in all wadeable situations. **B.** Battery Backpack Units: These are used under very restrictive guidelines and only in the smallest headwater streams. **C.** Boat-Mounted Units: These employ 3,500- to 5,000-watt generators with pulsators that produce 500-1,000 VDC at 10-20 amperes.

Figure 2.--Comparison chart of fish protocols.--Continued (page 2 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
SAMPLE AREA (for principal sample gear types)	Area sampled is the entire sampling reach.	Area sampled is the entire sampling reach.	Area sampled is the entire sampling reach.	Wading Methods: The best available habitat along one side of the reach is sampled. All microhabitats are included. Boat Methods: The best available shoreline and run habitat is sampled.
FIELD COLLECTION STEPS	**1.** Choose appropriate sample gear. *[Note: typically accomplished in a reconnaissance prior to sampling]* **2.** If electrofishing can be conducted, make 2 passes, proceeding from downstream to upstream at wadeable sites (upstream to downstream at nonwadeable sites). **3.** Collect seine samples as appropriate.	*No information provided*	Follow Standard Operating Procedures established by State Water Resources Agency. Trained individuals used to operate gear.	**1.** Select general site location during pre-survey planning. **2.** Electrofishing reach is established and marked for future passes. Wading sites are sampled in an upstream direction; boat sites are sampled downstream.

Figure 2.--Comparison chart of fish protocols.--Continued (page 3 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
FIELD PROCESSING STEPS	**1.** All fish that can be identified to species in the field by an ichthyologist are anesthetized (carbon dioxide), measured (total and standard length) and weighed (if more than 30 individuals of a species are present, then 30 are selected randomly for measurement and weight). **2.** All fish are checked for the presence of external anomalies (deformities, eroded fins, lesions, tumors, diseases, and parasites). **3.** Specimens that cannot be identified to species in the field are fixed in 10-percent buffered formalin for later identification in the laboratory. After 2 days to 1 week in formalin (to fix tissues), specimens are transferred (with an intermediate tap-water step) to 40-percent isopropyl alcohol or 70-percent ethanol for permanent preservation.	**1.** Identify all fish to species. **2.** Largest and smallest individuals are measured. **3.** Specimens are checked for anomalies. **4.** Voucher subsample of up to 25 specimens in 10-percent formalin. **5.** Preserve specimens that cannot be identified.	Follow Standard Operating Procedures. Fish are sorted, identified, and enumerated in the field. Voucher specimens or those difficult to identify are taken back to the laboratory for processing. Length/weight measurements and batch biomass are sometimes done. Visual inspections for disease and anomalies are done.	**1.** All fish are field identified to every practicable extent. Fish are identified, counted, weighed, and examined for external anomalies. An electric pump is used to recirculate and replenish live-well water. **2.** Specimens are retained for laboratory identification and (or) vouchers as necessary. All fish are fixed in 10-percent formalin and later washed in water and transferred to increasing concentrations of ethanol (in the laboratory, specimens are washed and transferred through a series of 35-, 50-, and 70-percent ethanol).

Figure 2.--Comparison chart of fish protocols.--Continued (page 4 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
ANCILLARY DATA AND COORDINATED SAMPLING ACTIVITIES	**A.** Ambient conductivity, water temperature, and dissolved-oxygen measurements made at the time of fish-community sampling **B.** Habitat data (reach, segment, basin) obtained for each Basic Fixed Site **C.** Invertebrate and algal community samples collected at each Basic Fixed Site **D.** Additional water, sediment, and tissue chemistry collected at each Basic Fixed Site **E.** Continuous stream discharge and field water-quality parameters (e.g., pH, temperature) collected at each Basic Fixed Site	**A.** Chemistry (qualitative streamside and quantitative laboratory), temperature, dissolved oxygen **B.** Physical habitat **C.** Invertebrates and algae **D.** Sediment metabolism **E.** Discharge	**A.** *In situ* measurements of temperature, pH, dissolved oxygen, and conductivity **B.** Physical measurements of velocity and (or) flow, sometimes particle size and channel morphological detail **C.** Habitat structure parameters either measured or estimated with visual-based technique **D.** Other assemblage collections as determined by Agency directive **E.** Water, sediment, and tissue chemistry collected as required for analytical and toxicological testing	**A.** Chemical analyses of water column, effluents, sediments **B.** Habitat evaluation conducted using the Qualitative Habitat Evaluation Index (QHEI) **C.** Invertebrate samples (*See* section on Invertebrates.) **D.** Other data may include: toxicity data from effluent or sediment testing, biomarkers, or fish contaminants.
LEVEL OF TAXONOMY	Species	Species	Lowest practicable taxon (usually species or genus)	Species and, if necessary, subspecies; all hybrids are identified.

Figure 2.--Comparison chart of fish protocols.--Continued (page 5 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
DATA ANALYSIS	**A.** Descriptive: species lists, richness, diversity, similarity **B.** Multivariate: differentiation of sites based on communities, gradient analysis -- evaluate changes in chemical (biological) characteristics associated with changes in biological (chemical) characteristics using, for example, two-way indicator species analysis (TWINSPAN), detrended correspondence analysis (DCA), and canonical correspondence analysis (CANOCO). **C.** Biological Indices: Data for calculating indices such as the Index of Biotic Integrity (IBI) will be available from NAWQA fish sampling. IBI's may be generated in study units where appropriate and with cooperator support.	Same as NAWQA.	Multimetric approach, where metrics and indices are calibrated by stream class and regional expectations, and represent key measures of assemblage elements and processes. Calibration may be done by multivariate techniques. In assessment, metrics are calculated, values converted to scores, and aggregated to provide an integrated assessment.	**A.** Descriptive: species, relative abundance (numbers, weights), and external anomalies, diseases, and parasites **B.** Multimetric: Index of Biotic Integrity (IBI) [3 versions modified for application in Ohio]; Modified Index of Well-being (MIWb) **C.** Multivariate analyses as needed

Figure 2.--Comparison chart of fish protocols.--Continued (page 6 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)	**A.** Standardized protocols and training of field personnel: Regional biologists provide coordination and oversight of study-unit sampling activities. **B.** Centralized unit: Biological Quality Assurance Unit at the USGS/ National Water Quality Laboratory oversees contractors and quality assurance/quality control. **C.** Reference collections: In-house reference collections are maintained by the Biological Quality Assurance Unit. **D.** Voucher collections: Voucher collections are maintained in outside repositories (e.g., museums). **E.** Collaboration with outside taxonomists includes verification of identifications, updating of taxonomic lists, and identification and description of new species. **F.** Taxonomic database (interagency): National Oceanographic Data Center (NODC) code facilitates tracking of name changes over time and sharing of data among agencies. **G.** Integrated database: National Water Information System-II contains physical (including habitat), chemical, and biological data, with facilitated data entry, verification, and analysis.	Voucher specimens and "unknowns" will be archived by professional ichthyologists at the National Museum of Natural History or by consultation with specialists in various taxonomic groups.	**A.** Documented Standard Operating Procedures **B.** Trained staff and delineation of responsibility **C.** Maintenance of a voucher collection **D.** Computerized database and analytical programs with verified entries **E.** Consistent reporting format and metric calculation	**A.** QA/QC is centrally organized in the Ecological Assessment Section. District surface-water units perform limited assessments. **B.** Reference collection is maintained in-house. **C.** Vouchers are deposited in the Ohio State University Museum of Biodiversity. **D.** All data are entered into the FINS subroutine of Ohio ECOS, a State-wide database organized and maintained by Ohio EPA.

Figure 2.--Comparison chart of fish protocols.--Continued (page 7 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
PROTOCOL DOCUMENT	**1. Meador, M.R.**, Cuffney, T.F., and Gurtz, M.E., 1993, Methods for collecting samples of fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93-104, 40 p.	**1. Klemm, D.J., and Lazorchak, J.M.**, eds., 1993, Environmental Monitoring and Assessment Program-Surface Waters and Region 3 Regional-EMAP 1993 Pilot Field Operations and Methods Manual--Streams: Cincinnati, Ohio, U.S. Environmental Protection Agency.	[*See* fig. 1: Program Design Documents.]	**1. Ohio Environmental Protection Agency**, 1987, Biological Criteria for the Protection of Aquatic Life, Volume II, Users manual for biological field evaluation of Ohio rivers and streams: Division of Water Quality Planning and Assessment. **2. Ohio Environmental Protection Agency**, 1989, Biological Criteria for the Protection of Aquatic Life, Volume III, Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities: Division of Water Quality Planning and Assessment.

Figure 2.--Comparison chart of fish protocols.--Continued (page 8 of 8)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
COMPONENT OBJECTIVE (Invertebrates) ***[Note: For NAWQA, EMAP, RBP's, and Ohio EPA, also see fig. 1 for Program Objectives and Design Features.]***	(Occurrence and Distribution Assessment): Evaluate the structure of benthic invertebrate communities within targeted habitats, and develop a taxa list of invertebrates of all habitats present, in selected sampling reaches. *[Note: This chart is most appropriate for the Occurrence and Distribution Assessment; modifications may be made to meet other Program objectives (e.g., trends and cause/effect case studies).]*	Evaluate benthic invertebrate community structure in streams of the U.S. to determine community responses to known impacts and to estimate the proportion of U.S. streams that are impaired.	Evaluate the structure of benthic macroinvertebrate assemblages within targeted habitats, develop taxonomic lists, and assess the functioning of the assemblage based on the potential functioning for a site. [geared toward Clean Water Act impairment level determinations and evaluation of management effects] • Site selection: Sites are selected non-randomly to represent homogeneous land-use and physiographic conditions. • Unit of sampling: representative geomorphic sequence or 20 channel widths, ca. 150-500 m • Location of sampling unit at site: similar to NAWQA • Replication: Multiple reaches (prefer 3) and multiple years (prefer 3) are sampled as money and manpower allow. • Sampling frequency: once or twice per year • Sampling season: same considerations as NAWQA	Evaluate the structure of benthic macroinvertebrate assemblages within targeted habitats, develop taxonomic lists, and assess the functioning of the assemblage based on knowledge of ecological processes.	Distribution and abundance of the macroinvertebrate assemblages of lotic habitats: evaluate the structural integrity of resident communities using artificial substrates supplemented with qualitative samples of the natural substrates.

Figure 3.--Comparison chart of invertebrate protocols. (page 1 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
SAMPLE TYPES	**A.** Richest Targeted Habitat (RTH): Semi-quantitative sample from the habitat type that (in the absence of human influences) is expected to support the richest assemblage of invertebrates in the sampling reach (e.g., riffles, snags). *[Relative Abundance Information]* **B.** Depositional Targeted Habitat (DTH): Semi-quantitative sample from a fine-grained, organically rich depositional habitat, usually a pool. *[Relative Abundance Information]* **C.** Qualitative Multihabitat (QMH): Qualitative sample composited from all (or as many as possible) habitat types present in the sampling reach. *[Taxa Presence Information]*	**A.** Riffle/run habitats: Composite sample **B.** Pool/glide habitats (depositional): Composite sample **C.** Qualitative Multihabitat: Composite sample	**A.** Riffle habitat: Semi-quantitative samples are taken from riffles to be consistent with past sampling efforts. **B.** Multihabitat: Semi-quantitative samples are collected from all major habitat types, e.g., snags, margins, vegetation beds, leaf packs, and depositional areas.	Sample type is based on targeted habitats and may include epifaunal and infaunal macroinvertebrates. Two approaches are utilized by States: **A.** Single Habitat: The decision on the targeted habitat of choice is based on the most productive and prevalent habitat representative of the stream type being assessed. Usually an epifaunal substrate is emphasized, i.e., riffle/run areas in high gradient streams, and snags and vegetation mats in low gradient streams. **B.** Multihabitat: Specific habitats are identified and categorized for sampling in some States. Candidate habitats used by States include cobble, shorezone, snags, sand/silt/clay, vegetation beds, and leaf packs.	**A.** Artificial substrates / run habitat: Run habitat with a continuous current speed of more than 0.3 feet per second. **B.** Qualitative / Multihabitat: Qualitative sampling is conducted by dip-net/hand picking from all representative habitats in the immediate sampling reach.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 2 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
SAMPLING STRATEGY	**A.** RTH: Composite of 5 samples from the richest targeted habitat type, distributed over multiple examples of the targeted habitat type within the sampling reach. Extreme habitat conditions (fastest/slowest velocities; largest/smallest substrates; etc.) are avoided. **B.** DTH: Composite of (a minimum of) 5 samples from the depositional targeted habitat type, distributed over multiple examples of the targeted habitat type within the sampling reach. Extreme examples of habitat conditions are avoided. C. QMH: Composite from as many habitat types as possible within the sampling reach, using equal sampling effort in each habitat type; limited to total sampling time of about 1 hour for most streams.	Nine transects are equally spaced along the sampling reach. A sample is collected at each transect and composited with other samples from similar habitats. Thus, all riffle/run samples are composited, and all pool/glide samples are composited. A total of 9 samples are collected. These are all riffle, all pool, or a mixture of these habitats. The qualitative multihabitat sample is a composite of all samples collected by this method from a reach.	**A.** Riffle habitat: Composite of 5 Surber or Hess samples or 1-m^2 kick net samples from riffle type habitat, distributed over multiple examples of this habitat type within the sampling reach. Extreme, atypical habitat conditions are avoided. **B.** Multihabitat: Composite sample from as many habitat types as possible within the sampling reach, using equal sampling effort in each habitat type. Riffle and multihabitat samples are kept separate.	**A.** Single Habitat: Composite of 2 samples from different velocity/depth regimes is done. Goal is aggregate over a relatively large area to get around the normal patchiness in the environment. **B.** Multihabitat: Composite may be done if standardized level of effort is employed. Alternatively, habitat samples kept separate, particularly for infauna versus epifauna.	**A.** Artificial substrates: Modified Hester-Dendy design using 5 sets of plates, each 1 ft^2 in surface area. Each sample is a composite of the entire cluster of 5 sets of plates. **B.** Qualitative / Multihabitat: Qualitative dip-net/hand-picked samples from all available habitats. Predominant taxa from each different habitat are noted on field sheets.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 3 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
SAMPLE GEAR	**RTH or DTH [425-µm mesh]:** **A.** Wadeable coarse-grained substrates: Slack sampler ("Surber-on-a-stick") **B.** Nonwadeable coarse-grained substrates: Dome sampler; artificial substrates; stovepipe samplers **C.**Wadeable fine-grained substrates: Grab samplers (Macan, Ekman, Ponar, Van Veen, or Petersen) **D.** Nonwadeable fine-grained substrates: Grab samplers (Ponar, Petersen, Van Veen, or Shipek) **E.** Woody snags and macrophytes: D-frame dip net; Slack sampler; snag sampler (Thorp and others) **QMH [210-µm mesh]:** D-frame kick net, supplemented by other techniques as appropriate for different habitat types. Also collect by visual inspection and hand-picking.	Transect samples: 595-µm-mesh Slack sampler -- standard "Surber" methods for fast waters; "sweep-kick" method for pools. Qualitative Multihabitat: same as for NAWQA	**A.** Riffle habitat: modified Surber or Hess samplers with 280-µm mesh, or kick nets with 280- to 425-µm mesh. 280-µm mesh is recommended to be consistent with past sampling efforts. **B.** Multihabitat: **1.** Depositional areas: Grab samplers (Ponar, Petersen) **2.** Woody snags and macrophyte beds: modified Surber or Hess samplers with 280-µm mesh, or kick nets with 280- to 425-µm mesh **3.** Stream margins: modified Surber, Hess, or kick net **4.** Leaf packs: grab samples, ca. 1 L	Sampling gear is habitat dependent, but consists of two basic categories: **A.** Kick nets: Kick nets (1 m^2) or D-frame dip nets (0.09 m^2) are used in streams where velocity will assist in the capture of organisms. A large composited sample is obtained with this gear. A standardized level of effort is employed to quantify the sample. **B.** D-frame dip nets: Sampling in various habitat types, such as shore-zone, depositional areas, vegetation snags, as well as riffles is done with a D-frame dip net. This gear is chosen for sampling low-gradient streams where low velocity requires a more active gear.	**A.** Artificial substrates: Modified Hester-Dendy consists of a cluster of 5 sets of masonite plates, each with 8 plates separated by 12 spacers and connected with an eyebolt. Each of the 5 plate-sets measures 1 ft^2 in surface area. Each sample is a composite of the entire cluster of 5 sets of plates. **B.** Qualitative / Multihabitat: Triangular-frame dip nets with No. 30 mesh. Also collect by visual inspection and hand-picking.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 4 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
SAMPLE AREA (for principal sample gear types)	Slack sampler: 0.5 m by 0.5 m (total area sampled is 5 times 0.25 m^2 = 1.25 m^2) Total area sampled for RTH or DTH samples using other gear is 5 times the area of an individual sample. QMH samples: not applicable	Same as for NAWQA; ca. 0.25 m^2 per transect. Total area sampled per site = 2.25 m^2	**A.** Modified Surber samplers: 0.093 m^2 **B.** Hess samplers: typically 0.086 m^2 **C.** Kick nets: 0.25 m^2 (0.5 m by 0.5 m) recommended Total area sampled for all composite samples is: (number of samples) times (sampling area)	Most States are focusing on a composited sample totaling approximately 2 m^2 of sampled area. When kick nets and dip nets are used, this is the relative sample size. Other gear typically samples a smaller area.	**A.** Artificial substrates: Total area of each composite sample (5 sets of plates) measures 1 ft^2. **B.** Qualitative / Multihabitat: not applicable

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 5 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
FIELD COLLECTION STEPS	**1.** Decide on RTH and DTH habitat types. **2.** Choose appropriate sample gear. *[Note: Steps 1 and 2 are typically accomplished in a reconnaissance prior to sampling.]* **3.** Collect 5 RTH samples in different examples of the targeted habitat distributed throughout the designated sampling reach. Composite these samples. **4.** Collect 5 DTH samples as in Step 3 and composite them. **5.** Collect QMH sample by compositing collections from all available instream habitat types in the sampling reach.	**1.** Mark transects. **2.** Select left, center, or right channel for sampling (random selection). **3.** Collect one sample from each transect and add to appropriate composite sample. **4.** Collect 210–µm qualitative sample.	**A.** Riffle habitat: **1.** Choose appropriate sampling gear. **2.** Collect 5 samples in different examples of the targeted habitat distributed throughout the sampling reach. Composite these samples. *[Note: If these data are to be compared with those previously collected using USFS Standard Operating Procedures, collect 3-5 samples in the same riffle (similar depth, velocity, substrate); do not composite these samples.]* **B.** Multihabitat: **1.** Choose appropriate sampling gear. **2.** Collect sample by compositing collections from all major habitat types in the sampling reach.	Follow Standard Operating Procedures established by State Water Resources Agency.	**1.** Select general site location during pre-survey planning. **2.** Set artificial substrates in run habitat at a sufficient depth to ensure water coverage. **3.** Retrieve artificial substrates after a 6-week colonization period. **4.** Collect qualitative dip-net/hand-picked sample from run, riffle, margin, and pool by sampling for a minimum of 30 minutes and thereafter until no new taxa are observed based on gross examination.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 6 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
FIELD PROCESSING STEPS	**1.** Elutriate, sieve, and split the composite samples until sample volume is less than 750 mL (use No. 40 sieve [425-µm mesh] for RTH and DTH; use No. 70 sieve [212-µm mesh] for QMH). **2.** Fix sample in 10-percent formalin. **3.** Label and ship samples (to be switched to 70-percent ethanol within 10 working days following receipt by contract laboratory).	**1.** If fewer than 3 transects are sampled for riffles or pools, the total sample is saved. **2.** If more than 3 transects are sampled for riffles or pools, then sample is split into thirds and one-third is saved. **3.** Samples are preserved with 70-percent ethanol. **4.** Samples are labeled and tracked before shipping to contract laboratory (no time limit specified).	**1.** Pick out and clean large pieces of organic matter (sticks, leaves) and rocks. **2.** Fix samples with 95-percent ethanol. If samples contain large amounts of organic matter, fix samples with 10-percent formalin. Replace formalin with 70- to 95-percent ethanol within 10 days. **3.** Label and ship samples along with sampling information and location information to the BLM Aquatic Ecosystem Laboratory, Logan, Utah, or a contract laboratory.	Follow Standard Operating Procedures. Two approaches for field processing of samples are adhered to: **1.** Sample sorting and subsampling in the field. Remainder of sample either discarded or preserved for quality control. **2.** Sample preserved in jar, labeled, and returned to laboratory for processing. Subsampling, whether done in the field or laboratory, generally follows the 100- or 300-organism technique. The sample is distributed in a homogeneous manner in a large gridded pan. Grids are randomly selected for processing. All organisms are removed from the selected grids. Once the targeted number of organisms (100 or 300) is reached, no more grids are processed. Some States (e.g., Ohio) use a Folsom sample splitter for all subsampling as an alternative to the RBP subsampling.	**1.** Place each set of plates in a container; preserve with 10-percent formalin. **2.** Deposit representative taxa from qualitative sample in a separate jar; preserve with 10-percent formalin. In Laboratory: **1.** Disassemble and scrape artificial substrates; screen with No. 40 and No. 30 sieves. Place samples in 70-percent alcohol. **2.** Place qualitative sample in 70-percent alcohol. **3.** Process subsamples with a Folsom sample splitter just prior to analysis.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 7 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
ANCILLARY DATA AND COORDINATED SAMPLING ACTIVITIES	**1.** Microhabitat data (current velocity, depth, substrate) collected for each semi-quantitative sample **2.** Habitat data (reach, segment, basin) obtained for each Basic Fixed Site **3.** Fish and algae community samples collected at each Basic Fixed Site **4.** Water, sediment, and tissue chemistry collected at each Basic Fixed Site **5.** Continuous stream discharge and field water-quality parameters (e.g., pH, temperature) collected at each Basic Fixed Site	**1.** Depth measured at 100 points along stream reach; substrate measured at 55 points in each reach; current velocity measured at 1 transect. **2.** Same as NAWQA **3.** Same as NAWQA **4.** Same as NAWQA	Specific supplemental data have not yet been determined. A minimum of a few standard habitat measurements should be collected at all sites, e.g., USEPA RBP's physical habitat assessment. **1.** Microhabitat data (water velocity, depth substrate) for each sample **2.** Meso- and macrohabitat data (reach, stream segment, basin) **3.** Sampling location information to facilitate regional comparisons: water body name, county, state, latitude, longitude, elevation, stream order, river mile, hydrologic unit code, ecoregion, sub-ecoregion, major land use, habitats sampled, sampling area, type of sampler	**A.** *In situ* measurements of temperature, pH, dissolved oxygen, and conductivity **B.** Physical measurements of velocity and (or) flow, sometimes particle size and channel morphological detail **C.** Habitat structure parameters either measured or estimated with visual-based technique **D.** Other assemblage collections as determined by Agency directives **E.** Water, sediment, and tissue chemistry collected as required for analytical and toxicological testing	**1.** Microhabitat data are recorded on a site description sheet. **2.** Site is identified by stream name, river code, river mile, and latitude/longitude. **2.** Fish, chemistry, and physical data are collected at all survey sites; chemistry data only and some flow information (USGS gages) are collected at NAWQMN sites.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 8 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
LEVEL OF TAXONOMY	Lowest practicable taxon (usually species or genus, as specified for various groups in the invertebrate processing protocol)	Same as NAWQA	**A.** Insects (non-Chironomidae: genus, some species **B.** Chironomidae: subfamily, hope to progress to genus level **C.** Non-insects: varies	Two levels -- (1) lowest practicable taxon (usually species or genus, as specified for various groups in the Standard Operating Procedures), or (2) family-level taxonomy	Lowest practicable level for most groups
DATA ANALYSIS	**A.** Descriptive: taxa lists, richness, diversity, similarity **B.** Multivariate: differentiation of sites based on communities, gradient analysis -- evaluate changes in biological characteristics associated with changes in chemical characteristics using, for example, two-way indicator species analysis (TWINSPAN), detrended correspondence analysis (DCA), and canonical correspondence analysis (CANOCO)	**A.** Descriptive: taxa lists, richness, diversity, similarity **B.** Multivariate: presently being evaluated **C.** Multimetric: Invertebrate Community Index (ICI), Biotic Index (BI), etc.; presently being evaluated	**A.** Descriptive: taxa lists, richness, diversity, similarity, biotic indices, multimetric approach, reference community comparison, ecoregional metric value comparisons **B.** Multivariate: differentiation of sites based on communities, gradient analysis, and changes in companion habitat and chemistry data	Multimetric approach, where metrics and indices are calibrated by stream class and regional expectations, and represent key measures of assemblage elements and processes. Calibration may be done by multivariate techniques. In assessment, metrics are calculated, and values are converted to scores and aggregated to provide an integrated assessment.	**A.** Descriptive: types and numbers; deformities are noted **B.** Multimetric: Invertebrate Community Index (ICI) **C.** Qualitative Community Tolerance Value (QCTV) [based on a weighted mean ICI value calculated for each taxon statewide] **D.** Multivariate analyses as needed

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 9 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)	A. Standardized protocols and training of field personnel. Regional biologists provide coordination and oversight of study-unit sampling activities. B. Centralized unit: Biological Quality Assurance Unit at the USGS/National Water Quality Laboratory oversees contractors and quality assurance/quality control. C. Field split samples: A percentage (e.g., 10 percent) of field split samples are processed. D. Reference collections: In-house reference collections are maintained by the Biological Quality-Assurance Unit. E. Voucher collections: Voucher collections are maintained in outside repositories (e.g., museums). F. Collaboration with outside taxonomists includes verification of identifications, updating of taxonomic lists, and identification and description of new species. G. Taxonomic database (interagency): National Oceanographic Data Center (NODC) code facilitates tracking of name changes over time and sharing of data among agencies. H. Integrated database (National Water Information System-II) contains physical (including habitat), chemical, and biological data, with facilitated data entry, verification, and analysis.	A. In general, 10 percent of samples will be retained by USEPA for QA/QC. B. All material is returned to USEPA following identification and enumeration. C. All samples are sent for verification and are tracked.	A. Documented, peer reviewed Standard Operating Procedures B. Trained staff C. Voucher collections, collaboration with outside taxonomists D. Computerized database and analytical programs with verified entries E. Consistent scientific reporting format and data summary calculations	A. Documented Standard Operating Procedures B. Trained staff and delineation of responsibility C. Maintenance of a voucher collection D. Computerized database and analytical programs with verified entries E. Consistent reporting format and metric calculation	A. QA/QC is centrally organized with the Ecological Assessment Section. B. In-house reference collections are maintained. C. Vouchers are retained for an extended period (10 years). D. Collaboration with outside taxonomists occurs as needed. E. All data are entered into the MIDGES subroutine of Ohio ECOS, a Statewide database organized and maintained by Ohio EPA.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 10 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	BLM / USFS	RBP's (State Programs)	Ohio EPA
PROTOCOL DOCUMENTS	**1. Cuffney, T.F.**, Gurtz, M.E., and Meador, M.R., 1993, Guidelines for the processing and quality assurance of benthic invertebrate samples collected as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93-407, 80 p. **2. Cuffney, T.F.**, Gurtz, M.E., and Meador, M.R., 1993, Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93-406, 66 p.	**1. Klemm, D.J.**, and Lazorchak, J.M., eds., 1993, Environmental Monitoring and Assessment Program-Surface Waters and Region 3 Regional-EMAP 1993 Pilot Field Operations and Methods Manual--Streams: Cincinnati, Ohio, U.S. Environmental Protection Agency.	Under development in collaboration with the USGS, USEPA, USFS, NPS, and State water quality agencies.	(*See* fig. 1: Program Design Documents.)	**1. Ohio Environmental Protection Agency**, 1987, Biological Criteria for the Protection of Aquatic Life, Volume II, Users manual for biological field evaluation of Ohio rivers and streams: Division of Water Quality Planning and Assessment, 21 p. **2. Ohio Environmental Protection Agency**, 1989, Biological Criteria for the Protection of Aquatic Life, Volume III, Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities: Division of Water Quality Planning and Assessment.

Figure 3.--Comparison chart of invertebrate protocols.--Continued (page 11 of 11)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)
COMPONENT OBJECTIVE (Algae) *[Note: Also see fig. 1 for Program Objectives and Design Features.]*	(Occurrence and Distribution Assessment): Evaluate the structure of benthic algal communities within targeted habitats and microhabitats; develop a list of taxa, relative abundances of taxa, and estimates of algal standing crop in selected sampling reaches. *[Note: This chart is most appropriate for the Occurrence and Distribution Assessment; modifications may be made to meet other Program objectives (e.g., trends and cause/effect case studies).]*	Evaluate algal community structure in streams of the U.S. to determine community responses to known impacts and to estimate the proportion of U.S. streams that are impaired.
SAMPLE TYPES	**A.** Richest Targeted Habitat (RTH): Semi-quantitative sample from the predominant periphyton microhabitat within the richest targeted habitat selected for invertebrate sampling within the sampling reach (e.g., epilithic periphyton microhabitat in riffles; epidendric or epiphytic periphyton microhabitat in coastal plain streams). *[Relative Abundance Information; Estimates of Algal Standing Crop]* **B.** Depositional Targeted Habitat (DTH): Semi-quantitative sample from a fine-grained, organically rich depositional habitat, e.g., epipsammic or epipelic periphyton microhabitat in a pool. *[Relative Abundance Information; Estimates of Algal Standing Crop]* **C.** Qualitative Multihabitat (QMH): A composite qualitative sample prepared by obtaining separate periphyton collections from each of all available periphyton microhabitats and compositing equivalent biovolume from each microhabitat in accordance with the total number of periphyton microhabitats present in the sampling reach. *[Taxa Presence Information]* *Optional (may be done in some study units):* **D.** Phytoplankton samples: Collection of quantitative whole-water sample of sufficient volume to ensure adequate phytoplankton material. *[Relative Abundance Information; Chlorophyll and Ash-free Dry Mass]*	Samples are collected from riffle/run habitats and composited. The same is true for pool/glide habitats (depositional). No qualitative multihabitat samples or phytoplankton samples are collected at present.

Figure 4.--Comparison chart of algae protocols. (page 1 of 6)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)
SAMPLING STRATEGY	**A.** RTH: Composite of 25 samples (approximately 3 cm^2 each) from the predominant periphyton microhabitat (natural substrates) in the richest targeted habitat type (total area ca. 75 cm^2) distributed over multiple examples of the targeted habitat type within the sampling reach. Extreme habitat conditions (fastest/slowest velocities; largest/smallest substrates; etc.) are avoided. **B.** DTH: Composite of a minimum of 5 Petri-dish samples (approximately 17 cm^2 each) from the epipsammic or epipelic microhabitats in a depositional habitat (total area approximately 85 cm^2), distributed over multiple examples of the depositional targeted habitat type within the sampling reach. Extreme examples of habitat conditions are avoided. C. QMH: Consists of 3 separate sample types: microalgae, macroalgae, and aquatic mosses. The microalgae sample is a composite from all available periphyton microhabitats within the sampling reach, using equal sampling effort in each microhabitat and compositing equal periphyton volume in accordance with the number of microhabitats; limited to total sampling time of about 0.5 hour total sampling effort in most streams. **D.** Phytoplankton samples *(study-unit option)*: Width- and depth-integrated samples collected during collection of water-chemistry samples.	Nine transects are equally spaced along the sampling reach. A sample is collected at each transect and composited with other samples from similar habitats. Thus, all riffle/run (erosional) samples are composited, and all pool/glide (depositional) samples are composited. A total of 9 samples are collected. These are all riffle, all pool, or a mixture of these habitats.
SAMPLE GEAR	**RTH samples:** **A.** Modified syringe sampling device (SG-92 periphyton sampler) **B.** PVC cylinder or pipe sampler **C.** Foil template method **D.** Surber/Hess sampler for macroalgae **E.** Artificial substrates **DTH Samples:** Petri-dish sampler **QMH samples:** Collection by scraping, brushing, suctioning, and hand-picking periphyton from all periphyton microhabitats **Phytoplankton samples** *(study-unit option)*: **A.** D-77 water sampler **B.** Kemmerer/Van Dorn samplers	Loose rock: brush (toothbrush), sample collection bottle Bedrock: scraper, suction device (blunt end of a bored-out [3/8 in.] 60-mL syringe) Soft beds: suction device (blunt end of a bored-out [3/8 in.] 60-mL syringe)

Figure 4.--Comparison chart of algae protocols.--Continued (page 2 of 6)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)
SAMPLE AREA (for principal sample gear types)	SG-92 periphyton sampler: approximately 3 cm^2 each (total area sampled per reach is 25 times 3 cm^2 = 75 cm^2) Petri-dish sampler: approximately 17 cm^2 each (total area sampled per reach is 5 times 17 cm^2 = 85 cm^2)	Area per sample (transect) = 12 cm^2 Total area sampled per reach (9 transects) = 108 cm^2
FIELD COLLECTION STEPS	Algal samples are typically collected in association with invertebrate sampling. **1.** Select predominant periphyton microhabitat in the RTH and DTH chosen for invertebrate sampling. **2.** Choose appropriate sample gear. *[Note: Steps 1 and 2 are typically accomplished in a reconnaissance prior to sampling.]* **3.** Collect replicate RTH samples from the selected periphyton microhabitat (typically epilithic, epidendric, or epiphytic) throughout the designated sampling reach. Composite the replicate samples and indicate total sampling area on field data sheet. **4.** Collect replicate DTH samples from the selected periphyton microhabitat (typically epipsammic or epipelic) throughout the designated sampling reach. Composite the replicate samples and indicate total sampling area on field data sheet. **5.** Collect QMH samples (microalgae, macroalgae, and aquatic mosses) from all available microhabitats within the designated sampling reach. Composite microalgal collections in accordance with the number of microhabitats present in the sampling reach.	Algal samples are typically collected in association with invertebrate sampling. **1.** Mark transects. **2.** Select left, center, or right channel for sampling (random selection). **3.** Collect one sample from each transect and add to appropriate composite sample (either erosional or depositional).

Figure 4.--Comparison chart of algae protocols.--Continued (page 3 of 6)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)
FIELD PROCESSING STEPS	Algal samples are processed for identification and enumeration of algal taxa; selected subsamples are prepared *(study-unit option)* for chlorophyll and ash-free dry mass determinations. **1.** Taxonomic Samples: **a.** Preserve samples with sufficient concentrated buffered formalin to constitute a 3- to 5-percent solution in the final sample. **b.** Apply an external sample label. **c.** Transport and store preserved samples in containers that prevent exposure to light. **2.** Chlorophyll and Ash-free Dry Mass *(study-unit option)*: **a.** Subsamples from (unpreserved) algal samples are filtered onto glass-fiber filters (0.7-µm pore size), using a filtration assembly and a hand-operated vacuum pump. **b.** Filters are folded, wrapped in foil, placed into filter containers, and properly labeled. Sample and subsample volumes are recorded on the sample label and field data sheet. **c.** Filter containers are transported to the analytical laboratory on dry ice, and determinations are made as soon as practicable.	**1.** Taxonomic samples: 50 mL of each composite sample (both erosional and depositional habitats) is preserved with formalin. **2.** Chlorophyll and Ash-free Dry Mass: 25 to 50 mL of each composite sample is filtered onto glass-fiber filters (0.45 µm) using a hand-operated vacuum pump. **a.** Chlorophyll Samples: Filter is folded, wrapped in foil, and frozen on dry ice. **b.** Ash-free Dry Mass Samples: Sample is filtered onto pre-leached, pre-ashed, pre-weighed glass-fiber filters (0.45 µm). Filter is wrapped unfolded in foil, and frozen on dry ice. **3.** Alkaline/Acid Phosphatase: 50 mL of each composite sample is frozen on dry ice.

Figure 4.--Comparison chart of algae protocols.--Continued (page 4 of 6)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)
ANCILLARY DATA AND COORDINATED SAMPLING ACTIVITIES	**1.** Microhabitat data (current velocity, depth, substrate) collected for each semi-quantitative (RTH and DTH) sample. **2.** Habitat data (reach, segment, basin) obtained for each Basic Fixed Site. **3.** Fish and invertebrate community samples collected at each Basic Fixed Site; coordinated algae-invertebrate sample collection recommended for synoptic survey sites *(study-unit option)*. **4.** Water, sediment, and tissue chemistry collected at each Basic Fixed Site. **5.** Continuous stream discharge and field water-quality parameters (e.g., pH, temperature) measured at each Basic Fixed Site.	**1.** Depth measured at 100 points along stream reach; substrate measured at 55 points in each reach; current velocity measured at 1 transect. **2.** Same as NAWQA **3.** Same as NAWQA **4.** Same as NAWQA
LEVEL OF TAXONOMY	RTH and DTH samples: Identification and enumeration (minimum of 500 live cells) of algal taxa at the lowest practicable taxon, generally species or variety. Biovolume calculated for dominant taxa. QMH samples: Identification of all algal taxa present in samples; HYRAX mounts are prepared for identification and verification of diatom species and varieties.	Same as for NAWQA, except: • EMAP is evaluating the use of 200 cell counts • No qualitative sample
DATA ANALYSIS	**A.** Descriptive: taxa lists, richness, diversity, similarity **B.** Multivariate: differentiation of sites based on communities, gradient analysis -- evaluate changes in chemical (biological) characteristics associated with changes in biological (chemical) characteristics using, for example, two-way indicator species analysis (TWINSPAN), detrended correspondence analysis (DCA), and canonical correspondence analysis (CANOCO).	**A.** Descriptive: taxa lists, richness, diversity, similarity **B.** Multivariate: presently being evaluated **C.** Analysis of Variance: for impact and regional trend analyses.

Figure 4.--Comparison chart of algae protocols.--Continued (page 5 of 6)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)
QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)	**A.** Standardized protocols and training of field personnel. Regional biologists provide coordination and oversight of study-unit sampling activities. **B.** Centralized unit: Biological Quality-Assurance Unit at the USGS/National Water Quality Laboratory oversees contractors and quality assurance/quality control. **C.** Field split samples: A percentage (e.g., 10 percent) of field split samples is processed. **D.** Reference collections: In-house reference collections are maintained by the Biological Quality-Assurance Unit. **E.** Voucher collections: Voucher collections are maintained in outside repositories (e.g., museums). **F.** Collaboration with outside taxonomists includes verification of identifications, updating of taxonomic lists, and identification and description of new species. **G.** Taxonomic database (interagency): National Oceanographic Data Center (NODC) code facilitates tracking of name changes over time and sharing of data among agencies. **H.** Integrated database (National Water Information System-II) contains physical (including habitat), chemical, and biological data, with facilitated data entry, verification, and analysis.	**A.** In general, 10 percent of samples will be retained by USEPA to be rechecked by an independent algalogist. **B.** All material is returned to USEPA following identification and enumeration. **C.** Reference collections will be maintained at USEPA-Cincinnati, Ohio. **D.** Voucher collections will be stored at USEPA-Cincinnati, Ohio. **E.** Sample-tracking of all samples sent for verification.
PROTOCOL DOCUMENTS	**1. Porter, S.D.**, Cuffney, T.F., Gurtz, M.E., and Meador, M.R., 1993, Methods for collecting algal samples as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93-409, 39 p.	**1. Klemm, D.J.**, and Lazorchak, J.M., eds., 1993, Environmental Monitoring and Assessment Program-Surface Waters and Region 3 Regional-EMAP 1993 Pilot Field Operations and Methods Manual--Streams: Cincinnati, Ohio, U.S. Environmental Protection Agency.

Figure 4.--Comparison chart of algae protocols.--Continued (page 6 of 6)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
COMPONENT OBJECTIVE (Habitat) *[Note: Also see fig. 1 for Program Objectives and Design Features.]*	(Occurrence and Distribution Assessment): Describe the environmental setting of selected sites and evaluate instream and riparian features within selected sampling reaches. *[Note: This chart is most appropriate for the Occurrence and Distribution Assessment; modifications may be made to meet other Program objectives (e.g., trends and cause/effect case studies).]*	**1.** Provide information on the environment and physical habitat structure of reaches to aid reach classification and predict expected biological condition. **2.** Provide physical habitat and environmental stressor information to diagnose possible causes of biotic impairment in reaches. **3.** Provide channel and riparian habitat data sufficiently precise to allow examination of responses of habitat structure to riparian, basin, and regional stressors.	Evaluate the structure of the habitat and relate it to the natural structure expected for the region. Use the evaluation of quality of habitat structure to enhance the bioassessment.	Describe and characterize salient macrohabitat attributes that most influence the aquatic life potential of a stream or river reach.
SAMPLING STRATEGY (General approach)	Habitat information is collected at four scales -- Basin, Segment, Reach, and Microhabitat: **A.** Basin: Basin-scale information is collected through geographic information system (GIS) databases. These data are collected at two scales: one that permits national coverage, and one that represents at the local, or study-unit, scale the most recent information and highest resolution available. These data include: **1.** Drainage area **2.** Drainage shape **3.** Drainage texture -- continued	Physical Habitat and Landscape Characterization information is collected at scales ranging from Region and Basin down to Microhabitat: **A.** National, Regional, Major Basin, and Catchment Scales: Landscape and larger scale information is collected from mapped data, remote imagery, and other sources (e.g., fish stocking and wastewater discharge records). It is compiled, accessed, and analyzed through geographic information system (GIS) databases, to be associated as ancillary information associated with each EMAP sample reach. These data are collected at varying resolution and generality appropriate for the scale under consideration and the desired use of -- continued	Most States use a visual-based assessment technique, where the quality of each parameter is evaluated as a continuum. Judgment criteria are established and tailored for specific regions and are succinctly defined to reduce investigator bias. A team approach to assessment is employed by most agencies. Specific parameters are assessed that represent important aspects of physical habitat structure. These parameters -- continued	Qualitative Habitat Evaluation Index (QHEI) information is collected at each fish sampling site. In the cases of multiple passes, the QHEI is initially completed during the first pass and reexamined and adjusted (if necessary) in subsequent passes. Information utilized is similar to that described for NAWQA.

Figure 5.--Comparison chart of habitat protocols. (page 1 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
SAMPLING STRATEGY (Continued)	**4.** Drainage density **5.** Storage **6.** Basin relief **7.** Ecoregion **8.** Physiographic province **9.** Land use **10.** Geologic type **11.** Soil type **12.** Potential natural vegetation **13.** Wetlands **14.** Precipitation **B.** Segment: Segment-scale data are obtained using GIS and 7.5-minute topographic maps. Segment is defined as that part of a stream bounded by ("major") tributary junctions or major discontinuities such as waterfalls, landform features, significant changes in gradient, or point-source discharges. These data include: **1.** Segment gradient **2.** Elevation **3.** Channel sinuosity **4.** Stream order **5.** Hydrologic features (dams, outfalls, bridges, etc.) -- continued	the information (differing, for example, if the objective is to classify the site, diagnose possible causes for reach-level changes, or track trends in land use or vegetation cover). These data include: **1.** Topographic drainage area **2.** Drainage density, basin morphometry (e.g, basin relief, elevation, etc.) **3.** Geologic type, soil type **4.** Potential natural vegetation **5.** Land use/land cover, including wetlands **6.** Precipitation, evaporation, runoff **7.** Atmospheric deposition rates of selected substances **8.** Ecoregion, physiographic province **9.** Zoogeographic regions (at least for fish, birds) **10.** Potential barriers/corridors to biotic dispersal/migration (dams, wastewater inputs, etc.) **11.** Road density, human/livestock population density **12.** Waste discharge permit density **B.** Segment: Segments are defined by tributary junctions on 1:100,000-scale stream traces contained in the River Reach File, Version 3 (RF3). Segment-scale data are obtained from a variety of sources in addition to RF3 itself, including 7.5-minute maps (1:24,000 scale), State resource agency records, satellite imagery, and in some regions aerial photography and potentially low-elevation videography. These data include: **1.** Segment gradient, elevation, valley bottom type and constraint **2.** Segment length and channel sinuosity -- continued	relate to instream habitat features, channel morphology, and bank and riparian vegetative structure. The parameters may be regionally specific or designed for specific State programs. Some parameters are assessed for the immediate sampling location, and others pertain to the reach or stream segment, or perhaps to the entire catchment. However, they generally include the following: **1.** Extent and abundance of instream cover for fish **2.** Quality of the benthic or epifaunal substrate **3.** Extent of embeddedness of the epifaunal substrate **4.** Representation of various velocity and depth regimes **5.** Presence or absence of channel alteration **6.** Extent of sediment deposition **7.** Sinuosity or frequency of riffles in a reach **8.** Status of channel fullness or wetted width **9.** Condition of banks **10.** Extent of vegetative protection on banks -- continued	

Figure 5.--Comparison chart of habitat protocols.--Continued (page 2 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
SAMPLING STRATEGY (Continued)	C. Reach: Reach characterizations are conducted to collect data on instream and riparian features. Two levels of reach-scale habitat data are collected. The first level consists of a relatively rapid assessment of habitat conditions. The second level consists of a detailed, surveyed map of the reach. These habitat data are obtained directly from field measurements (see "Field Collection Steps" below). -- continued	**3.** Stream order (1:100,000 and 1:24,000 map scale) **4.** Riparian vegetation -- likely in arid and agricultural regions **5.** Potential barriers/corridors (e.g., dams, culverts, temperature barriers, etc.) **6.** Ditching, channelization, flow rerouting and canalization C. Reach: The reach physical habitat assessment employs a randomized, systematic spatial sampling design that minimizes bias in the placement and positioning of individual measurements. Sampling areas or points are placed systematically at spacings that are proportional to base-flow channel width. Measurements are made where practicable (or, if necessary, attributes that are otherwise measureable can be estimated), rather than interpreting quality or importance of the attribute to biota or its importance as an indicator of disturbance. Channel and riparian habitat are characterized based on field data collected at 100 positions along the channel midline (e.g., habitat class, thalweg depth, width), at 11 evenly spaced cross sections (e.g., riparian vegetation cover, substrate size, fish cover), and at 3 evenly spaced valley cross sections (elevation profile, land use). Reach attributes estimated by the data analysis include: **1.** Channel dimensions and their variability **2.** Reach gradient, sinuosity, channel shape -- continued	**11.** Extent of grazing or other disruptive pressure **12.** Riparian vegetative zone width The judgment of these parameters depends on the reference condition for the region and stream type, and the assessed quality of the physical habitat structure is adjusted accordingly.	

Figure 5.--Comparison chart of habitat protocols.--Continued (page 3 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
SAMPLING STRATEGY (Continued)	**D.** Microhabitat: Microhabitat data (velocity, substrate type, and depth) are collected at the locations where invertebrate and algal samples are taken.	**3.** Channel-unit scale habitat classifications **4.** Residual pool frequency and dimensions **5.** Riparian vegetation structure/cover in three layers **6.** Canopy cover over stream **7.** Distribution of substrate sizes and embeddedness **8.** Fish cover **9.** Human disturbances/land use in channel, banks, and riparian area **10.** Valley form and channel incision **D.** Microhabitat: Microhabitat data (e.g., substrate, depth, fish cover, etc.) are collected at 11 cross-section transects spaced evenly along the sampling reach. These transects are also the locations where macroinvertebrate and periphyton samples are taken.		
SAMPLE GEAR	**1.** Tape measure and rangefinder **2.** Brunton compass **3.** Clinometer **4.** Surveying rod and level **5.** Velocity meter **6.** Tree diameter tape measure	**1.** Tape measure (50-m fiberglass) and rangefinder **2.** Bearing compass (Silva Ranger-type) **3.** Clinometer **4.** Spherical canopy densiometer **5.** Surveyor's rod (telescoping 1.5 m to 5 m or 7 m) **6.** Calibrated staff (1.2 m marked in centimeters) and hip chain **7.** Velocity meter	Mostly visual-based technique. Shading densiometers, flow meters, staff gage, and measuring tapes sometimes used. Data sheets with lists of parameters and descriptions of judgment criteria are prepared for specific regions or ecoregions, or stream types.	**1.** QHEI field sheet **2.** QHEI procedures **3.** Depth pole graduated at 10-cm intervals **4.** Hip chain

Figure 5.--Comparison chart of habitat protocols.--Continued (page 4 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
SAMPLE AREA	Field habitat measurements are made in the entire sampling reach.	Field habitat measurements are made throughout the sampling reach. "Office" or remote measures are made on the mapped segment, drainage basin, or region, as appropriate.	Biological sampling site or area used to assess specific instream parameters. Reach is expanded beyond site to appropriately assess parameters related to channel morphology, bank stability, and riparian vegetation.	Entire sampling reach (150-200 m for wading sites; 500 m for boat sites)
FIELD COLLECTION STEPS	Only data for reach characterizations are collected in the field: **A.** First-Level Reach Characterization -- All data are collected with respect to a known reference location (permanent structure such as a gage station or bridge). This location is important for linking habitat data at the various scales. Collection of data is based on the establishment of a minimum of 6 transects, systematically chosen to represent conditions in geomorphic units (pools, riffles, runs). Data collected include: **1.** Channel width **2.** Bank width **3.** Flood-plain width **4.** Stream depth **5.** Velocity **6.** Bed substrate (Wentworth scale) **7.** Embeddedness -- continued	The Field Physical Habitat Protocol consists of 5 different components (see also Table 1 from the habitat protocol document): **A.** Thalweg Profile: A longitudinal survey of depth, width, and habitat class at 100 equally spaced points along the centerline between the two ends of the sample stream reach. *[This provides residual pool volume, stream size, channel complexity, and proportions of habitat types.]* **B.** Large Woody Debris Tally: Count and estimate dimensions of woody debris in the active channel along the full length of the sample reach. Estimate the portion of each piece that is presently inundated by water. *[This component allows precise estimate of wood and its distribution within the reach.]* -- continued	Habitat assessments are usually conducted after biological collections, to minimize disturbance and to benefit from a direct exposure of the investigator to instream features. Parameters in State programs are taken from the following list: **1.** Instream cover **2.** Epifaunal substrate characterization **3.** Channel alteration **4.** Sediment deposition **5.** Bottom scouring **6.** Channel sinuosity **7.** Lower bank channel capacity -- continued	The general site location is selected during pre-survey planning. The sampling site is established, measured, and marked for future passes. QHEI sheet is completed by the fish crew leader after the site has been electrofished. Data include: **1.** Substrate: quality and quantity, with condition (silt, embeddedness) **2.** Cover: types and quantity **3.** Channel: sinuosity, development, stability, condition **4.** Riparian Zone: width, quality, bank erosion **5.** Pool Quality: maximum depth, current types, morphology **6.** Riffle/Run Quality: depth, stability, embeddedness **7.** Local Gradient: attenuated by stream size

Figure 5.--Comparison chart of habitat protocols.--Continued (page 5 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
FIELD COLLECTION STEPS (continued)	**A.** First-Level Reach Characterization -- continued: **8.** Sun angle **9.** Stream heading **10.** Bank angle **11.** Bank height **12.** Bank vegetative stability **13.** Bank shape **14.** Bank erosion **15.** Habitat features (categories of instream cover such as woody snags, undercut banks, etc.) -- measured within a zone 2 m wide on either side of each transect line. **16.** Bank woody vegetation -- evaluated at the ends of each transect line using a point-quarter method. **B.** Second-Level Reach Characterization -- Data collected for second-level reach characterizations include: **1.** Planimetric map of the reach based on surveyed profiles of the flood plain, water surface, and channel bed **2.** A minimum of 3 monumented cross sections **3.** A quantified particle-size analysis of the channel-bed substrate **4.** Permanent plot vegetation analysis **5.** Photodocumentation	**C.** Channel/riparian cross sections: At each of 11 evenly spaced cross sections, measure and estimate substrate, bank characteristics, riparian vegetation structure, canopy cover, channel gradient, channel compass bearing, fish cover, and presence/proximity of human disturbance types. *[This component allows calculation of many attributes contributing to habitat quality and riparian disturbance.]* **D.** Valley transects: Rough measure of valley elevation changes and landform classification at three 40-m cross sections (20 m out from both left and right banks at bottom, middle, and top of sample reach). *[This component allows classification of valley type.]* **E.** Discharge: Measure instantaneous discharge at the time of sampling, using flow meter and velocity-area method where possible; portable weir or timed filling of bucket in very small streams. *[Discharge gives another measure of the stream size, and is helpful for interpreting many chemical and biological measures.]*	**8.** Channel flow status **9.** Channel shape **10.** Width to depth ratio **11.** Bank stability **12.** Bank vegetative protection **13.** Grazing or other disruptive pressure **14.** Riparian vegetative zone width **15.** Canopy cover In addition to the above, data are collected on the following: High-gradient streams: • Riffle/pool sequence • Ratio of pool dimensions to riffle dimensions • Velocity/depth regime • Embeddedness • Percent fines *or* Low-gradient streams: • Pool variability • Pool substrate characterization	

Figure 5.--Comparison chart of habitat protocols.--Continued (page 6 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
ANCILLARY DATA AND COORDINATED SAMPLING ACTIVITIES	**A.** Fish, invertebrate and algal community samples are collected at each Basic Fixed Site. **B.** Water, sediment, and tissue chemistry are collected at each Basic Fixed Site. **C.** Continuous stream discharge and field water-quality parameters (e.g., pH, temperature) are measured at each Basic Fixed Site.	**A.** The primary data upon which stream ecological responses will be determined are biological. Fish, macroinvertebrate, and algal assemblage samples are collected at all the EMAP sample reaches. **B.** Fish tissue contaminants and sediment oxygen demand are collected at EMAP sample reaches. In the Mid-Appalachian and Oregon Pilots, Qualitative Rapid Bioassessment Habitat Assessment forms were also filled out to compare with more quantitative methods. **C.** Field pH, conductivity, and temperature are measured, and water sample is collected, for chemistry at each EMAP sample reach. **D.** As part of pilot activities, sediments were collected for toxicity bioassay at selected sites.	**A.** Biological assemblage collections are made as determined by Agency directives. **B.** Water, sediment, and tissue chemistry are collected as required for analytical and toxicological testing. **C.** *In situ* measurements of temperature, pH, dissolved oxygen, and conductivity are made.	**A.** QHEI is conducted concurrently with fish sampling. **B.** Invertebrate, chemistry, and physical information is obtained for each site.

Figure 5.--Comparison chart of habitat protocols.--Continued (page 7 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
DATA ANALYSIS	Raw data are summarized to describe each sampling reach. Multivariate techniques are used to group sites according to common habitat features.	Raw data from multiple field measurement locations within each sample reach will be aggregated or averaged to yield single-reach summary values for a number of habitat characteristics (refer to "Field Collection Steps" above for primary analyses of each of the 5 field protocol components).	Scores are totaled for all parameters. The total score is compared to the reference to obtain a percentage comparison. Some States have developed a percentage or score threshold for attaining acceptable conditions. Individual parameter values are used to identify habitat degradation and to relate to biological condition.	QHEI scores are calculated based on a numeric index ranging from 20-100. Major habitat attributes that correspond to high and low quality habitat have been identified (Rankin, 1989). A matrix of these attributes is produced for each site, which allows further interpretation of the causes of habitat impairment.
Quality Assurance/ Quality Control (QA/QC)	**A.** Standardized protocols and training of field personnel. Regional biologists provide coordination and oversight of study-unit sampling activities. **B.** Integrated database (National Water Information System-II) contains physical (including habitat), chemical, and biological data, with facilitated data entry, verification, and analysis.	Data quality control by standardized protocols and field forms, comprehensive crew training, field audits, database verification and validation. Data quality assessment by sampling replication to estimate components of variance and precision ("signal-to-noise" ratio).	**A.** Documented Standard Operating Procedures **B.** Trained staff and delineation of responsibility **C.** Maintenance of a photodocumentation record **D.** Computerized database and analytical programs with verified entries **E.** Consistent reporting format and parameter calculation	**A.** Extensive training to help standardize between raters **B.** One-year training refresher required **C.** Visual aids important

Figure 5.--Comparison chart of habitat protocols.--Continued (page 8 of 9)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	RBP's (State Programs)	Ohio EPA
PROTOCOL DOCUMENT	**1. Meador, M.R.**, Hupp, C.R., Cuffney, T.F., and Gurtz, M.E., 1993, Methods for characterizing stream habitat as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93-408, 48 p.	**1. Kaufmann, P.R.**, and Robison, E.G., 1993, Physical habitat assessment, Section 6, *in* Klemm, D.J., and Lazorchak, J.M., eds., Environmental Monitoring and Assessment Program-Surface Waters and Region 3 Regional-EMAP 1993 Pilot Field Operations and Methods Manual-- Streams: Cincinnati, Ohio, U.S. Environmental Protection Agency.	**1. Barbour, M.T.**, and Stribling, J.B., 1991, Use of habitat assessment in evaluating the biological integrity of stream communities, *in* Biological criteria: Research and regulation, EPA-440/5-91-005: Washington, D.C., USEPA, Office of Water, p. 25-38. **2. Hayslip, G.A.**, ed., 1993, EPA Region 10 in-stream biological monitoring handbook, EPA 910/9-92-013: Seattle, Wash., USEPA, Region 10, Environmental Services Division. **3. Mid-Atlantic Coastal Streams Workgroup**, (In preparation), Standard operating procedures and technical basis: Macroinvertebrate collection and habitat assessment for low gradient, nontidal streams: Wheeling, W.Va., USEPA, Region III. **4. Plafkin, J.L.**, Barbour, M.T., Porter, K.D., Gross, S.K., and Hughes, R.M., 1989, Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish, EPA/440/4-89-001: Washington, D.C., USEPA, Office of Water.	**1. Ohio Environmental Protection Agency**, 1989, Biological Criteria for the Protection of Aquatic Life, Volume III, Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities: Division of Water Quality Planning and Assessment. **2. Rankin, E.T.**, 1989, The Qualitative Habitat Evaluation Index (QHEI): Rationale, Methods, and Application: Division of Water Quality Planning and Assessment.

Figure 5.--Comparison chart of habitat protocols.--Continued (page 9 of 9)

Physical habitat characteristic	NAWQA (USGS)	EMAP (USEPA)	STATES	BLM	USFS
Channel Width					
a. Wetted Width -- at several places	X	X		X	X
b. Wetted Width -- one estimate			X		
Channel Depth					
a. Via Transect	X	X			
b. Via Habitat		X	X	X	X
c. Thalweg Depth	X	X			
Channel Gradient	X	X		X	X
Bed Substrate (Visual)	X	X	X	X	X
a. Pebble Counts	X	X		X	X
b. Surface Fines (P/A=Presence/Absence)	P/A	X		X	X
Embeddedness	X	X	X	X	X
Habitat Features / Cover (Instream; e.g., snags, boulders, cobble, pools, etc.)	X	X	X	X	X
Large Woody Debris	Indirect	X	Indirect	X	X
Sun Angle	X				
Shading (Canopy Cover)		X	X	X	
Stream Heading (Aspect)	X	X	Indirect	Indirect	Indirect
Channel Shape	Indirect	Indirect	Indirect		Optional
Velocity	X	X	X	X	X
Discharge (Flow)	X	X	Indirect	Indirect	Indirect
Bank Angle (Hor=Horizontal, Ver=Vertical)	X(Ver)	X(Ver)		X(Hor)	X(Hor)
Bank Height	X	X			
Bank Vegetative Stability (Platts)	X	X	X	X	
Bank Shape	X	Indirect	Indirect		Optional
Bank Erosion	X	X	X	X	X
Bank Width	X	X		Indirect	Indirect
Bankfull Width	Indirect			X	X
Flood-plain Width	X	X	Indirect	Indirect	Indirect
Riparian Zone					
a. Vegetation Type	X	X	Indirect	X	X
b. Density	X	Indirect			Indirect
c. Dominance	Indirect	X	Indirect	X	Indirect
d. Vigor / Biomass	X	Indirect	X		Indirect
e. Areal Extent / Width	Indirect	X	X	X	Indirect
f. Successional Stage (Size Class)	Indirect	Indirect		X	Indirect
Human Disturbance		X	X		

Figure 6.--Comparison chart of physical and vegetative habitat characteristics.

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
COMPONENT OBJECTIVE (Tissues) *[Note: For NAWQA and EMAP, also see fig. 1 for Program Objectives and Design Features.]*	(Occurrence and Distribution Assessment): Determine the occurrence of trace elements and organic compounds in relation to contaminant sources; provide improved geographic coverage with respect to priority constituents whose occurrences have been detected. Establish baseline concentrations at sites thought to be minimally affected by human activity. *[Note: This chart is most appropriate for the Occurrence and Distribution Assessment; modifications may be made to meet other Program objectives (e.g., trends and cause/effect case studies).]*	Estimate, with known uncertainty, the extent and levels of sociologically important contaminants, with a focus on the potential impact to wildlife and human consumers (primary focus is impact to wildlife). As the only contaminants indicator (for EMAP), it has the function of registering presence (absence) of important contaminants.	To formulate guidance for States on how to sample and analyze fish and shellfish and to conduct risk assessment, risk management, and risk communication activities related to the issuance of consumption advisories. Specific objectives (*see* Reference 1, p. 76) are to recommend: • A tiered monitoring strategy • Target species • Target analytes in fish and shellfish tissue • Risk-based procedures for estimating target analyte screening values • Standard field procedures • Analytical methods • Procedures for data management, analysis, and reporting • QA/QC procedures All sampling and analysis will be done at the discretion of and by the States. USEPA's role will be restricted to providing guidance related to methods and procedures.	Determine the prevalence of selected bioaccumulative pollutants in fish and to identify correlations with sources of these pollutants. Also estimate human health risks from those pollutants studied for which cancer potency factors and (or) reference doses have been established. Samples collected at 388 sites and analyzed for 60 bioaccumulative pollutants.	**A.** Document temporal and geographic trends in the concentrations of bioaccumulative contaminants in fish and avian wildlife. **B.** Provide feedback to the regulatory process with respect to bioaccumulative contaminants. **C.** Provide frame-of-reference against which to compare findings from other investigations. **D.** Provide an archive of tissue for retrospective analyses, searches for new and previously unrecognized contaminants, and methods development. • The NCBP was composed of three networks--freshwater fish, duck wings, and starlings--which operated more or less independently. •The NCBP originated in 1965 as a component of the multiagency National Pesticide Monitoring Program (NPMP), which focused largely on organochlorine insecticides (especially DDT). • The components were managed as a partnership between research and operational elements within the USFWS.	Determine the current status and detect any changes in the environmental quality of estuarine and coastal waters by using contaminant levels (major and trace elements and organic contaminants) in sediments and tissues. **A.** Benthic Surveillance Project: bottomfish livers and sediment (93 stations) **B.** Mussel Watch Project: mussels, oysters and sediments (250 stations) **C.** Quality Assurance Project **D.** Specimen Bank **E.** Bioeffects Surveys and others	Determine the occurrence of certain contaminants in fish from the Ohio River and selected tributaries.

Figure 7.--Comparison chart of tissue protocols. (page 1 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
SITE SELECTION STRATEGY	In each study unit, tissues are collected at: **A.** Occurrence Survey sites selected to help define which contaminants occur in biota in the study unit. These sites include the Basic Fixed Sites, and selected other sites where contamination is likely. The occurrence survey should include sites where bed sediments are collected for chemical analyses, reference sites (where little or no contamination is expected), and ecological synoptic survey sites wherever possible. **B.** Spatial Distribution Survey sites to improve geographic coverage, with particular emphasis on assessment of priority constituents identified in the occurrence survey. (*Also see* fig. 1: Program Objectives and Design Features.)	Chosen by EMAP probability design (*Also see* fig. 1: Program Objectives and Design Features.)	**A.** Screening Study -- Sites should be located in frequently fished areas near: **1.** Point sources such as: industrial or municipal discharges, combined sewer overflows, urban storm drains **2.** Nonpoint source inputs such as: • landfills, RCRA or Superfund CERCLA sites • areas of intense agricultural, silvicultural, or resource extraction activities or urban land development • areas receiving inputs through multimedia mechanisms such as hydrogeologic connections or atmospheric deposition **3.** Areas acting as potential pollutant sinks where contaminated sediments accumulate and bioaccumulation potential might be enhanced **4.** Areas where sediments are disturbed by dredging activities **5.** Unpolluted areas that can serve as reference sites for subsequent intensive studies **B.** Intensive Study -- conducted at all screening sites where the selected screening value (SV) for one or more target analytes was exceeded.	**A.** Targeted Sites: sites where contamination was known or suspected, including locations near pulp and paper mills, refineries using the catalytic reforming process, Superfund sites, former wood preserving operations, other industrial sites, publicly owned treatment works, and agricultural and urban areas (314 sites). **B.** NASQAN Reference Sites (39 sites) **C.** Background Sites (35 sites)	**A.** Fish. **1.** Based on the assumption that fish would integrate pesticide exposure over broad expanses of time and space, the original fish network was composed of 50 fixed stations situated at key features and nodal points in the major flowing-water systems of the U.S. and the Great Lakes (coincident with USGS NASQAN sites). **2.** Beginning in 1970, the number of stations was doubled (i.e., to 100), but the collection frequency was halved (fall only). **3.** In 1975, due to the demise of most other aspects of the NPMP, 17 additional stations more oriented to the needs of the USFWS were added. Some of the original 100 were discontinued; 114 stations were sampled from 1976-86. **B.** Starlings: Randomly selected locations within blocks of 5 degrees latitude/longitude in contiguous United States. **C.** Duck Wings. No specified physical locations. ***[Note: Starlings and Duck Wings not discussed further in this comparison chart.]***	Sites are representative of bays or estuaries, and are not near known hot spots. Sites are revisited on a yearly basis once established.	Tissue samples collected from fish population sampling sites, i.e., electrofishing or lockchamber sites. Additional sites identified based on historic information or lack thereof. Input received from fish and wildlife and State health agencies.

Figure 7.--Comparison chart of tissue protocols.--Continued (page 2 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
UNIT OF SAMPLING	Sampling reach, as defined for ecological surveys, plus upstream and (or) downstream areas if needed to obtain sample, as long as there are no intervening contaminant sources. (*See* fig. 1: Program Objectives and Design Features.)	Stream reach, 150 m to 500 m (*See* fig. 1: Program Objectives and Design Features.)	None prescribed	None prescribed	Fish: Station locations were loosely defined and generally were composed of a reach or, in the Great Lakes, a large open-water area (e.g., Saginaw Bay).	None prescribed	Consistent with population study reaches -- lockchamber or 500- to 1,000-m "electrozones."
SAMPLING STRATEGY (General approach, including sample types and compositing)	Collect a representative composite sample of target taxon (taxa) in the sampling reach, near the location of sampling for analyses of water-column and bed-sediment chemistry and for ecological studies. At each site, 2 types of samples are collected: one for analyses of trace elements, and another sample for organic compounds. The same taxon is collected at as many sites as possible within a study unit; national consistency is provided by a national list of target taxa and decision trees that guide selection from that list. Collection of samples of more than one taxon (preferably a fish and an invertebrate taxon) is suggested for up to 50 percent of the Spatial Distribution Survey sites.	A subsample (or in some cases the entire sample) of a number of individuals of a target species is chosen from the final catch for that stream reach, or lake, for later compositing and analyses. **A.** Streams: Primary Target taxa are small fish, because they are more ubiquitous (compared with large fish), have more representative value for contaminants level in a stream reach, and are more relevant to wildlife consumers. Secondary Target taxa (large fish) target predators (over bottom feeders), should have greater bioaccumulation of contaminants, and are more relevant to human consumers (fishability). **B.** Lakes: Primary Target taxa are predators, and Secondary Target taxa are bottom feeders.	Composite samples of fish fillets (skin on, belly flap retained) or the edible portions of shellfish. **A.** Screening Study: One composite sample of each of 2 target species at each screening site. **B.** Intensive Study: Whenever possible, the target species in the screening study found to have elevated tissue concentrations of one or more of the target analytes should be resampled in the intensive study.	Collect a representative composite sample of target taxon for analyses of studied pollutants. Sample types include: Bottom Feeders (whole body) and Game Species (fillet). Both sample types analyzed for all pollutants.	Samples of fish are composited from whole, adult specimens, as alike in size and weight as possible, from taxa representing 2 broadly-defined categories -- predatory and bottom-dwelling fishes. Strategy is based on the assumption that fish collected from nodal points would provide space- and time-integrated average concentrations of accumulative contaminants.	Collect target species and sediment, and analyze each sample for the list of target analytes.	Collect composite samples of fish from the following groups: • Bottom feeder • Omnivore • Piscivore

Figure 7.--Comparison chart of tissue protocols.--Continued (page 3 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
REPLICATION	**A.** Duplicate samples (of the same taxon) for organics analyses at 10 percent of the sites. **B.** Three replicate samples (of the same taxon) for trace element analyses at all sites (recommended). **C.** Three replicate samples (of the same taxon) for both trace elements and organic compounds recommended at sites selected for trends studies.	Replication generally not done since EMAP sample will usually consist of most or all of the individuals of that species from a given stream reach or lake. There may not be enough tissue mass (streams) or large individuals of equal size (lakes) for 2 samples. Replication could, and perhaps should, be done at those locations where the catch of a target species is large enough. Repeat visits, however, are being done. Because EMAP design is probabilistic, each plot is a replicate within each class of stream (i.e., 1st order, 2nd order, 3rd order).	**A.** Screening Study: Replication strongly encouraged, but optional. **B.** Intensive Study: USEPA recommends that States analyze replicate composite samples of each target species at each sampling site. All replicate composite samples for a given sampling site should be collected within 1 month of each other so that temporal changes in contaminant concentrations associated with the reproductive cycle of the target species are minimized. *(Also see* Sample Size below.)	No field replicates collected	Beginning in 1970, one fish species from each fish station (usually the bottom-dwelling species) was collected in duplicate. About 10 percent of the samples were analyzed in duplicate.	Three stations per site; one sample per station.	None specified (funding constrained)

Figure 7.--Comparison chart of tissue protocols.--Continued (page 4 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
SAMPLING FREQUENCY	**A.** Occurrence Survey: Once; usually done during the year preceding the 3-year intensive period (cycle repeated every 9 years). **B.** Spatial Distribution Survey: Once; usually done during the first year of the 3-year intensive period (cycle repeated every 9 years). May include a second sample for a subset of sites previously sampled for the Occurrence Survey. **C.** Trends sites: Once each year recommended for trends sites	Once each year, during the index period (*Also see* fig. 1: Program Objectives and Design Features.)	**A.** Screening Study: Biennial screening of water bodies where commercial, recreational, or subsistence harvesting is commonly practiced. **B.** Intensive Study: As determined by results of screening study.	One-time only sampling (1988)	Fish were collected spring and fall every year from 1967-69. From 1970-74, they were collected each fall. From 1976-1986, fish were collected every other year.	Annually for Benthic Surveillance and Mussel Watch Projects	Once each year
SAMPLING SEASON (and other temporal considerations)	Late summer or early fall (base-flow conditions preferred; avoid spawning periods of target taxa)	Index period chosen from overlap of best sampling periods for each indicator; for streams, this is usually during base-flow conditions in the spring.	**A.** Screening Study: Sampling should be conducted during the period when the target species is most frequently harvested, preferably late summer to early fall. **B.** Intensive Study: Should be conducted during the same period or periods during which screening studies were conducted (i.e., when the target species are most frequently harvested for consumption) and should be conducted preferably within 1 year of the screening studies.	Avoid spawning and migration periods.	Beginning in 1970, fish were collected in the fall.	Based on water temperature; therefore, sampling time varies around the country.	Late summer

Figure 7.--Comparison chart of tissue protocols.--Continued (page 5 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
SAMPLE GEAR	As necessary to collect sufficient number and mass of specimens of target taxa, including: electrofishing apparatus, seines, clam rakes, and forceps.	Mostly electrofishing; seining in some cases (*also see* section on Fish).	Not specified except that chemical methods should not be used. Methods should simulate sport, subsistence, or commercial fishing practices used in the study area.	As necessary to collect sufficient number and mass of specimens of target taxon.	Fish could be captured by any means except poisoning (which was relaxed to allow the use of rotenone). Collectors were warned to avoid the use of chemically treated (i.e., tarred) nets.	• Fish: various trawls. • Bivalves: dredge or by hand, depending on water depth. • Sediment: grab samplers or box corers.	• Electrofishing • Lockchamber rotenone (lockchamber program currently on hold; no collections)
SAMPLE SIZE	**A.** Mollusks: 10 or more individuals **1.** Trace elements: minimum 5 g wet weight soft tissues (10 g optimal) **2.** Organics: Minimum 50 g wet weight soft tissues (100 g optimal) **B.** Aquatic insects (trace elements only): 20 or more individuals; minimum 5 g wet weight (10 g optimal) C. Fish: 5 or more individuals (8 optimal) **1.** Trace elements (livers): minimum 5 g wet weight (of livers) (10 g optimal) **2.** Organics (whole fish): minimum 50 g wet weight (100 g optimal) **D.** Aquatic plants (trace elements only): apical 5 cm of several individual plants; minimum 5 g wet weight (10 g optimal)	Fish (stream pilot study) • Small fish (primary target): 20 to 100 small fish (above a certain minimum length) per composite • Large fish (secondary target, where available): 3 to 5 individuals per composite **A.** Streams (pilot study): 20 to 100 small fish (primary target) are collected above a certain minimum length; where available, 3 to 5 large fish (secondary target) are also collected. **B.** Lakes: 3 to 5 large individuals of one predator (primary target) or bottom feeder (secondary target) species above a certain minimum length.	USEPA does not recommend one set of sample size requirements (e.g., number of replicate composite samples per site and the number of individuals per composite sample) for all fish contaminant monitoring studies. Rather, USEPA presents a more general approach to sample size determination that is both scientifically defensible and cost effective. At each site, States must determine the appropriate number of replicate composite samples and individuals per composite sample based on: • Site-specific estimations of the population variance of the target analyte concentration • Fisheries management considerations • Statistical power considerations	Fish • 3 to 5 individuals (500 g wet weight sample mass)	Fish: • 5 whole adult specimens, as alike in size and weight as possible; composite sample to weigh at least 1 pound, not to exceed 25 pounds, with no individual specimen weighing more than 5 pounds.	**A.** Mussel Watch: 3 tissue composite samples per site. Number of specimens per composite varies with the size of the animals -- usually: **1.** Organics analyses: 20 oysters or 30 mussels **2.** Elemental analyses: 20 oysters or 30 mussels **3.** Gonadal index: 10 specimens **4.** Histopathology: 10 specimens **B.** Fish: 30 fish livers. Each liver is split into samples for metals, organics, histopathology, aryl hydrocarbon hydroxylase (AHH) activity and DNA adducts. The various subsamples of the livers are composited for organic analysis. If the livers are large enough, individual metal analysis is done.	Fish • 5 to 10 individuals per composite • Species specific • Smallest fish no less than 75 percent of total length of largest fish • Right-side fillets only

Figure 7.--Comparison chart of tissue protocols.--Continued (page 6 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
TARGET TAXA	Prioritized groups of taxa: A. Trace elements: 1. Corbicula fluminea (Asiatic clam) *[soft tissues]* 2. Aquatic insects (immatures) 3. Fish (livers) 4. Aquatic plants B. Organic compounds: 1. Corbicula fluminea (Asiatic clam) *[soft tissues]* 2. Fish (whole fish) NAtional Target Taxa (NATT): A. Mollusks: Corbicula fluminea (Asiatic clam) B. Aquatic insects (immatures): Trichoptera Hydropsyche sp. Brachycentrus sp. Limnephilus sp. Chironomidae Chironomus sp. Plecoptera Perlidae Perlodidae Pteronarcyidae -- continued	Depends on ecoregion --taxa will usually consist of members of the groups shown below: A. Streams 1. Primary target group Cyprinidae (insectivorous) Percidae (darters) Cottidae 2. Secondary target group Salmonidae Centrarchidae Catostomidae Ictaluridae Cyprinus carpio (Common carp) B. Lakes 1. Primary target group Salmonidae (trout) Micropterus salmoides (Largemouth bass) Micropterus dolomieui (Smallmouth bass) Esocidae Perca flavescens (Yellow perch) Centrarchidae 2. Secondary target group Catostomidae Ictaluridae	A. Recommended Target Species for Inland Fresh Waters: Morone chrysops (White bass) Micropterus salmoides (Largemouth bass) Pomoxis nigromaculatus (Black crappie) Pomoxis annularis (White crappie) Perca flavescens (Yellow perch) Stizostedion vitreum (Walleye) Cyprinus carpio (Common carp) Catostomus commersoni (White sucker) Ictalurus punctatus (Channel catfish) Pylodictis olivaris (Flathead catfish) Esox lucius (Northern pike) Salvelinus namaycush (Lake trout) Oncorhynchus mykiss (Rainbow trout) Salmo trutta (Brown trout) B. Recommended Target Species for Great Lakes Waters: Morone chrysops (White bass) Micropterus dolomieui (Smallmouth bass) Stizostedion vitreum (Walleye) -- continued	Fish: Bottom Feeders Cyprinus carpio (Common carp) Catostomus commersoni (White sucker) Ictalurus punctatus (Channel catfish) Game Species -- Selected by USEPA Regional or State Personnel. Suggested Species include: Morone chrysops (White bass) Esox lucius (Northern pike) Stizostedion vitreum (Walleye) Micropterus salmoides (Largemouth bass) Pomoxis sp. (Crappie)	Fish. Fish collectors were provided with a list of preferred taxa within each category (predatory or bottom-dwelling) organized by habitat (cold-, cool-, or warm-water) from which to select. The most frequently collected taxa included: 1. Predators Micropterus salmoides (Largemouth bass) Micropterus dolomieui (Smallmouth bass) Perca flavescens (Yellow perch) Stizostedion vitreum (Walleye) Stizostedion canadense (Sauger) Salvelinus namaycush (Lake trout) 2. Bottom dwellers Cyprinus carpio (Common carp) Ictalurus punctatus (Channel catfish) Catostomus sp. (suckers)	A. Mussel Watch: Mytilus edulis (Blue Mussel) M. californianus (Californian Mussel) Crassostrea virginica (American Oyster) Ostrea sandvicensis (Hawaiian Oyster) B. Benthic Surveillance. Arius felis (Hardhead Catfish) Cheilotrema saturnum (Black Croaker) Coregonus sardinella (Least Cisco) Cynoscion arenarius (Sand Seatrout) Cynoscion nebulosus (Spotted Seatrout) Genyonemus lineatus (White Croaker) Hippoglossoides elassodon (Flathead Sole) Hypsopsetta guttulata (Diamond Turbot) Lagodon rhomboides (Pinfish) Leiostomus xanthurus (Spot) Lepidopsetta bilineata (Rock Sole) Leptocottus armatus (Pacific Staghorn Sculpin) Limanda aspera (Yellowfin Sole) Liopsetta glacialis (Arctic Flounder) -- continued	Fish. 1. Bottom feeder Cyprinus carpio (Common carp) 2. Omnivore Ictalurus punctatus (Channel catfish) 3. Piscivore -- one of the following: Stizostedion canadense (Sauger) Micropterus dolomieui (Smallmouth bass) Stizostedion vitreum (Walleye) Morone chrysops (White bass) -- and hybrids (White bass X striped bass) Micropterus salmoides (Largemouth bass) Selection usually subject to availability.

Figure 7.--Comparison chart of tissue protocols.--Continued (page 7 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
TARGET TAXA (continued)	C. Fish: Cyprinus carpio (Carp) Catostomus commersoni (White sucker) Catostomus catostomus (Longnose sucker) Catostomus macrocheilus (Large-scale sucker) Ictalurus punctatus (Channel catfish) Micropterus salmoides (Largemouth bass) Lepomis macrochirus (Bluegill) Salvelinus fontinalis (Brook trout) Salmo trutta (Brown trout) D. Vascular plants: Potamogeton sp. (Pondweed) Hydrilla verticillata (Hydrilla) Elodea sp. (Waterweed)		*Great Lakes Waters Target Species (continued):* Cyprinus carpio (Common carp) Catostomus commersoni (White sucker) Ictalurus punctatus (Channel catfish) Esox masquinongy (Muskellunge) Oncorhynchus tschawytscha (Chinook salmon) Salvelinus namaycush (Lake trout) Oncorhynchus mykiss (Rainbow trout) Salmo trutta (Brown trout) Other Lists of Target Species Available for the following Estuaries and Marine Waters: • Northeast Atlantic (Maine through Connecticut) • Mid-Atlantic (New York through Virginia) • Southeast Atlantic (North Carolina through Florida) • Gulf of Mexico (West Coast of Florida through Texas) • Southern California (Santa Monica Bay to Tijuana Estuary) • Pacific Northwest (Alaska through Oregon) • Northern California (Klamath River through Morro Bay)			Micropogonias undulatus (Atlantic Croaker) Myoxocephalus octodecemspinosus (Longhorn Sculpin) Myoxocephalus quadricornis (Four-horn Sculpin) Paralabrax nebulifer (Barred Sand Bass) Paralichthys californicus (California Halibut) Parophrys vetulus (English Sole) Phanerodon furcatus (White Surf Perch) Platichthys stellatus (Starry Flounder) Pleuroichthys ritteri (Spotted Turbot) Pleuronichthys verticalis (Hornyhead Turbot) Pogonias cromis (Black Drum) Pseudopleuronectes americanus (Winter Flounder) Morone americana (White Perch) Sciaenops ocellatus (Red Drum) Scophthalmus aquosus (Windowpane Flounder) Seriphus politus (Queenfish) Symphurus atricauda (California Tonguefish)	

Figure 7.--Comparison chart of tissue protocols.--Continued (page 8 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
LEVEL OF TAXONOMY	Species	Species	Species	Species	Usually to species; exception was the redhorses (suckers of the genus Moxostoma)	Species	Species
TARGET ANALYTES FOR CHEMICAL ANALYSES	A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls (total; plan to add congeners) 3. Dioxins/furans (limited number of sites) B. Polycyclic aromatic hydrocarbons (invertebrates only) C. Major metals and trace elements *Note: Crawford and Luoma (1993) includes a chart comparing target analytes of national programs.*	A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls (individual PCB congeners, including coplanars, and total) B. Major metals and trace elements: Ag, Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, Sn, Zn C. Other chlorinated organics and metals--may be added based upon regional concerns	A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls: total PCB congeners 3. Dioxins/furans B. Organophosphate pesticide C. Chlorophenoxy herbicides D. Polycyclic aromatic hydrocarbons E. Major metals and trace elements: Cd, Hg, and Se only	A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls 3. Dioxins/furans 4. Other B. Polycyclic aromatic hydrocarbons: Biphenyl only C. Major metals and trace elements: Hg only	Fish. A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls (selected Aroclors and total PCB's) 3. Other B. Major metals and trace elements: As, Cd, Cu, Pb, Hg, Se, and Zn only The number of analytes varied, but generally increased over time. Residues in 10 percent of the samples were confirmed by GC/MS.	A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls and congeners B. Polycyclic aromatic compounds C. Major metals and trace elements D. Tributyl tin species	A. Chlorinated organic compounds: 1. Organochlorine pesticides 2. Polychlorinated biphenyls B. Polycyclic aromatic compounds: infrequent (every 3 years) C. Major metals and trace elements: Cd, Pb, and Hg only
FIELD COLLECTION STEPS	As necessary to obtain an adequate sample.	In accordance with field protocol for fish community collections (*see* fig. 2).	Collect specimens by active or passive means that will ensure chemical integrity of the sample.	As necessary to obtain an adequate sample.	As necessary to obtain an adequate sample.	As necessary depending on site conditions.	None provided

Figure 7.--Comparison chart of tissue protocols.--Continued (page 9 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
FIELD PROCESSING STEPS	A. Corbicula: 1. Rinse in ambient stream water. 2. Hold in stream water at 10±2 °C for 24 hours. 3. Measure shell length. 4. Place entire organism in container. 5. Label; freeze on dry ice; ship to laboratory. B. Aquatic insects: 1. Remove insects from cases if necessary. 2. Rinse in ambient stream water. 3. Hold in stream water at 10±2 °C for 4 to 6 hours. 4. Place in labeled bag; freeze on dry ice in the field. 5. Identify specimens and select target species. 6. Count individuals; obtain composite wet weight; refreeze; ship to laboratory. C. Fish livers (trace elements): 1. Capture and sacrifice fish. 2. Rinse using ambient water. 3. Obtain total length (mm) and weight (g). -- continued	1. Subsample obtained from fish community sample. 2. Sample (whole fish only) wrapped in aluminum foil, labeled, put in zip-lock bag. 3. Freeze on dry ice (or place on ice) in field; ship to laboratory.	A. Fish: 1. Identify to species. 2. Rinse in ambient water. 3. Measure total body length. 4. Determine sex if possible (optional). 5. Identify morphological abnormalities (optional). 6. Wrap individually in heavy aluminum foil. 7. Cool on ice immediately (use dry ice if to be held for more than 24 hours). 8. Ship to laboratory. B. Shellfish: 1. Identify to species. 2. Scrub with nylon or natural fiber brush. 3. Rinse in ambient water. -- continued	1. Capture and sacrifice fish. 2. Obtain total length and weight. 3. Wrap fish in aluminum foil, dull side next to fish. 4. Place in plastic bag; freeze on dry ice; ship to laboratory.	A. Fish: 1. Sort and identify fish. 2. Group fish into samples by species. 3. Rinse in ambient water. 4. Weigh and measure specimens. 5. Wrap in aluminum foil (shiny side out). 6. Place sample in polyethylene bag. 7. Bag together the 5 specimens comprising a sample. 8. Chill sample; then freeze for storage and shipment.	A. Mussel Watch bivalves: 1. Animals scrubbed using seawater. 2. Animals packaged in aluminum foil or plastic depending on analyses. 3. Any histopathology data obtained. 4. Specimens frozen and shipped to laboratory. 5. (Oysters were shucked in the field and the tissues frozen.) 6. Shell length, volume, etc., recorded. B. Benthic Surveillance 1. Fish collected using trawl nets. 2. Livers excised and tissue samples taken for various analyses. -- continued	1. Scale fish as appropriate. 2. Skin catfish. 3. Remove edible fillet from right side. *(Do not puncture gut in process.)* 4. Wrap in food grade aluminum foil. 5. Freeze as soon as possible.

Figure 7.--Comparison chart of tissue protocols.--Continued (page 10 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
FIELD PROCESSING STEPS (continued)	C. Fish livers (cont.): **4.** Take scale (or spine) sample. **5.** Open body cavity; record maturity, sex. **6.** Excise liver; place in precleaned glass jar. **7.** Composite liver sample; weigh; place jar in labeled bag; freeze on dry ice; ship to laboratory. D. Whole fish (organics): Follow steps **C.1.** through **C.5.**: **6.** Wrap fish in aluminum foil, dull side next to fish. **7.** Wrap again. **8.** Place in labeled plastic bag; freeze on dry ice; ship to laboratory. E. Aquatic plants: **1.** Rinse sample (apical 5 cm) in ambient stream water. **2.** Soak in ambient stream water at 10±2 °C for 1 hour. **3.** Change water; soak for additional 1 hour. **4.** Remove plants from water; obtain wet weight. **5.** Place in labeled bag; freeze on dry ice; ship to laboratory.		**B.** Shellfish (continued): **4.** Measure body size. **5.** Determine sex if possible (crustaceans). **6.** Identify morphological abnormalities (optional). **7.** Wrap individually in heavy aluminum foil. (Specimens may be grouped in waterproof plastic bags.) **8.** Place on ice; ship to laboratory.			**B.** Benthic Surveillance (continued): **3.** Liver samples composited and frozen. **4.** Age, sex, health condition, and size determined. All sample processing done under clean conditions. Details of sample processing are in Lauenstein and Cantillo (1993). C. Sediments: Composite samples prepared and kept refrigerated and frozen.	

Figure 7.--Comparison chart of tissue protocols.--Continued (page 11 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
ANCILLARY DATA AND COORDINATED SAMPLING ACTIVITIES	A. Fish 1. Species 2. Individual length and weight 3. External anomalies 4. Sex 5. Age 6. Percent moisture 7. Lipid content [organics only] B. Insects 1. Species 2. Composite weight 3. Percent moisture C. Mollusks 1. Species 2. Shell length 3. Composite weight 4. Percent moisture 5. Lipid content [organics only] D. Plants 1. Species 2. Composite weight 3. Percent moisture E. Bed-sediment samples for chemical analyses of trace elements and organic compounds collected -- continued	Fish Tissue Sample Tracking Form includes the following: 1. Fish lengths (large fish) 2. Number of fish (small fish) 3. Species: code, common name 4. Were fish distributed over entire stream reach (or from all lake sampling stations)? As a diagnostic indicator, tissue contaminant levels can be used with data from all the indicators to establish probable cause of impact.	*(See* Field Processing Steps above.)	A. Fish 1. Species 2. Individual length and weight 3. Lipid content (laboratory)	A. Fish Fish collectors were asked to identify nearby contaminant sources. Some recorded age of the fish. Lipid and moisture content were determined for each composite sample.	A. Fish 1. Species 2. Size and weight 3. Gross pathology 4. Sex 5. Age 6. Percent moisture 7. Lipid content B. Bivalves 1. Species 2. Size 3. Gonadal stage 4. Histopathology 5. Perkinsus marinus presence C. Sediments 1. Grain size 2. Total Organic Carbon, Total Inorganic Carbon 3. Coprostonal D. Site 1. Salinity 2. Temperature 3. Tidal horizon	A. Fish 1. Species 2. Total length and weight 3. External anomalies 4. Percent moisture 5. Lipid content [organics only]

Figure 7.--Comparison chart of tissue protocols.--Continued (page 12 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
ANCILLARY DATA AND COORDINATED SAMPLING ACTIVITIES (continued)	at same sites as tissues, including Basic Fixed Sites. Water-chemistry samples collected at each Basic Fixed Site. F. Fish, invertebrate and algal community samples collected at each Basic Fixed Site. G. Continuous stream discharge and field water-quality parameters (e.g., pH, temperature) measured at each Basic Fixed Site.						
DATA ANALYSIS	A. Contaminant concentrations: means and ranges, among stations and within stations B. Statistical comparisons of stations: t-tests, Analysis of Variance (ANOVA) C. Graphical comparisons of contaminant concentrations D. Analysis of trends	Data can be presented in several different ways. EMAP's primary means will be by Cumulative Distribution Function (CDF). Histograms and maps, showing percent of sites in a region where each contaminant is found above a certain level (detection limit or level of concern), and geographic distributions of these sites also can be used.	A. Target analytes (means and ranges) B. Lipid analysis	A. Contaminant concentrations B. Statistical analysis to determine source correlations	A. Fish Fish results were subjected to analysis of variance and covariance to test for temporal trends. Geographic trends were reported subjectively.	Same as NAWQA	Used by State agencies for consumption advisory purposes. Compared against U.S. Department of Agriculture Food and Drug Administration Action Levels.

Figure 7.--Comparison chart of tissue protocols.--Continued (page 13 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)	**A.** Standardized protocols and training of field personnel. Regional biologists provide coordination and oversight of study-unit sampling activities. **B.** Centralized unit: Biological Quality Assurance Unit at the USGS/National Water Quality Laboratory verifies taxonomic identifications of taxa collected for tissue analyses. C. Field QA/QC: • Clean equipment and instruments • High-purity reagents • Gloves for field dissections • Field duplicates for 10 percent of sites. **D.** Laboratory QA/QC: • Split samples • Standard reference samples • Spiked samples • Blank samples • Interlaboratory comparisons **E.** Integrated database National Water Information System-II contains physical, chemical, and biological data, with facilitated data entry, verification, and analysis.	**A.** Sample tracking system ("chain of custody"). **B.** Laboratory QA/QC: • Duplicates • Spikes • Check standards • Standard reference materials • Laboratory blanks • Control charts for QC samples	**A.** Each laboratory must have an adequate QA/QC program. **B.** Methods of analysis must be documented and validated. **C.** Laboratory QA/QC: • Calibration standards • Spiked method blanks • Matrix spikes • Matrix spike replicates • Laboratory replicates • Analytical replicates • Isotopically labeled internal standards • Surrogate spikes • Accuracy-based performance evaluation samples • Split samples	Laboratory QA/QC: Dioxin/furans • 1 Method blank • 1 Spiked blank • 1 Detection limit verification • 1 Duplicate sample • 8 Environmental samples Xenobiotics • 1 Method blank • 1 Spiked blank • 1 Duplicate sample • 9 Environmental samples	Fish Analyses of fish samples were cross-checked by independent laboratories. Identifications of some residues were confirmed by GC/MS. Analytical QA/QC included the analysis of procedural blanks, internal and external standards, reference materials, and blind replicates. Aliquots of samples were retained for confirmatory analyses.	National Status and Trends (NS&T) QA Project oversees the analyses by NS&T cooperating laboratories. Performance-based so no methods are specified. Use of standard reference materials, certified reference materials, blanks, duplicates, and participation in intercomparison exercise is required.	Field instruments cleaned between composite samples (not individuals within a composite). Laboratory QA/QC as provided by Texas A&M University. Spikes and duplicates on 10 percent of samples.

Figure 7.--Comparison chart of tissue protocols.--Continued (page 14 of 15)

	NAWQA (USGS)	EMAP--Surface Waters (USEPA)	Fish Contamination Program (USEPA)	National Study of Chemical Residues in Fish (USEPA)	National Contaminant Biomonitoring Program (NBS)	National Status and Trends Program (NOAA)	ORSANCO
PROTOCOL DOCUMENT	**1. Crawford, J.K.**, and Luoma, S.N., 1993, Guidelines for studies of contaminants in biological tissues for the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 92-494, 69 p.	**1. Baker, J.R.**, Merritt, G.D., and Sutlar, D.W., eds., 1993, EMAP-Surface Waters Lake Field Operations, Volumes I and II: Las Vegas, Nev., U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. **2. Klemm, D.J.**, and Lazorchak, J.M., eds., Environmental Monitoring and Assessment Program-Surface Waters and Region 3 Regional-EMAP 1993 Pilot Field Operations and Methods Manual--Streams: Cincinnati, Ohio, U.S. Environmental Protection Agency.	**1. U.S. Environmental Protection Agency**, 1993, Guidance for assessing chemical contaminant data for use in fish advisories: Volume I: Fish sampling and analysis: Office of Water, EPA 823-R-93-002, variously paged.	**1. U.S. Environmental Protection Agency**, 1984, Sampling guidance manual for the National Dioxin Study. **2. U.S. Environmental Protection Agency**, 1986, Work/Quality Assurance Project Plan for the Bioaccumulation Study. **3. U.S. Environmental Protection Agency**, 1992, National study of chemical residues in fish (vols. I and II): Office of Science and Technology, Standards and Applied Science Division, EPA 823-R-92-008a and b, variously paged.	**1. Schmitt, C.J.**, 1988, Instructions for the collection and shipment of National Contaminant Biomonitoring Program (NCBP) freshwater fish samples: U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, Columbia, Mo., Standard Operating Procedure No. F5.18, 18 p. Standard Operating Procedures for collection of starlings and duck wings, for the preparation of samples, and for the analytical chemistry are part of the internal documentation maintained at the Patuxent Wildlife Research Center. Summaries of these procedures are provided in the published reports from these networks.	**1. Lauenstein, G.L.**, and Cantillo, A.Y., eds., 1993, Sampling and analytical methods of the NOAA National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992, Volumes I -IV: Silver Spring, Md., Technical Memorandum NOAA/NOS/ORCA/71.	*No information provided*

Figure 7.--Comparison chart of tissue protocols.--Continued (page 15 of 15)

APPENDIXES

APPENDIX I: Names and Addresses of Workshop Participants

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Interagency Biological Methods Workshop

June 22-23, 1993
U.S. Geological Survey
Reston, Virginia

Agenda

Tuesday, June 22

8:30 AM Welcome and Opening Remarks
-- Dallas Peck, Director, U.S. Geological Survey

8:45 AM An Overview of NAWQA Biology and Workshop Objectives
-- Marty Gurtz, U.S. Geological Survey

9:20 AM The ITFM Perspective on Biological Indicators
-- Chris Yoder, Ohio Environmental Protection Agency

9:40 AM Overview of State Agency Biomonitoring Programs
-- Mike Barbour, Tetra Tech, Inc.

10:00 AM ***Break***

10:20 AM Brief remarks from participating agencies and programs

11:00 AM Objectives for Working Groups

11:15 AM Working Group Session I (assigned meeting rooms: Fish, Invertebrates, Algae, Habitat, Tissues)
- ❍ Introductions and backgrounds of participants

11:45 AM -- 1:00 PM ***Lunch*** (with your Working Group)

1:00 PM Working Group Session II (assigned meeting rooms)
- ❍ Compilation of protocol charts
- ❍ Element-by-element discussion of protocol charts: note similarities, highlight differences; discuss reasons for differences among protocols
- ❍ Prepare brief narratives discussing similarities and differences

4:30 PM Reconvene in Auditorium: Review progress of Working Groups

Wednesday, June 23

8:30 AM Working Group Session III (assigned meeting rooms)
- ❍ Continue discussions of protocol charts, if necessary
- ❍ Future directions: opportunities for research and collaboration

11:45 AM -- 1:00 PM ***Lunch***

1:00 PM Reconvene in Auditorium
- ❍ Working Group Reports and Discussion
- ❍ Wrap-Up and Open Discussion

4:00 PM Adjourn

APPENDIX III: List of Participants in Each Workgroup

Fish Workgroup

Mike Meador *(Facilitator)*
Frank McCormick *(Recorder/Reporter)*
Ron Preston
Mike Rexrode
Charlie Saylor
Terry Short
Chris Yoder
Steve Zylstra

Invertebrates Workgroup

Tom Cuffney *(Facilitator/Reporter)*
Marc Sylvester *(Recorder)*
Steve Ahlstedt
Ted Angradi
Marjorie Coombs
Wayne Davis
Chris Faulkner
Brian Hill
Roy Irwin
Dave Lenat
Mark Nelson
Mark Vinson

Algae Workgroup (June 22 only)

Stephen Porter *(Facilitator/Reporter)*
Brian Hill *(Recorder)*
Marty Gurtz
Steve Sorenson

Habitat Workgroup

Cliff Hupp *(Facilitator)*
Mike Barbour *(Recorder/Reporter)*
George Gibson *(Reporter)*
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Ron Parker
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Tissues Workgroup

Kent Crawford *(Facilitator)*
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Adriana Cantillo
Ryan Childs
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