Biological Criteria

National Program Guidance for Surface Waters

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Contents

Acknowledgments ......................................................... iv
Dedication .............................................................. iv
Definitions ............................................................... v
Executive Summary ..................................................... vii

Part I: Program Elements

1. Introduction ......................................................... 3
   Value of Biological Criteria ........................................ 4
   Process for Implementation ........................................ 6
   Independent Application of Biological Criteria .................. 7
   How to Use This Document ......................................... 7

2. Legal Authority ...................................................... 9
   Section 303 .......................................................... 9
   Section 304 .......................................................... 10
   Potential Applications Under the Act .............................. 10
   Potential Applications Under Other Legislation .................. 10

3. The Conceptual Framework ......................................... 13
   Premise for Biological Criteria .................................... 13
   Biological Integrity .................................................. 14
   Biological Criteria .................................................. 14
      Narrative Criteria ............................................... 15
      Numeric Criteria ................................................ 16
   Refining Aquatic Life Use Classifications ....................... 17
   Developing and Implementing Biological Criteria ................ 18
4. Integrating Biological Criteria in Surface Water Management
   Implementing Biological Criteria ............................................. 21
   Biological Criteria in State Programs ..................................... 22
   Future Directions ................................................................... 24

Part II: The Implementation Process

5. The Reference Condition ....................................................... 27
   Site-specific Reference Condition .......................................... 28
      The Upstream-Downstream Reference Condition ................... 28
      The Near Field-Far Field Reference Condition ..................... 28
   The Regional Reference Condition ......................................... 29
      Paired Watershed Reference Condition ............................... 29
      Ecoregional Reference Condition ...................................... 29

6. The Biological Survey .......................................................... 33
   Selecting Aquatic Community Components ............................. 34
   Biological Survey Design ...................................................... 35
      Selecting the Metric ........................................................... 35
      Sampling Design ................................................................ 36

7. Hypothesis Testing: Biological Criteria and the Scientific Method
   Hypothesis Testing ................................................................. 37
   Diagnosis ............................................................................. 38

References ............................................................................. 43

Appendix A: Common Questions and Their Answers .................. 45
Appendix B: Table of Contents; Biological Criteria—Technical Reference Guide 49
Appendix C: Table of Contents; Biological Criteria—Development By States 51
Appendix D: Contributors and Reviewers .................................. 53
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Special recognition goes to the Steering Committee who helped develop document goals and made a significant contribution toward the final guidance. Members of the Steering Committee include:

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James Plafkin, Ph.D.    Dave Courtemanch
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Suzanne K. Macy Marcy, Ph.D.
Editor

In Memory of

James L. Plafkin, Ph.D.
Definitions

To effectively use biological criteria, a clear understanding of how these criteria are developed and applied in a water quality standards framework is necessary. This requires, in part, that users of biological criteria start from the same frame of reference. To help form this frame of reference, the following definitions are provided. Please consider them carefully to ensure a consistent interpretation of this document.

Definitions

- **An AQUATIC COMMUNITY** is an association of interacting populations of aquatic organisms in a given waterbody or habitat.

- **A BIOLOGICAL ASSESSMENT** is an evaluation of the biological condition of a waterbody using biological surveys and other direct measurements of resident biota in surface waters.

- **BIOLOGICAL CRITERIA**, or biocriteria, are numerical values or narrative expressions that describe the reference biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use.

- **BIOLOGICAL INTEGRITY** is functionally defined as the condition of the aquatic community inhabiting unimpaired waterbodies of a specified habitat as measured by community structure and function.

- **BIOLOGICAL MONITORING** is the use of a biological entity as a detector and its response as a measure to determine environmental conditions. Toxicity tests and biological surveys are common biomonitoring methods.

- **A BIOLOGICAL SURVEY**, or biosurvey, consists of collecting, processing and analyzing representative portions of a resident aquatic community to determine the community structure and function.

- **A COMMUNITY COMPONENT** is any portion of a biological community. The community component may pertain to the taxonomic group (fish, invertebrates, algae), the taxonomic category (phylum, order, family, genus, species), the feeding strategy (herbivore, omnivore, carnivore) or organizational level (individual, population, community association) of a biological entity within the aquatic community.

- **REGIONS OF ECOLOGICAL SIMILARITY** describe a relatively homogeneous area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variable. Regions of ecological similarity help define the potential for designated use classifications of specific waterbodies.

- **DESIGNATED USES** are those uses specified in water quality standards for each waterbody or segment whether or not they are being attained.

- **An IMPACT** is a change in the chemical, physical or biological quality or condition of a waterbody caused by external sources.

- **An IMPAIRMENT** is a detrimental effect on the biological integrity of a waterbody caused by an impact that prevents attainment of the designated use.

- **A POPULATION** is an aggregate of interbreeding individuals of a biological species within a specified location.

- **A WATER QUALITY ASSESSMENT** is an evaluation of the condition of a waterbody using biological surveys, chemical-specific analyses of pollutants in waterbodies, and toxicity tests.

- **An ECOLOGICAL ASSESSMENT** is an evaluation of the condition of a waterbody using water quality and physical habitat assessment methods.
Executive Summary

The Clean Water Act (Act) directs the U.S. Environmental Protection Agency (EPA) to develop programs that will evaluate, restore and maintain the chemical, physical, and biological integrity of the Nation's waters. In response to this directive, States and EPA implemented chemically based water quality programs that successfully addressed significant water pollution problems. However, these programs alone cannot identify or address all surface water pollution problems. To create a more comprehensive program, EPA is setting a new priority for the development of biological water quality criteria. The initial phase of this program directs State adoption of narrative biological criteria as part of State water quality standards. This effort will help States and EPA achieve the objectives of the Clean Water Act set forth in Section 101 and comply with statutory requirements under Sections 303 and 304. The Water Quality Standards Regulation provides additional authority for biological criteria development.

In accordance with priorities established in the FY 1991 Agency Operating Guidance, States are to adopt narrative biological criteria into State water quality standards during the FY 1991-1993 triennium. To support this priority, EPA is developing a Policy on the Use of Biological Assessments and Criteria in the Water Quality Program and is providing this program guidance document on biological criteria.

This document provides guidance for development and implementation of narrative biological criteria. Future guidance documents will provide additional technical information to facilitate development and implementation of narrative and numeric criteria for each of the surface water types.

When implemented, biological criteria will expand and improve water quality standards programs, help identify impairment of beneficial uses, and help set program priorities. Biological criteria are valuable because they directly measure the condition of the resource at risk, detect problems that other methods may miss or underestimate, and provide a systematic process for measuring progress resulting from the implementation of water quality programs.
Biological criteria require direct measurements of the structure and function of resident aquatic communities to determine biological integrity and ecological function. They supplement, rather than replace chemical and toxicological methods. It is EPA's policy that biological survey methods be fully integrated with toxicity and chemical-specific assessment methods and that chemical-specific criteria, whole-effluent toxicity evaluations and biological criteria be used as independent evaluations of non-attainment of designated uses.

Biological criteria are narrative expressions or numerical values that describe the biological integrity of aquatic communities inhabiting waters of a given aquatic life use. They are developed under the assumptions that surface waters impacted by anthropogenic activities may contain impaired aquatic communities (the greater the impact the greater the expected impairment) and that surface waters not impacted by anthropogenic activities are generally not impaired. Measures of aquatic community structure and function in unimpaired surface waters functionally define biological integrity and form the basis for establishing the biological criteria.

Narrative biological criteria are definable statements of condition or attainable goals for a given use designation. They establish a positive statement about aquatic community characteristics expected to occur within a waterbody (e.g., "Aquatic life shall be as it naturally occurs" or "A natural variety of aquatic life shall be present and all functional groups well represented"). These criteria can be developed using existing information. Numeric criteria describe the expected attainable community attributes and establish values based on measures such as species richness, presence or absence of indicator taxa, and distribution of classes of organisms. To implement narrative criteria and develop numeric criteria, biota in reference waters must be carefully assessed. These are used as the reference values to determine if, and to what extent, an impacted surface waterbody is impaired.

Biological criteria support designated aquatic life use classifications for application in standards. The designated use determines the benefit or purpose to be derived from the waterbody; the criteria provide a measure to determine if the use is impaired. Refinement of State water quality standards to include more detailed language about aquatic life is essential to fully implement a biological criteria program. Data collected from biosurveys can identify consistently distinct characteristics among aquatic communities inhabiting different waters with the same designated use. These biological and ecological characteristics may be used to define separate categories within a designated use, or separate one designated use into two or more use classifications.

To develop values for biological criteria, States should (1) identify unimpaired reference waterbodies to establish the reference condition and (2) characterize the aquatic communities inhabiting reference surface waters. Currently, two principal approaches are used to establish reference sites: (1) the site-specific approach, which may require upstream-downstream or near field-far field evaluations, and (2) the regional approach, which identifies similarities in the physico-chemical characteristics of watersheds that influence aquatic ecology. The basis for choosing reference sites depends on classifying the habitat type and locating unimpaired (minimally impacted) waters.
Once reference sites are selected, their biological integrity must be evaluated using quantifiable biological surveys. The success of the survey will depend in part on the careful selection of aquatic community components (e.g., fish, macroinvertebrates, algae). These components should serve as effective indicators of high biological integrity, represent a range of pollution tolerances, provide predictable, repeatable results, and be readily identified by trained State personnel. Well-planned quality assurance protocols are required to reduce variability in data collection and to assess the natural variability inherent in aquatic communities. A quality survey will include multiple community components and may be measured using a variety of metrics. Since multiple approaches are available, factors to consider when choosing possible approaches for assessing biological integrity are presented in this document and will be further developed in future technical guidance documents.

To apply biological criteria in a water quality standards program, standardized sampling methods and statistical protocols must be used. These procedures must be sensitive enough to identify significant differences between established criteria and tested communities. There are three possible outcomes from hypothesis testing using these analyses: (1) the use is impaired, (2) the biological criteria are met, or (3) the outcome is indeterminate. If the use is impaired, efforts to diagnose the cause(s) will help determine appropriate action. If the use is not impaired, no action is required based on these analyses. The outcome will be indeterminate if the study design or evaluation was incomplete. In this case, States would need to re-evaluate their protocols.

If the designated use is impaired, diagnosis is the next step. During diagnostic evaluations three main impact categories must be considered: chemical, physical, and biological stress. Two questions are posed during initial diagnosis: (1) what are obvious potential causes of impairment, and (2) what possible causes do the biological data suggest? Obvious potential causes of impairment are often identified during normal field biological assessments. When an impaired use cannot be easily related to an obvious cause, the diagnostic process becomes investigative and iterative. Normally the diagnoses of biological impairments are relatively straightforward; States can use biological criteria to confirm impairment from a known source of impact.

There is considerable State interest in integrating biological assessments and criteria in water quality management programs. A minimum of 20 States now use some form of standardized biological assessments to determine the status of biota in State waters. Of these, 15 States are developing biological assessments for future criteria development. Five States use biological criteria to define aquatic life use classifications and to enforce water quality standards. Several States have established narrative biological criteria in their standards. One State has instituted numeric biological criteria.

Whether a State is just beginning to establish narrative biological criteria or is developing a fully integrated biological approach, the programmatic expansion from source control to resource management represents a natural progression in water quality programs. Implementation of biological criteria will provide new options for expanding the scope and application of ecological perspectives.
Part I

Program Elements
The principal objectives of the Clean Water Act are "to restore and maintain the chemical, physical and biological integrity of the Nation's waters" (Section 101). To achieve these objectives, EPA, States, the regulated community, and the public need comprehensive information about the ecological integrity of aquatic environments. Such information will help us identify waters requiring special protection and those that will benefit most from regulatory efforts.

To meet the objectives of the Act and to comply with statutory requirements under Sections 303 and 304, States are to adopt biological criteria in State standards. The Water Quality Standards Regulation provides additional authority for this effort. In accordance with the FY 1991 Agency Operating Guidance, States and qualified Indian tribes are to adopt narrative biological criteria into State water quality standards during the FY 1991-1993 triennium. To support this effort, EPA is developing a Policy on the Use of Biological Assessments and Criteria in the Water Quality Program and providing this program guidance document on biological criteria.

Like other water quality criteria, biological criteria identify water quality impairments, support regulatory controls that address water quality problems, and assess improvements in water quality from regulatory efforts. Biological criteria are numerical values or narrative expressions that describe the reference biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use. They are developed through the direct measurement of aquatic community components inhabiting unimpaired surface waters.

Biological criteria complement current programs. Of the three objectives identified in the Act (chemical, physical, and biological integrity), current water quality programs focus on direct measures of...
chemical integrity (chemical-specific and whole-effluent toxicity) and, to some degree, physical integrity through several conventional criteria (e.g., pH, turbidity, dissolved oxygen). Implementation of these programs has significantly improved water quality. However, as we learn more about aquatic ecosystems it is apparent that other sources of waterbody impairment exist. Biological impairments from diffuse sources and habitat degradation can be greater than those caused by point source discharges (Judy et al. 1987; Miller et al. 1989). In Ohio, evaluation of instream biota indicated that 36 percent of impaired stream segments could not be detected using chemical criteria alone (see Fig. 1). Although effective for their purpose, chemical-specific criteria and whole-effluent toxicity provide only indirect evaluations and protection of biological integrity (see Table 1).

To effectively address our remaining water quality problems we need to develop more integrated and comprehensive evaluations. Chemical and physical integrity are necessary, but not sufficient conditions to attain biological integrity, and only when chemical, physical, and biological integrity are achieved, is ecological integrity possible (see Fig. 2). Biological criteria provide an essential third element for water quality management and serve as a natural progression in regulatory programs. Incorporating biological criteria into a fully integrated program directly protects the biological integrity of surface waters and provides indirect protection for chemical and physical integrity (see Table 2). Chemical-specific criteria, whole-effluent toxicity evaluations, and biological criteria, when used together, complement the relative strengths and weaknesses of each approach.

Table 1.—Current Water Quality Program Protection of the Three Elements of Ecological Integrity.

<table>
<thead>
<tr>
<th>ELEMENTS OF ECOLOGICAL INTEGRITY</th>
<th>PROGRAM THAT DIRECTLY PROTECTS</th>
<th>PROGRAM THAT INDIRECTLY PROTECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Integrity</td>
<td>Chemical Specific Criteria (toxics)</td>
<td>Whole Effluent Toxicity (toxics)</td>
</tr>
<tr>
<td>Physical Integrity</td>
<td>Criteria for Conventionals (pH, DO, turbidity)</td>
<td></td>
</tr>
<tr>
<td>Biological Integrity</td>
<td></td>
<td>Chemical/Whole Effluent Toxicity (biotic response in lab)</td>
</tr>
</tbody>
</table>

Table 1: Current programs focus on chemical specific and whole-effluent toxicity evaluations. Both are valuable approaches for the direct evaluation and protection of chemical integrity. Physical integrity is also directly protected to a limited degree through criteria for conventional pollutants. Biological integrity is only indirectly protected under the assumption that by evaluating toxicity to organisms in laboratory studies, estimates can be made about the toxicity to other organisms inhabiting ambient waters.
Table 2.—Water Quality Programs that Incorporate Biological Criteria to Protect Elements of Ecological Integrity.

<table>
<thead>
<tr>
<th>ELEMENTS OF ECOCLOGICAL INTEGRITY</th>
<th>DIRECTLY PROTECTS</th>
<th>INDIRECTLY PROTECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Integrity</td>
<td>Chemical Specific Criteria (toxics)</td>
<td>Biocriteria (identification of impairment)</td>
</tr>
<tr>
<td></td>
<td>Whole Effluent Toxicity (toxics)</td>
<td></td>
</tr>
<tr>
<td>Physical Integrity</td>
<td>Criteria for conventinals (pH, temp., DO)</td>
<td>Biocriteria (habitat evaluation)</td>
</tr>
<tr>
<td>Biological Integrity</td>
<td>Biocriteria (biotic response in surface water)</td>
<td>Chemical/Whole Effluent Testing (biotic response in lab)</td>
</tr>
</tbody>
</table>

Table 2: When biological criteria are incorporated into water quality programs the biological integrity of surface waters may be directly evaluated and protected. Biological criteria also provide additional benefits by requiring an evaluation of physical integrity and providing a monitoring tool to assess the effectiveness of current chemically based criteria.

Figure 2.—The Elements of Ecological Integrity

![Figure 2](image)

Fig. 2: Ecological Integrity is attainable when chemical, physical, and biological integrity occur simultaneously.

Value of Biological Criteria

Biological criteria provide an effective tool for addressing remaining water quality problems by directing regulatory efforts toward assessing the biological resources at risk from chemical, physical or biological impacts. A primary strength of biological criteria is the detection of water quality problems that other methods may miss or underestimate. Biological criteria can be used to determine to what extent current regulations are protecting the use.

Biological assessments provide integrated evaluations of water quality. They can identify impairments from contamination of the water column and sediments from unknown or unregulated chemicals, non-chemical impacts, and altered physical habitat. Resident biota function as continual monitors of environmental quality, increasing the likelihood of detecting the effects of episodic events (e.g., spills, dumping, treatment plant malfunctions, nutrient enrichment), toxic nonpoint source pollution (e.g., agricultural pesticides), cumulative pollution (i.e., multiple impacts over time or continuous low-level stress), or other impacts that periodic chemical sampling is unlikely to detect. Impacts on the physical habitat such as sedimentation from stormwater runoff and the effects of physical or structural habitat alterations (e.g., dredging, filling, channelization) can also be detected.

Biological criteria require the direct measure of resident aquatic community structure and function to determine biological integrity and ecological function. Using these measures, impairment can be detected and evaluated without knowing the impact(s) that may cause the impairment.

Biological criteria provide a regulatory framework for addressing water quality problems and offer additional benefits, including providing:

- the basis for characterizing high quality waters and identifying habitats and community components requiring special protection under State anti-degradation policies;
- a framework for deciding 319 actions for best control of nonpoint source pollution;
- an evaluation of surface water impairments predicted by chemical analyses, toxicity
testing, and fate and transport modeling (e.g., wasteload allocation);

- improvements in water quality standards (including refinement of use classifications);
- a process for demonstrating improvements in water quality after implementation of pollution controls;
- additional diagnostic tools.

The role of biological criteria as a regulatory tool is being realized in some States (e.g., Arkansas, Maine, Ohio, North Carolina, Vermont). Biological assessments and criteria have been useful for regulatory, resource protection, and monitoring and reporting programs. By incorporating biological criteria in programs, States can improve standards setting and enforcement, measure impairments from permit violations, and refine wasteload allocation models. In addition, the location, extent, and type of biological impairments measured in a waterbody provide valuable information needed for identifying the cause of impairment and determining actions required to improve water quality. Biological assessment and criteria programs provide a cost-effective method for evaluating water quality when a standardized, systematic approach to study design, field methods, and data analysis is established (Ohio EPA 1988a).

### Process for Implementation

The implementation of biological criteria will follow the same process used for current chemical-

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>EPA GUIDANCE</th>
<th>STATE IMPLEMENTATION</th>
<th>STATE APPLICATION</th>
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</thead>
<tbody>
<tr>
<td>Chemical Specific</td>
<td>Pollutant specific numeric criteria</td>
<td>State Standards</td>
<td>Permit limits Monitoring</td>
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<td></td>
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<td>use designation</td>
<td>Best Management Practices</td>
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<tr>
<td></td>
<td></td>
<td>numeric criteria</td>
<td>Wasteload allocation</td>
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<td></td>
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<td>antidegradation</td>
<td></td>
</tr>
<tr>
<td>Narrative Free Forms</td>
<td>Whole effluent toxicity guidance</td>
<td>Water Quality Narrative</td>
<td>Permit limits Monitoring</td>
</tr>
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<td></td>
<td></td>
<td>no toxic amounts translator</td>
<td>Wasteload allocation</td>
</tr>
<tr>
<td>Biological</td>
<td>Biosurvey minimum requirement guidance</td>
<td>State Standards</td>
<td>Permit conditions Monitoring</td>
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<td>Wasteload allocation</td>
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<tr>
<td></td>
<td></td>
<td>narrative/numeric criteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>antidegradation</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Similar to chemical specific criteria and whole effluent toxicity evaluations, EPA is providing guidance to States for the adoption of biological criteria into State standards to regulate sources of water quality impairment.
and training for State personnel. In Phase II, States are to develop plans necessary to implement biological criteria for each surface water type.

- Phase III requires full implementation and integration of biological criteria in water quality standards. This requires using biological surveys to derive biological criteria for classes of surface waters and designated uses. These criteria are then used to identify nonattainment of designated uses and make regulatory decisions.

Narrative biological criteria can be developed for all five surface water classifications with little or no data collection. Application of narrative criteria in seriously degraded waters is possible in the short term. However, because of the diversity of surface waters and the biota that inhabit these waters, significant planning, data collection, and evaluation will be needed to fully implement the program. Criteria for each type of surface water are likely to be developed at different rates. The order and rate of development will depend, in part, on the development of EPA guidance for specific types of surface water. Biological criteria technical guidance for streams will be produced during FY 1991. The tentative order for future technical guidance documents includes guidance for rivers (FY 1992), lakes (FY 1993), wetlands (FY 1994) and estuaries (FY 1995). This order and timeline for guidance does not reflect the relative importance of these surface waters, but rather indicates the relative availability of research and the anticipated difficulty of developing guidance.

Independent Application of Biological Criteria

Biological criteria supplement, but do not replace, chemical and toxicological methods. Water chemistry methods are necessary to predict risks (particularly to human health and wildlife), and to diagnose, model, and regulate important water quality problems. Because biological criteria are able to detect different types of water quality impairments and, in particular, have different levels of sensitivity for detecting certain types of impairment compared to toxicological methods, they are not used in lieu of, or in conflict with, current regulatory efforts.

As with all criteria, certain limitations to biological criteria make independent application essential. Study design and use influences how sensitive biological criteria are for detecting community impairment. Several factors influence sensitivity: (1) State decisions about what is significantly different between reference and test communities, (2) study design, which may include community components that are not sensitive to the impact causing impairment, (3) high natural variability that makes it difficult to detect real differences, and (4) types of impacts that may be detectable sooner by other methods (e.g., chemical criteria may provide earlier indications of impairment from a bioaccumulative chemical because aquatic communities require exposure over time to incur the full effect).

Since each type of criteria (biological criteria, chemical-specific criteria, or whole-effluent toxicity evaluations) has different sensitivities and purposes, a criterion may fail to detect real impairments when used alone. As a result, these methods should be used together in an integrated water quality assessment, each providing an independent evaluation of nonattainment of a designated use. If any one type of criteria indicates impairment of the surface water, regulatory action can be taken to improve water quality. However, no one type of criteria can be used to confirm attainment of a use if another form of criteria indicates nonattainment (see Hypothesis Testing: Biological Criteria and the Scientific Method, Chapter 7). When these three methods are used together, they provide a powerful, integrated, and effective foundation for waterbody management and regulations.

How to Use this Document

The purpose of this document is to provide EPA Regions, States and others with the conceptual framework and assistance necessary to develop and implement narrative and numeric biological criteria and to promote national consistency in application. There are two main parts of the document. Part One (Chapters 1, 2, 3, and 4) includes the essential concepts about what biological criteria are
and how they are used in regulatory programs. Part Two (Chapters 5, 6, and 7) provides an overview of the process that is essential for implementing a State biological criteria program. Specific chapters include the following:

Part I: PROGRAM ELEMENTS

- Chapter 2, Legal Authority, reviews the legal basis for biological criteria under the Clean Water Act and includes possible applications under the Act and other legislation.

- Chapter 3, Conceptual Framework, discusses the essential program elements for biological criteria, including what they are and how they are developed and used within a regulatory program. The development of narrative biological criteria is discussed in this chapter.

- Chapter 4, Integration, discusses the use of biological criteria in regulatory programs.

Part II: THE IMPLEMENTATION PROCESS

- Chapter 5, The Reference Condition, provides a discussion on alternative forms of reference conditions that may be developed by a State based on circumstances and needs.

- Chapter 6, The Biological Survey, provides some detail on the elements of a quality biological survey.

- Chapter 7, Hypothesis Testing: Biological Criteria and the Scientific Method, discusses how biological surveys are used to make regulatory and diagnostic decisions.

- Appendix A includes commonly asked questions and their answers about biological criteria.

Two additional documents are planned in the near term to supplement this program guidance document.

1. "Biological Criteria Technical Reference Guide" will contain a cross reference of technical papers on available approaches and methods for developing biological criteria (see tentative table of contents in Appendix B).

2. "Biological Criteria Development by States" will provide a summary of different mechanisms several States have used to implement and apply biological criteria in water quality programs (see tentative outline in Appendix C).

Both documents are planned for FY 1991. As previously discussed, over the next triennium technical guidance for specific systems (e.g., streams, wetlands) will be developed to provide guidance on acceptable biological assessment procedures to further support State implementation of comprehensive programs.

This biological criteria program guidance document supports development and implementation of biological criteria by providing guidance to States working to comply with requirements under the Clean Water Act and the Water Quality Standards Regulation. This guidance is not regulatory.
Legal Authority


The general authority for biological criteria comes from Section 101(a) of the Act which establishes as the objective of the Act the restoration and maintenance of the chemical, physical, and biological integrity of the Nation's waters. To meet this objective, water quality criteria must include criteria to protect biological integrity. Section 101(a)(2) includes the interim water quality goal for the protection and propagation of fish, shellfish, and wildlife. Propagation includes the full range of biological conditions necessary to support reproducing populations of all forms of aquatic life and other life that depend on aquatic systems. Sections 303 and 304 provide specific directives for the development of biological criteria.

Section 303

Under Section 303(c) of the Act, States are required to adopt protective water quality standards that consist of uses, criteria, and antidegradation. States are to review these standards every three years and to revise them as needed.

Section 303(c)(2)(A) requires the adoption of water quality standards that "serve the purposes of the Act," as given in Section 101. Section 303(c)(2)(B), enacted in 1987, requires States to adopt numeric criteria for toxic pollutants for which EPA has published 304(a)(1) criteria. The section further requires that, where numeric 304(a) criteria are not available, States should adopt criteria based on biological assessment and monitoring methods, consistent with information published by EPA under 304(a)(8).

These specific directives do not serve to restrict the use of biological criteria in other settings where they may be helpful. Accordingly, this guidance document provides assistance in implementing various sections of the Act, not just 303(c)(2)(B).
Section 304

Section 304(a) directs EPA to develop and publish water quality criteria and information on methods for measuring water quality and establishing water quality criteria for toxic pollutants on bases other than pollutant-by-pollutant, including biological monitoring and assessment methods which assess:

- the effects of pollutants on aquatic community components ("... plankton, fish, shellfish, wildlife, plant life ...") and community attributes ("... biological community diversity, productivity, and stability ...") in any body of water and;
- factors necessary "... to restore and maintain the chemical, physical, and biological integrity of all navigable waters ..." for "... the protection of shellfish, fish, and wildlife for classes and categories of receiving waters ...".

Potential Applications Under the Act

Development and use of biological criteria will help States to meet other requirements of the Act, including:

- lists of waters that cannot attain designated uses without nonpoint source controls [Sec. 319];
- development of management plans and conducting monitoring in estuaries of national significance [Sec. 320];
- issuing permits for ocean discharges and monitoring ecological effects [Sec. 403(c) and 301(h)(3)];
- determination of acceptable sites for disposal of dredge and fill material [Sec. 404];

Potential Applications Under Other Legislation

Several legislative acts require an assessment of risk to the environment (including resident aquatic communities) to determine the need for regulatory action. Biological criteria can be used in this context to support EPA assessments under:

- Toxic Substances Control Act (TSCA) of 1976
- Resource Conservation and Recovery Act (RCRA),
- Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA),
- Superfund Amendments and Reauthorization Act of 1986 (SARA),
- Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA);
- National Environmental Policy Act (NEPA);
- Federal Lands Policy and Management Act (FLPMA).
- The Fish and Wildlife Conservation Act of 1960
- Marine Protection, Research, and Sanctuaries Act
- Coastal Zone Management Act
Wild and Scenic Rivers Act

Fish and Wildlife Coordination Act, as Amended in 1965

A summary of the applicability of these Acts for assessing ecological impairments may be found in Risk Assessment Guidance for Superfund-Environmental Evaluation Manual (Interim Final) 1989.

Other federal and State agencies can also benefit from using biological criteria to evaluate the biological integrity of surface waters within their jurisdiction and to the effects of specific practices on surface water quality. Agencies that could benefit include:


- Department of Commerce (National Oceanic and Atmospheric Administration, National Marine Fisheries Service),

- Department of Transportation (Federal Highway Administration)

- Department of Agriculture (U.S. Forest Service, Soil Conservation Service)

- Department of Defense,

- Department of Energy,

- Army Corps of Engineers,

- Tennessee Valley Authority.
The Conceptual Framework

Biological integrity and the determination of use impairment through assessment of ambient biological communities form the foundation for biological criteria development. The effectiveness of a biological criteria program will depend on the development of quality criteria, the refinement of use classes to support narrative criteria, and careful application of scientific principles.

Premise for Biological Criteria

Biological criteria are based on the premise that the structure and function of an aquatic biological community within a specific habitat provide critical information about the quality of surface waters. Existing aquatic communities in pristine environments not subject to anthropogenic impact exemplify biological integrity and serve as the best possible goal for water quality. Although pristine environments are virtually non-existent (even remote waters are impacted by air pollution), minimally impacted waters exist. Measures of the structure and function of aquatic communities inhabiting unimpared (minimally impacted) waters provide the basis for establishing a reference condition that may be compared to the condition of impacted surface waters to determine impairment.

Based on this premise, biological criteria are developed under the assumptions that: (1) surface waters subject to anthropogenic disturbance may contain impaired populations or communities of aquatic organisms—the greater the anthropogenic

Aquatic communities assessed in unimpaired waterbodies (top) provide a reference for evaluating impairments in the same or similar waterbodies suffering from increasing anthropogenic impacts (bottom).
disruption, the greater the likelihood and magnitude of impairment; and (2) surface waters not subject to anthropogenic disturbance generally contain unimpaired (natural) populations and communities of aquatic organisms exhibiting biological integrity.

Biological Integrity

The expression "biological integrity" is used in the Clean Water Act to define the Nation's objectives for water quality. According to Webster's New World Dictionary (1966), integrity is, "the quality or state of being complete; unimpaired." Biological integrity has been defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitats within a region" (Karr and Dudley 1981). For the purposes of biological criteria, these concepts are combined to develop a functional definition for evaluating biological integrity in water quality programs. Thus, biological integrity is functionally defined as:

the condition of the aquatic community inhabiting the unimpaired waterbodies of a specified habitat as measured by community structure and function.

It will often be difficult to find unimpaired waters to define biological integrity and establish the reference condition. However, the structure and function of aquatic communities of high quality waters can be approximated in several ways. One is to characterize aquatic communities in the most protected waters representative of the regions where such sites exist. In areas where few or no unimpaired sites are available, characterization of least impaired systems approximates unimpaired systems. Concurrent analysis of historical records should supplement descriptions of the condition of least impaired systems. For some systems, such as lakes, evaluating paleoecological information (the record stored in sediment profiles) can provide a measure of less disturbed conditions.

Surface waters, when inhabited by aquatic communities, are exhibiting a degree of biological integrity. However, the best representation of biological integrity for a surface water should form the basis for establishing water quality goals for those waters. When tied to the development of biological criteria, the realities of limitations on biological integrity can be considered and incorporated into a progressive program to improve water quality.

Biological Criteria

Biological criteria are narrative expressions or numerical values that describe the biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use. While biological integrity describes the ultimate goal for water quality, biological criteria are based on aquatic community structure and function for waters within a variety of designated uses. Designated aquatic life uses serve as general statements of attained or attainable uses of State waters. Once established for a designated use, biological criteria are quantifiable values used to determine whether a use is impaired, and if so, the level of impairment. This is done by specifying what aquatic community structure and function should exist in waters of a given designated use, and then comparing this condition with the condition of a site under evaluation. If the existing aquatic community measures fail to meet the criteria, the use is considered impaired.

Since biological surveys used for biological criteria are capable of detecting water quality problems (use impairments) that may not be detected by chemical or toxicity testing, violation of biological criteria is sufficient cause for States to initiate regulatory action. Corroborating chemical and toxicity testing data are not required (though they may be desirable) as supporting evidence to sustain a determination of use impairment. However, a finding that biological criteria fail to indicate use impairment does not mean the use is automatically attained. Other evidence, such as violation of physical or chemical criteria, or results from toxicity tests, can also be used to identify impairment. Alternative forms of criteria provide independent assessments of nonattainment.

As stated above, biological criteria may be narrative statements or numerical values. States can establish general narrative biological criteria early in program development without conducting biological assessments. Once established in State standards, narrative biological criteria form the legal and
programmatic basis for expanding biological assessment and biosurvey programs needed to implement narrative criteria and develop numeric biological criteria. Narrative biological criteria should become part of State regulations and standards.

**Narrative Criteria**

Narrative biological criteria are general statements of attainable or attained conditions of biological integrity and water quality for a given use designation. Although similar to the "free from" chemical water quality criteria, narrative biological criteria establish a positive statement about what should occur within a water body. Narrative criteria can take a number of forms but they must contain several attributes to support the goals of the Clean Water Act to provide for the protection and propagation of fish, shellfish, and wildlife. Thus, narrative criteria should include specific language about aquatic community characteristics that (1) must exist in a waterbody to meet a particular designated aquatic life use, and (2) are quantifiable. They must be written to protect the use. Supporting statements for the criteria should promote water quality to protect the most natural community possible for the designated use. Mechanisms should be established in the standard to address potentially conflicting multiple uses. Narratives should be written to protect the most sensitive use and support antidegradation.

Several States currently use narrative criteria. In Maine, for example, narrative criteria were established for four classes of water quality for streams and rivers (see Table 4). The classifications were based on the range of goals in the Act from "no discharge" to "protection and propagation of fish, shellfish, and wildlife" (Courtemanch and Davies 1987). Maine separated its "high quality water" into two categories, one that reflects the highest goal of the Act (no discharge, Class AA) and one that reflects high integrity but is minimally impacted by human activity (Class A). The statement "The aquatic life . . . shall be as naturally occurs" is a narrative biological criterion for both Class AA and A waters. Waters in Class B meet the use when the life stages of all indigenous aquatic species are supported and no detrimental changes occur in community composition (Maine DEP 1986). These criteria directly support refined designated aquatic life uses (see Section D, Refining Aquatic Life Use Classifications).

These narrative criteria are effective only if, as Maine has done, simple phrases such as "as naturally occurs" and "nondetrimental" are clearly operationally defined. Rules for sampling procedures and data analysis and interpretation should become part of the regulation or supporting documentation. Maine was able to develop these criteria and their supporting statements using avail-

<table>
<thead>
<tr>
<th>RIVERS AND STREAMS</th>
<th>MANAGEMENT PERSPECTIVE</th>
<th>LEVEL OF BIOLOGICAL INTEGRITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class AA</td>
<td>High quality water for preservation of recreational and ecological interests. No discharges of any kind permitted. No impoundment permitted.</td>
<td>Aquatic life shall be as naturally occurs.</td>
</tr>
<tr>
<td>Class A</td>
<td>High quality water with limited human interference. Discharges restricted to noncontact process water or highly treated wastewater of quality equal to or better than the receiving water. Impoundment permitted.</td>
<td>Aquatic life shall be as naturally occurs.</td>
</tr>
<tr>
<td>Class B</td>
<td>Good quality water. Discharges of well treated effluents with ample dilution permitted.</td>
<td>Ambient water quality sufficient to support life stages of all indigenous aquatic species. Only nondetrimental changes in community composition may occur.</td>
</tr>
<tr>
<td>Class C</td>
<td>Lowest quality water. Requirements consistent with interim goals of the federal Water Quality Law (fishable and swimmable).</td>
<td>Ambient water quality sufficient to support the life stages of all indigenous fish species. Changes in species composition may occur but structure and function of the aquatic community must be maintained.</td>
</tr>
</tbody>
</table>

Table 4.—Aquatic Life Classification Scheme for Maine's Rivers and Streams.
able data from water quality programs. To implement the criteria, aquatic life inhabiting unimpaired waters must be measured to quantify the criteria statement.

Narrative criteria can take more specific forms than illustrated in the Maine example. Narrative criteria may include specific classes and species of organisms that will occur in waters for a given designated use. To develop these narratives, field evaluations of reference conditions are necessary to identify biological community attributes that differ significantly between designated uses. For example in the Arkansas use class Typical Gulf Coastal Ecoregion (i.e., South Central Plains) the narrative criterion reads:

"Streams supporting diverse communities of indigenous or adapted species of fish and other forms of aquatic life. Fish communities are characterized by a limited proportion of sensitive species; sunfishes are distinctly dominant, followed by darters and minnows. The community may be generally characterized by the following fishes: Key Species—Redfin shiner, Spotted sucker, Yellow bullhead, Flier, Slough darter, Grass pickerel; Indicator Species—Pirate perch, Warmouth, Spotted sunfish, Dusky darter, Creek chubsucker, Banded pygmy sunfish (Arkansas DPCE 1988).

In Connecticut, current designated uses are supported by narratives in the standard. For example, under Surface Water Classifications, Inland Surface Waters Class AA, the Designated Use is: "Existing or proposed drinking water supply; fish and wildlife habitat; recreational use; agricultural, industrial supply, and other purposes (recreation uses may be restricted)."

The supporting narratives include:

*Benthic invertebrates which inhabit lotic waters: A wide variety of macroinvertebrate taxa should normally be present and all functional groups should normally be well represented... Water quality shall be sufficient to sustain a diverse macroinvertebrate community of Indigenous species. Taxa within the Orders Plecoptera (stoneflies), Ephemeroptera (mayflies), Coleoptera (beetles), Tricoptera (caddisflies) should be well represented (Connecticut DEP 1987).

For these narratives to be effective in a biological criteria program expressions such as "a wide variety" and "functional groups should normally be well represented" require quantifiable definitions that become part of the standard or supporting documentation. Many States may find such narratives in their standards already. If so, States should evaluate current language to determine if it meets the requirements of quantifiable narrative criteria that support refined aquatic life uses.

Narrative biological criteria are similar to the traditional narrative "free froms" by providing the legal basis for standards applications. A sixth "free from" could be incorporated into standards to help support narrative biological criteria such as "free from activities that would impair the aquatic community as it naturally occurs." Narrative biological criteria can be used immediately to address obvious existing problems.

**Numeric Criteria**

Numerical indices that serve as biological criteria should describe expected attainable community attributes for different designated uses. It is important to note that full implementation of narrative criteria will require similar data as that needed for developing numeric criteria. At this time, States may or may not choose to establish numeric criteria but may find it an effective tool for regulatory use.

To derive a numeric criterion, an aquatic community's structure and function is measured at reference sites and set as a reference condition. Examples of relative measures include similarity indices, coefficients of community loss, and comparisons of lists of dominant taxa. Measures of existing community structure such as species richness, presence or absence of indicator taxa, and distribution of trophic feeding groups are useful for establishing the normal range of community components to be expected in unimpaired systems. For example, Ohio uses criteria for the warmwater habitat use class based on multiple measures in different reference sites within the same ecoregion. Criteria are set as the 25th percentile of all biological index scores recorded at established reference
sites within the ecoregion. Exceptional warmwater habitat index criteria are set at the 75th percentile (Ohio EPA 1988a). Applications such as this require an extensive data base and multiple reference sites for each criteria value.

To develop numeric biological criteria, careful assessments of biota in reference sites must be conducted (Hughes et al. 1988). There are numerous ways to assess community structure and function in surface waters. No single index or measure is universally recognized as free from bias. It is important to evaluate the strengths and weaknesses of different assessment approaches. A multi-metric approach that incorporates information on species richness, trophic composition, abundance or biomass, and organism condition is recommended. Evaluations that measure multiple components of communities are also recommended because they tend to be more reliable (e.g., measures of fish and macroinvertebrates combined will provide more information than measures of fish communities alone). The weaknesses of one measure or index can often be compensated by combining it with the strengths of other community measurements.

The particular indices used to develop numeric criteria depend on the type of surface waters (streams, rivers, lakes, Great Lakes, estuaries, wetlands, and nearshore marine) to which they must be applied. In general, community-level indices such as the Index of Biotic Integrity developed for mid-western streams (Karr et al. 1986) are more easily interpreted and less variable than fluctuating numbers such as population size. Future EPA technical guidance documents will include evaluations of the effectiveness of different biological survey and assessment approaches for measuring the biological integrity of surface water types and provide guidance on acceptable approaches for biological criteria development.

Refining Aquatic Life Use Classifications

State standards consist of (1) designated aquatic life uses, (2) criteria sufficient to protect the designated and existing use, and (3) an antidegradation clause. Biological criteria support designated aquatic life use classifications for application in State standards. Each State develops its own designated use classification system based on the generic uses cited in the Act (e.g., protection and propagation of fish, shellfish, and wildlife). Designated uses are intentionally general. However, States may develop subcategories within use designations to refine and clarify the use class. Clarification of the use class is particularly helpful when a variety of surface waters with distinct characteristics fit within the same use class, or do not fit well into any category. Determination of nonattainment in these waters may be difficult and open to alternative interpretations. If a determination is in dispute, regulatory actions will be difficult to accomplish. Emphasizing aquatic community structure within the designated use focuses the evaluation of attainment/nonattainment on the resource of concern under the Act.

Flexibility inherent in the State process for designating uses allows the development of subcategories of uses within the Act's general categories. For example, subcategories of aquatic life uses may be on the basis of attainable habitat (e.g., cold versus warmwater habitat); innate differences in community structure and function (e.g., high versus low species richness or productivity); or fundamental differences in important community components (e.g., warmwater fish communities dominated by bass versus catfish). Special uses may also be designated to protect particularly unique, sensitive, or valuable aquatic species, communities, or habitats.

Refinement of use classes can be accomplished within current State use classification structures. Data collected from biosurveys as part of a developing biocriteria program may reveal unique and consistent differences among aquatic communities inhabiting different waters with the same designated use. Measurable biological attributes could then be used to separate one class into two or more classes. The result is a refined aquatic life use. For example, in Arkansas the beneficial use Fisheries "provides for the protection and propagation of fish, shellfish, and other forms of aquatic life" (Arkansas DPCE 1988). This use is subdivided into Trout, Lakes and Reservoirs, and Streams. Recognizing that stream characteristics across regions of the State differed ecologically, the State further subdivided the stream designated uses into eight additional uses based on regional characteristics (e.g., Springwater-influenced Gulf Coastal Ecoregion, Ouachita Mountains Ecotogion). Within this classification system, it was relatively straightforward for
Arkansas to establish detailed narrative biological criteria that list aquatic community components expected in each ecoregion (see Narrative Criteria section). These narrative criteria can then be used to establish whether the use is impaired.

States can refine very general designated uses such as high, medium, and low quality to specific categories that include measurable ecological characteristics. In Maine, for example, Class AA waters are defined as "the highest classification and shall be applied to waters which are outstanding natural resources and which should be preserved because of their ecological, social, scenic, or recreational importance." The designated use includes "Class AA waters shall be of such quality that they are suitable . . . as habitat for fish and other aquatic life. The habitat shall be characterized as free flowing and natural." This use supports development of narrative criteria based on biological characteristics of aquatic communities (Maine DEP 1986; see the Narrative Criteria section).

Biological criteria that include lists of dominant or typical species expected to live in the surface water are particularly effective. Descriptions of impaired conditions are more difficult to interpret. However, biological criteria may contain statements concerning which species dominate disturbed sites, as well as those species expected at minimally impacted sites.

Most States collect biological data in current programs. Refining aquatic life use classifications and incorporating biological criteria into standards will enable States to evaluate these data more effectively.

### Developing and Implementing Biological Criteria

Biological criteria development and implementation in standards require an understanding of the selection and evaluation of reference sites, measurement of aquatic community structure and function, and hypothesis testing under the scientific method. The developmental process is important for State water quality managers and their staff to understand to promote effective planning for resource and staff needs. This major program element deserves careful consideration and has been separated out in Part II by chapter for each developmental step as noted below. Additional guidance will be provided in future technical guidance documents.

The developmental process is illustrated in Figure 3. The first step is establishing narrative criteria in standards. However, to support these narratives, standardized protocols need to be developed to quantify the narratives for criteria implementation. They should include data collection procedures, selection of reference sites, quality assurance and quality control procedures, hypothesis testing, and statistical protocols. Pilot studies should be conducted using these standard protocols to ensure they meet the needs of the program, test the hypotheses, and provide effective measures of the biological integrity of surface waters in the State.

**Figure 3.—Process for the Development and Implementation of Biological Criteria**

Develop Standard Protocols  
(Test protocol sensitivity)

- Identify and Conduct Biosurveys at Unimpaired Reference Sites
- Establish Biological Criteria
- Conduct Biosurveys at Impacted Sites  
(Determine impairment)

If impairment is found, diagnosis of cause will lead to the implementation of a control. Continued monitoring should accompany control implementation to determine the effectiveness of intervention. Monitoring is also recommended where no impairment is found to ensure that the surface water maintains or improves in quality.
The next step is establishing the reference condition for the surface water being tested. This reference may be site specific or regional but must establish the unimpaired baseline for comparison (see Chapter 5, The Reference Condition). Once reference sites are selected, the biological integrity of the site must be evaluated using carefully chosen biological surveys. A quality biological survey will include multiple community components and may be measured using a variety of metrics (see Chapter 6, The Biological Survey). Establishing the reference condition and conducting biological surveys at the reference locations provide the necessary information for establishing the biological criteria.

To apply biological criteria, impacted surface waters with comparable habitat characteristics are evaluated using the same procedures as those used to establish the criteria. The biological survey must support standardized sampling methods and statistical protocols that are sensitive enough to identify biologically relevant differences between established criteria and the community under evaluation. Resulting data are compared through hypothesis testing to determine impairment (see Chapter 7, Hypothesis Testing).

When water quality impairments are detected using biological criteria, they can only be applied in a regulatory setting if the cause for impairment can be identified. Diagnosis is iterative and investigative (see Chapter 7, Diagnosis). States must then determine appropriate actions to implement controls. Monitoring should remain a part of the biological criteria program whether impairments are found or not. If an impairment exists, monitoring provides a mechanism to determine if the control effort (intervention) is resulting in improved water quality. If there is no impairment, monitoring ensures the water quality is maintained and documents any improvements. When improvements in water quality are detected through monitoring programs two actions are recommended. When reference condition waters improve, biological criteria values should be recalculated to reflect this higher level of integrity. When impaired surface waters improve, states should reclassify those waters to reflect a refined designated use with a higher level of biological integrity. This provides a mechanism for progressive water quality improvement.
Integrating biological criteria into existing water quality programs will help to assess use attainment/nonattainment, improve problem discovery in specific waterbodies, and characterize overall water resource condition within a region. Ideally, biological criteria function in an iterative manner. New biosurvey information can be used to refine use classes. Refined use classes will help support criteria development and improve the value of data collected in biosurveys.

Implementing Biological Criteria

As biological survey data are collected, these data will increasingly support current use of biomonitoring data to identify water quality problems, assess their severity, and set planning and management priorities for remediation. Monitoring data and biological criteria should be used at the outset to help make regulatory decisions, develop appropriate controls, and evaluate the effectiveness of controls once they are implemented.

The value of incorporating biological survey information in regulatory programs is illustrated by evaluations conducted by North Carolina. In response to amendments of the Federal Water Pollution Control Act requiring secondary effluent limits for all wastewater treatment plants, North Carolina became embroiled in a debate over whether meeting secondary effluent limits (at considerable cost) would result in better water quality. North Carolina chose to test the effectiveness of additional treatment by conducting seven chemical and biological surveys before and after facility upgrades (North
Biological Criteria in State Programs

Biological criteria are used within water programs to refine use designations, establish criteria for determining use attainment/nonattainment, evaluate effectiveness of current water programs, and detect and characterize previously unknown impairments. Twenty States are currently using some form of standardized ambient biological assessments to determine the status of biota within State waters. Levels of effort vary from bioassessment studies to fully developed biological criteria programs.

Fifteen States are developing aspects of biological assessments that will support future development of biological criteria. Colorado, Illinois, Iowa, Kentucky, Massachusetts, Tennessee, and Virginia conduct biological monitoring to evaluate biological conditions, but are not developing biological criteria. Kansas is considering using a community metric for water resource assessment. Arizona is planning to refine ecoregions for the State. Delaware, Minnesota, Texas, and Wisconsin are developing sampling and evaluation methods to apply to future biological criteria programs. New York is proposing to use biological criteria for site-specific evaluations of water quality impairment. Nebraska and Vermont use informal biological criteria to support existing aquatic life narratives in their water quality standards and other regulations. Vermont recently passed a law requiring that biological criteria be used to regulate through permitting the indirect discharge of sanitary effluents.

Florida incorporated a specific biological criterion into State standards for invertebrate species diversity. Species diversity within a waterbody, as measured by a Shannon diversity index, may not fall below 75 percent of reference values. This criterion has been used in enforcement cases to obtain injunctions and monetary settlements. Florida's approach is very specific and limits alternative applications.

Four States—Arkansas, North Carolina, Maine, and Ohio—are currently using biological criteria to define aquatic life use classifications and enforce water quality standards. These states have made biological criteria an integral part of comprehensive water quality programs.

- Arkansas rewrote its aquatic life use classifications for each of the State's ecoregions. This has allowed many cities to design wastewater treatment plants to meet realistic attainable dissolved oxygen conditions as determined by the new criteria.

- North Carolina developed biological criteria to assess impairment to aquatic life uses written as narratives in the State water quality standards. Biological data and criteria are used extensively to identify waters of special concern or those with exceptional water quality. In addition to the High Quality Waters (HQW) and Outstanding Resource Waters (ORW) designations, Nutrient Sensitive Waters (NSW) at risk for eutrophication are assessed using biological
criteria. Although specific biological measures are not in the regulations, strengthened use of biological monitoring data to assess water quality is being proposed for incorporation in North Carolina's water quality standards.

Maine has enacted a revised Water Quality Classification Law specifically designed to facilitate the use of biological assessments. Each of four water classes contains descriptive aquatic life conditions necessary to attain that class. Based on a statewide database of macroinvertebrate samples collected above and below outfalls, Maine is now developing a set of dichotomous keys that serve as the biological criteria. Maine's program is not expected to have a significant role in permitting, but will be used to assess the degree of protection afforded by effluent limitations.

Ohio has instituted the most extensive use of biological criteria for defining use classifications and assessing water quality. Biological criteria were developed for Ohio rivers and streams using an ecoregional reference site approach. Within each of the State's five ecoregions, criteria for three biological indices (two for fish communities and one for macroinvertebrates) were derived. Ohio successfully uses biological criteria to demonstrate attainment of aquatic life uses and discover previously unknown or unidentified environmental degradation (e.g., twice as many impaired waters were discovered using biological criteria and water chemistry together than were found using chemistry alone). The upgraded use designations based on biological criteria were upheld in Ohio courts and the Ohio EPA successfully proposed their biological criteria for inclusion in the State water quality standards regulations.

States and EPA have learned a great deal about the effectiveness of integrated biological assessments through the development of biological criteria for freshwater streams. This information is particularly valuable in providing guidance on developing biological criteria for other surface water types. As previously discussed, EPA plans to produce supporting technical guidance for biological criteria development in streams and other surface waters. Production of these guidance documents will be contingent on technical progress made on each surface water type by researchers in EPA, States and the academic community.

EPA will also be developing outreach workshops to provide technical assistance to Regions and States working toward the implementation of biological criteria programs in State water quality management programs. In the interim, States should use the technical guidance currently available in the Technical Support Manual(s): Waterbody Surveys and Assessments for Conducting Use Attainability Analysis (U.S. EPA 1983b, 1984a,b).

During the next triennium, State effort will be focused on developing narrative biological criteria. Full implementation and integration of biological criteria will require several years. Using available guidance, States can complement the adoption of narrative criteria by developing implementation plans that include:

1. Defining program objectives, developing research protocols, and setting priorities;
2. Determining the process for establishing reference conditions, which includes developing a process to evaluate habitat characteristics;
3. Establishing biological survey protocols that include justifications for surface water classifications and selected aquatic community components to be evaluated; and
4. Developing a formal document describing the research design, quality assurance and quality control protocols, and required training for staff.

Whether a State begins with narrative biological criteria or moves to fully implement numeric criteria, the shift of the water quality program focus from source control to resource management represents a natural progression in the evolution from the technology-based to water quality-based approaches in water quality management. The addition of a biological perspective allows water quality programs to more directly address the objectives of the Clean Water Act and to place their efforts in a context that is more meaningful to the public.
Future Directions

Biological criteria now focus on resident aquatic communities in surface waters. They have the potential to expand in scope toward greater ecological integration. Ecological criteria may encompass the ambient aquatic communities in surface waters, wildlife species that use the same aquatic resources, and the aquatic community inhabiting the gravel and sediments underlying the surface waters and adjacent land (hyporheic zone); specific criteria may apply to physical habitat. These areas may represent only a few possible options for biological criteria in the future.

Many wildlife species depend on aquatic resources. If aquatic population levels decrease or if the distribution of species changes, food sources may be sufficiently altered to cause problems for wildlife species using aquatic resources. Habitat degradation that impairs aquatic species will often impact important wildlife habitat as well. These kinds of impairments are likely to be detected using biological criteria as currently formulated. In some cases, however, uptake of contaminants by resident aquatic organisms may not result in altered structure and function of the aquatic community. These impacts may go undetected by biological criteria, but could result in wildlife impairments because of bioaccumulation. Future expansion of biological criteria to include wildlife species that depend on aquatic resources could provide a more integrative ecosystem approach.

Rivers may have a subsurface flood plain extending as far as two kilometers from the river channel. Preliminary mass transport calculations made in the Flathead River basin in Montana indicate that nutrients discharged from this subsurface flood plain may be crucial to biotic productivity in the river channel (Stanford and Ward 1988). This is an unexplored dimension in the ecology of gravel river beds and potentially in other surface waters.

As discussed in Chapter 1, physical integrity is a necessary condition for biological integrity. Establishing the reference condition for biological criteria requires evaluation of habitat. The rapid bioassessment protocol provides a good example of the importance of habitat for interpreting biological assessments (Plafkin et al. 1989). However, it may be useful to more fully integrate habitat characteristics into the regulatory process by establishing criteria based on the necessary physical structure of habitats to support ecological integrity.
Part II

The Implementation Process
The implementation of biological criteria requires: (1) selection of unimpaired (minimal impact) surface waters to use as the reference condition for each designated use, (2) measurement of the structure and function of aquatic communities in reference surface waters to establish biological criteria, and (3) establishment of a protocol to compare the biological criteria to biota in impacted waters to determine whether impairment has occurred. These elements serve as an interactive network that is particularly important during early development of biological criteria where rapid accumulation of information is effective for refining both designated uses and developing biological criteria values. The following chapters describe these three essential elements.
A key step in developing values for supporting narrative and creating numeric biological criteria is to establish reference conditions; it is an essential feature of environmental impact evaluations (Green 1979). Reference conditions are critical for environmental assessments because standard experimental controls are rarely available. For most surface waters, baseline data were not collected prior to an impact, thus impairment must be inferred from differences between the impact site and established references. Reference conditions describe the characteristics of waterbody segments least impaired by human activities and are used to define attainable biological or habitat conditions.

Wide variability among natural surface waters across the country resulting from climatic, landform, and other geographic differences prevents the development of nationwide reference conditions. Most States are also too heterogeneous for single reference conditions. Thus, each State, and when appropriate, groups of States, will be responsible for selecting and evaluating reference waters within the State to establish biological criteria for a given surface water type or category of designated use. At least seven methods for estimating attainable conditions for streams have been identified (Hughes et al. 1986). Many of these can apply to other surface waters. References may be established by defining models of attainable conditions based on historical data or unimpaired habitat (e.g., streams in old growth forest). The reference condition established as before-after comparisons or concurrent measures of the reference water and impact sites can be based on empirical data (Hall et al. 1989).

Currently, two principal approaches are used for establishing the reference condition. A State may opt to (1) identify site-specific reference sites for each evaluation of impact or (2) select ecologically similar regional reference sites for comparison with impacted sites within the same region. Both approaches depend on evaluations of habitats to ensure that waters with similar habitats are compared. The designation of discrete habitat types is more fully developed for streams and rivers. Development of habitat types for lakes, wetlands, and estuaries is ongoing.
Site-Specific Reference Condition

A site-specific reference condition, frequently used to evaluate the impacts from a point discharge, is best for surface waters with a strong directional flow such as in streams and rivers (the upstream-downstream approach). However, it can also be used for other surface waters where gradients in contaminant concentration occur based on proximity to a source (the near field-far field approach). Establishment of a site-specific reference condition requires the availability of comparable habitat within the same waterbody in both the reference location and the impacted area.

A site-specific reference condition is difficult to establish if (1) diffuse nonpoint source pollution contaminates most of the water body; (2) modifications to the channel, shoreline, or bottom substrate are extensive; (3) point sources occur at multiple locations on the waterbody; or (4) habitat characteristics differ significantly between possible reference locations and the impact site (Hughes et al. 1986; Plafkin et al. 1989). In these cases, site-specific reference conditions could result in underestimates of impairment. Despite limitations, the use of site-specific reference conditions is often the method of choice for point source discharges and certain waterbodies, particularly when the relative impairments from different local impacts need to be determined.

The Upstream-Downstream Reference Condition

The upstream-downstream reference condition is best applied to streams and rivers where the habitat characteristics of the waterbody above the point of discharge are similar to the habitat characteristics of the stream below the point of discharge. One standard procedure is to characterize the biotic condition just above the discharge point (accounting for possible upstream circulation) to establish the reference condition. The condition below the discharge is also measured at several sites. If significant differences are found between these measures, impairment of the biota from the discharge is indicated. Since measurements of resident biota taken in any two sites are expected to differ because of natural variation, more than one biological assessment for both upstream and downstream sites is often needed to be confident in conclusions drawn from these data (Green, 1979). However, as more data are collected by a State, and particularly if regional characteristics of the waterbodies are incorporated, the basis for determining impairment from site-specific upstream-downstream assessments may require fewer individual samples. The same measures made below the "recovery zone" downstream from the discharge will help define where recovery occurs.

The upstream-downstream reference condition should be used with discretion since the reference condition may be impaired from impacts upstream from the point source of interest. In these cases it is important to discriminate between individual point source impact versus overall impairment of the system. When overall impairment occurs, the resident biota may be sufficiently impaired to make it impossible to detect the effect of the target point source discharger.

The approach can be cost effective when one biological assessment of the upstream reference condition adequately reflects the attainable condition of the impacted site. However, routine comparisons may require assessments of several upstream sites to adequately describe the natural variability of reference biota. Even so, measuring a series of site-specific references will likely continue to be the method of choice for certain point source discharges, especially where the relative impairments from different local impacts need to be determined.

The Near Field-Far Field Reference Condition

The near field-far field reference condition is effective for establishing a reference condition in surface waters other than rivers and streams and is particularly applicable for unique waterbodies (e.g., estuaries such as Puget Sound may not have comparable estuaries for comparison). To apply this method, two variables are measured (1) habitat characteristics, and (2) gradient of impairment. For reference waters to be identified within the same waterbody, sufficient size is necessary to separate the reference from the impact area so that a gradient of impact exists. At the same time, habitat characteristics must be comparable.
Although not fully developed, this approach may provide an effective way to establish biological criteria for estuaries, large lakes, or wetlands. For example, estuarine habitats could be defined and possible reference waters identified using physical and chemical variables like those selected by the Chesapeake Bay Program (U.S. EPA 1987a, e.g., substrate type, salinity, pH) to establish comparable subhabitats in an estuary. To determine those areas least impaired, a "mussel watch" program like that used in Narragansett Bay (i.e., captive mussels are used as indicators of contamination, (Phelps 1988)) could establish impairment gradients. These two measures, when combined, could form the basis for selecting specific habitat types in areas of least impairment to establish the reference condition.

Regional Reference Conditions

Some of the limitations of site-specific reference conditions can be overcome by using regional reference conditions that are based on the assumption that surface waters integrate the character of the land they drain. Waterbodies within the same watershed in the same region should be more similar to each other than to those within watersheds in different regions. Based on these assumptions, a distribution of aquatic regions can be developed based on ecological features that directly or indirectly relate to water quality and quantity, such as soil type, vegetation (land cover), land-surface form, climate, and land use. Maps that incorporate several of these features will provide a general purpose broad scale ecoregional framework (Gallant et al. 1989).

Regions of ecological similarity are based on hydrologic, climatic, geologic, or other relevant geographic variables that influence the nature of biota in surface waters. To establish a regional reference condition, surface waters of similar habitat type are identified in definable ecological regions. The biological integrity of these reference waters is determined to establish the reference condition and develop biological criteria. These criteria are then used to assess impacted surface waters in the same watershed or region. There are two forms of regional reference conditions: (1) paired watersheds and (2) ecoregions.

Paired Watershed Reference Conditions

Paired watershed reference conditions are established to evaluate impaired waterbodies, often impacted by multiple sources. When the majority of a waterbody is impaired, the upstream-downstream or near field-far field reference condition does not provide an adequate representation of the unimpaired condition of aquatic communities for the waterbody. Paired watershed reference conditions are established by identifying unimpaired surface waters within the same or very similar local watershed that is of comparable type and habitat. Variables to consider when selecting the watershed reference condition include absence of human disturbance, waterbody size and other physical characteristics, surrounding vegetation, and others as described in the "Regional Reference Site Selection" feature.

This method has been successfully applied (e.g., Hughes 1985) and is an approach used in Rapid Bioassessment Protocols (Plafkin et al. 1989). State use of this approach results in good reference conditions that can be used immediately in current programs. This approach has the added benefit of promoting the development of a database on high quality waters in the State that could form the foundation for establishing larger regional references (e.g., ecoregions.)

Ecoregional Reference Conditions

Reference conditions can also be developed on a larger scale. For these references, waterbodies of similar type are identified in regions of ecological similarity. To establish a regional reference condition, a set of surface waters of similar habitat type are identified in each ecological region. These sites must represent similar habitat type and be representative of the region. As with other reference conditions, the biological integrity of selected reference waters is determined to establish the reference. Biological criteria can then be developed and used to assess impacted surface waters in the same region. Before reference conditions may be established, regions of ecological similarity must be defined.
Regional Reference Site Selection

To determine specific regional reference sites for streams, candidate watersheds are selected from the appropriate maps and evaluated to determine if they are typical for the region. An evaluation of level of human disturbance is made and a number of relatively undisturbed reference sites are selected from the candidate sites. Generally, watersheds are chosen as regional reference sites when they fall entirely within typical areas of the region. Candidate sites are then selected by aerial and ground surveys. Identification of candidate sites is based on: (1) absence of human disturbance, (2) stream size, (3) type of stream channel, (4) location within a natural or political refuge, and (5) historical records of resident biota and possible migration barriers.

Final selection of reference sites depends on a determination of minimal disturbance derived from habitat evaluation made during site visits. For example, indicators of good quality streams in forested ecoregions include: (1) extensive, old, natural riparian vegetation; (2) relatively high heterogeneity in channel width and depth; (3) abundant large woody debris, coarse bottom substrate, or extensive aquatic or overhanging vegetation; (4) relatively high or constant discharge; (5) relatively clear waters with natural color and odor; (6) abundant diatom, insect, and fish assemblages; and (7) the presence of piscivorous birds and mammals.

One frequently used method is described by Omernik (1987) who combined maps of land-surface form, soil, potential natural vegetation, and land use within the conterminous United States to generate a map of aquatic ecoregions for the country. He also developed more detailed regional maps. The ecoregions defined by Omernik have been evaluated for streams and small rivers in Arkansas (Rohm et al. 1987), Ohio (Larsen et al. 1988; Whittier et al. 1987), Oregon (Whittier et al. 1988), Colorado (Gallant et al. 1989), and Wisconsin (Lyons 1989) and for lakes in Minnesota (Heiskary et al. 1987). State ecoregion maps were developed for Colorado (Gallant et al. 1989) and Oregon (Clarke et al. mss). Maps for the national ecoregions and six multi-state maps of more detailed ecoregions are available from the U.S. EPA Environmental Research Laboratory, Corvallis, Oregon.

Ecoregions such as those defined by Omernik (1987) provide only a first step in establishing regional reference sites for development of the reference condition. Field site evaluation is required to account for the inherent variability within each ecoregion. A general method for selecting reference sites for streams has been described (Hughes et al. 1986). These are the same variables used for comparable watershed reference site selection. Regional and on-site evaluations of biological factors help determine specific sites that best represent typical but unimpaired surface water habitats within the region. Details on this approach for streams is described in the "Regional Reference Site Selection" feature. To date, the regional approach has been tested on streams, rivers, and lakes. The method appears applicable for assessing other inland ecosystems. To apply this approach to wetlands and estuaries will require additional evaluation based on the relevant ecological features of these ecosystems (e.g., Brooks and Hughes, 1988).

Ideally, ecoregional reference sites should be as little disturbed as possible, yet represent waterbodies for which they are to serve as reference waters. These sites may serve as references for a large number of similar waterbodies (e.g., several reference streams may be used to define the reference condition for numerous physically separate streams if the reference streams contain the same range of stream morphology, substrate, and flow of the other streams within the same ecological region).

An important benefit of a regional reference system is the establishment of a baseline condition for the least impacted surface waters within the dominant land use pattern of the region. In many areas a return to pristine, or presettlement, conditions is impossible, and goals for waterbodies in extensively developed regions could reflect this. Regional reference sites based on the least impacted sites within a region will help water quality programs restore and protect the environment in a way that is ecologically feasible.
This approach must be used with caution for two reasons. First, in many urban, industrial, or heavily developed agricultural regions, even the least impacted sites are seriously degraded. Basing standards or criteria on such sites will set standards too low if these high levels of environmental degradation are considered acceptable or adequate. In such degraded regions, alternative sources for the regional reference may be needed (e.g., measures taken from the same region in a less developed neighboring State or historical records from the region before serious impact occurred). Second, in some regions the minimally-impacted sites are not typical of most sites in the region and may have remained unimpaired precisely because they are unique. These two considerations emphasize the need to select reference sites very carefully, based on solid quantitative data interpreted by professionals familiar with the biota of the region.

Each State, or groups of States, can select a series of regional reference sites that represent the attainable conditions for each region. Once biological criteria are established using this approach, the cost for evaluating local impairments is often lower than a series of measures of site-specific reference sites. Using paired watershed reference conditions immediately in regulatory programs will provide the added benefit of building a database for the development of regions of ecological similarity.
A critical element of biological criteria is the characterization of biological communities inhabiting surface waters. Use of biological data is not new; biological information has been used to assess impacts from pollution since the 1890s (Forbes 1928), and most States currently incorporate biological information in their decisions about the quality of surface waters. However, biological information can be obtained through a variety of methods, some of which are more effective than others for characterizing resident aquatic biota. Biological criteria are developed using biological surveys; these provide the only direct method for measuring the structure and function of an aquatic community.

Different subhabitats within the same surface water will contain unique aquatic community components. In fast-flowing stream segments species such as (1) black fly larva; (2) brook trout; (3) water penny; (4) crane fly larva; and (5) water moss occur.

However, in slow-flowing stream segments, species like (1) water strider; (2) smallmouth bass; (3) crayfish; and (4) fingermall clams are abundant.

Biological survey study design is of critical importance to criteria development. The design must be scientifically rigorous to provide the basis for legal action, and be biologically relevant to detect problems of regulatory concern. Since it is not financially or technically feasible to evaluate all organisms in an entire ecosystem at all times, careful selection of community components, the time and place chosen for assessments, data gathering methods used, and the consistency with which these variables are applied will determine the success of the biological criteria program. Biological surveys must therefore be carefully planned to meet scientific and legal requirements, maximize information, and minimize cost.
Biosurveys can range from collecting samples of a single species to comprehensive evaluations of an entire ecosystem. The first approach is difficult to interpret for community assessment; the second approach is expensive and impractical. A balance between these extremes can meet program needs. Current approaches range between detailed ecological surveys, biosurveys of targeted community components, and biological indicators (e.g., keystone species). Each of these biosurveys has advantages and limitations. Additional discussion will be provided in technical guidance under development.

No single type of approach to biological surveys is always best. Many factors affect the value of the approach, including seasonal variation, waterbody size, physical boundaries, and other natural characteristics. Pilot testing alternative approaches in State waters may be the best way to determine the sensitivity of specific methods for evaluating biological integrity of local waters. Due to the number of alternatives available and the diversity of ecological systems, individuals responsible for research design should be experienced biologists with expertise in the local and regional ecology of target surface waters. States should develop a data management program that includes data analysis and evaluation and standard operating procedures as part of a Quality Assurance Program Plan.


Selecting Aquatic Community Components

Aquatic communities contain a variety of species that represent different trophic levels, taxonomic groups, functional characteristics, and tolerance ranges. Careful selection of target taxonomic groups can provide a balanced assessment that is sufficiently broad to describe the structural and functional condition of an aquatic ecosystem, yet be sufficiently practical to use on a daily basis (Plafkin et al. 1989; Lenat 1988). When selecting community components to include in a biological assessment, primary emphasis should go toward including species or taxa that (1) serve as effective indicators of high biological integrity (i.e., those likely to live in unimpaired waters), (2) represent a range of pollution tolerances, (3) provide predictable, repeatable results, and (4) can be readily identified by trained State personnel.

Fish, macroinvertebrates, algae, and zooplankton are most commonly used in current bioassessment programs. The taxonomic groups chosen will vary depending on the type of aquatic ecosystem being assessed and the type of expected impairment. For example, benthic macroinvertebrate and fish communities are taxonomic groups often chosen for flowing fresh water. Macroinvertebrates and fish both provide valuable ecological information, while fish correspond to the regulatory and public perceptions of water quality and reflect cumulative environmental stress over longer time frames. Plants are often used in wetlands, and algae are useful in lakes and estuaries to assess eutrophication. In marine systems, benthic macroinvertebrates and submerged aquatic vegetation may provide key community components. Amphipods, for example, dominate many aquatic communities and are more sensitive than other invertebrates such as polychaetes and molluscs to a wide variety of pollutants including hydrocarbons and heavy metals (Reich and Hart 1979; J.D. Thomas, pers. comm.).

It is beneficial to supplement standard groups with additional community components to meet specific goals, objectives, and resources of the assessment program. Biological surveys that use two or three taxonomic groups (e.g., fish, macroinvertebrates, algae) and, where appropriate, include different trophic levels within each group (e.g., primary, secondary, and tertiary consumers) will
provide a more realistic evaluation of system biological integrity. This is analogous to using species from two or more taxonomic groups in bioassays. Impairments that are difficult to detect because of the temporal or spatial habits or the pollution tolerances of one group may be revealed through impairments in different species or assemblages (Ohio EPA 1988a).

Selection of aquatic community components that show different sensitivities and responses to the same perturbation will aid in identifying the nature of a problem. Available data on the ecological function, distribution, and abundance of species in a given habitat will help determine the most appropriate target species or taxa for biological surveys in the habitat. The selection of community components should also depend on the ability of the organisms to be accurately identified by trained State personnel. Attendant with the biological criteria program should be the development of identification keys for the organisms selected for study in the biological survey.

**Biological Survey Design**

Biological surveys that measure the structure and function of aquatic communities will provide the information needed for biological criteria development. Elements of community structure and function may be evaluated using a series of metrics. Structural metrics describe the composition of a community, such as the number of different species, relative abundance of specific species, and number and relative abundance of tolerant and intolerant species. Functional metrics describe the ecological processes of the community. These may include measures such as community photosynthesis or respiration. Function may also be estimated from the proportions of various feeding groups (e.g., omnivores, herbivores, and insectivores, or shredders, collectors, and grazers). Biological surveys can offer variety and flexibility in application. Indices currently available are primarily for freshwater streams. However, the approach has been used for lakes and can be developed for estuaries and wetlands.

**Selecting the metric**

Several methods are currently available for measuring the relative structural and functional well-being of fish assemblages in freshwater streams, such as the Index of Biotic Integrity (IBI); Karr 1981; Karr et al. 1986; Miller et al. 1988) and the Index of Well-being (IWB; Gammon 1976, Gammon et al. 1981). The IBI is one of the more widely used assessment methods. For additional detail, see the "Index of Biotic Integrity" feature.

### Index of Biotic Integrity

The Index of Biotic Integrity (IBI) is commonly used for fish community analysis (Karr 1981). The original IBI was comprised of 12 metrics:

- six metrics evaluate species richness and composition
  - number of species
  - number of darter species
  - number of sucker species
  - number of sunfish species
  - number of intolerant species
  - proportion of green sunfish
- three metrics quantify trophic composition
  - proportion of omnivores
  - proportion of insectivorous cyprinids
  - proportion of piscivores
- three metrics summarize fish abundance and condition information
  - number of individuals in sample
  - proportion of hybrids
  - proportion of individuals with disease

Each metric is scored 1 (worst), 3, or 5 (best), depending on how the field data compare with an expected value obtained from reference sites. All 12 metric values are then summed to provide an overall index value that represents relative integrity. The IBI was designed for midwestern streams; substitute metrics reflecting the same structural and functional characteristics have been created to accommodate regional variations in fish assemblages (Miller et al. 1988).
Several indices that evaluate more than one community characteristic are also available for assessing stream macroinvertebrate populations. Taxa richness, EPT taxa (number of taxa of the insect orders Ephemeroptera, Plecoptera, and Tricoptera), and species pollution tolerance values are a few of several components of these macroinvertebrate assessments. Example indices include the Invertebrate Community Index (ICI; Ohio EPA, 1988) and Hilsenhoff Biotic Index (HBI; Hilsenhoff, 1987).

Within these metrics specific information on the pollution tolerances of different species within a system will help define the type of impacts occurring in a waterbody. Biological indicator groups (intolerant species, tolerant species, percent of diseased organisms) can be used for evaluating community biological integrity if sufficient data have been collected to support conclusions drawn from the indicator data. In marine systems, for example, amphipods have been used by a number of researchers as environmental indicators (McCall 1977; Botton 1979; Mearns and Word 1982).

**Sampling design**

Sampling design and statistical protocols are required to reduce sampling error and evaluate the natural variability of biological responses that are found in both laboratory and field data. High variability reduces the power of a statistical test to detect real impairments (Sokal and Rohlf, 1981). States may reduce variability by refining sampling techniques and protocol to decrease variability introduced during data collection, and increase the power of the evaluation by increasing the number of replications. Sampling techniques are refined, in part, by collecting a representative sample of resident biota from the same component of the aquatic community from the same habitat type in the same way at sites being compared. Data collection protocols should incorporate (1) spatial scales (where and how samples are collected) and (2) temporal scales (when data are collected) (Green, 1979):

- **Spatial Scales** refer to the wide variety of subhabitats that exist within any surface water habitat. To account for subhabitats, adequate sampling protocols require selecting (1) the location within a habitat where target groups reside and (2) the method for collecting data on target groups. For example, if fish are sampled only from fast flowing riffles within stream A, but are sampled from slow flowing pools in stream B, the data will not be comparable.

- **Temporal Scales** refer to aquatic community changes that occur over time because of diurnal and life-cycle changes in organism behavior or development, and seasonal or annual changes in the environment. Many organisms go through seasonal life-cycle changes that dramatically affect their presence and abundance in the aquatic community. For example, macroinvertebrate data collected from stream A in March and stream B in May, would not be comparable because the emergence of insect adults after March would significantly alter the abundance of subadults found in stream B in May. Similar problems would occur if algae were collected in lake A during the dry season and lake B during the wet season.

Field sampling protocols that produce quality assessments from a limited number of site visits greatly enhance the utility of the sampling technique. Rapid bioassessment protocols, recently developed for assessing streams, use standardized techniques to quickly gather physical, chemical, and biological quantitative data that can assess changes in biological integrity (Plafkin et al. 1989). Rapid bioassessment methods can be cost-effective biological assessment approaches when they have been verified with more comprehensive evaluations for the habitats and region where they are to be applied.

Biological survey methods such as the IBI for fish and ICI for macroinvertebrates were developed in streams and rivers and have yet to be applied to many ecological regions. In addition, further research is needed to adapt the approach to lakes, wetlands, and estuaries, including the development of alternative structural or functional endpoints. For example, assessment methods for algae (e.g. measures of biomass, nuisance bloom frequency, community structure) have been used for lakes. Assessment metrics appropriate for developing biological criteria for lakes, large rivers, wetlands, and estuaries are being developed and tested so that a multi-metric approach can be effectively used for all surface waters.
Hypothesis Testing: Biological Criteria and the Scientific Method

Biological criteria are applied in the standards program by testing hypotheses about the biological integrity of impacted surface waters. These hypotheses include the null hypothesis—the designated use of the waterbody is not impaired—and alternative hypotheses such as the designated use of the waterbody is impaired (more specific hypotheses can also be generated that predict the type(s) of impairment). Under these hypotheses specific predictions are generated concerning the kinds and numbers of organisms representing community structure and function expected or found in unimpaired habitats. The kinds and numbers of organisms surveyed in unimpaired waters are used to establish the biological criteria. To test the alternative hypotheses, data collection and analysis procedures are used to compare the criteria to comparable measures of community structure and function in impacted waters.

Hypothesis Testing

To detect differences of biological and regulatory concern between biological criteria and ambient biological integrity at a test site, it is important to establish the sensitivity of the evaluation. A 10 percent difference in condition is more difficult to detect than a 50 percent difference. For the experimental/survey design to be effective, the level of detection should be predetermined to establish sample size requirements for data collection (Sokal and Rohlf 1981). Knowledge of expected natural variation, experimental error, and the kinds of detectable differences that can be expected will help determine sample size requirements for data collection.
size and location. This forms the basis for defining
data quality objectives, standardizing data collection
procedures, and developing quality assurance/
quality control standards.

Once data are collected and analyzed, they are
used to test the hypotheses to determine if character­
istics of the resident biota at a test site are sig­
nificantly different from established criteria values
for a comparable habitat. There are three possible
outcomes:

1. The use is impaired when survey design and
data analyses are sensitive enough to detect
changes of regulatory importance, and
significant differences were detected. The
next step is to diagnose the cause(s) and
source(s) of impairment.

2. The biological criteria are met when survey
design and data analyses are sensitive
enough to detect differences of regulatory
significance, but no differences were found.
In this case, no action is required by States
based on these measures. However, other
evidence may indicate impairment (e.g.,
chemical criteria are violated; see below).

3. The outcome is indeterminate when survey
design and data analyses are not sensitive
enough to detect differences of regulatory
significance, and no differences were
detected. If a State or Region determines
that this is occurring, the development of
study design and evaluation for biological
criteria was incomplete. States must then
determine whether they will accept the
sensitivity of the survey or conduct
additional surveys to increase the power of
their analyses. If the sensitivity of the
original survey is accepted, the State should
determine what magnitude of difference the
survey is capable of detecting. This will aid
in re-evaluating research design and desired
detection limits. An indeterminate outcome
may also occur if the test site and the
reference conditions were not comparable.
This variable may also require re-evaluation.

As with all scientific studies, when implementing
biological criteria, the purpose of hypothesis testing
is to determine if the data support the conclusion
that the null hypothesis is false (i.e., the designated
use is not impaired in a particular waterbody).

Biological criteria cannot prove attainment. This
reasoning provides the basis for emphasizing inde­
pendent application of different assessment
methods (e.g., chemical versus biological criteria).
No type of criteria can "prove" attainment; each type
of criteria can disprove attainment.

Although this discussion is limited to the null
and one alternative hypothesis, it is possible to
generate multiple working hypotheses (Popper,
1968) that promote the diagnosis of water quality
problems when they exist. For example, if physical
habitat limitations are believed to be causing impair­
ment (e.g., sedimentation) one alternative
hypothesis could specify the loss of community
components sensitive to this impact. Using multiple
hypotheses can maximize the information gained
from each study. See the Diagnosis section for addi­
tional discussion.

**Diagnosis**

When impairment of the designated use is
found using biological criteria, a diagnosis of prob­
able cause of impairment is the next step for im­
plementation. Since biological criteria are primarily
designed to detect water quality impairment,
problems are likely to be identified without a known
cause. Fortunately the process of evaluating test
sites for biological impairment provides significant
information to aid in determining cause.

During diagnostic evaluations, three main im­
pact categories should be considered: chemical,
physical, and biological. To begin the diagnostic
process two questions are posed:

- What are the obvious causes of impairment?
- If no obvious causes are apparent, what
  possible causes do the biological data
  suggest?

Obvious causes such as habitat degradation,
point source discharges, or introduced species are
often identified during the course of a normal field
biological assessment. Biomonitoring programs nor­
mally provide knowledge of potential sources of im­
pact and characteristics of the habitat. As such,
diagnosis is partly incorporated into many existing
State field-oriented bioassessment programs. If
more than one impact source is obvious, diagnosis
will require determining which impact(s) is the cause of impairment or the extent to which each impact contributes to impairment. The nature of the biological impairment can guide evaluation (e.g., chemical contamination may lead to the loss of sensitive species, habitat degradation may result in loss of breeding habitat for certain species).

Case studies illustrate the effectiveness of biological criteria in identifying impairments and possible sources. For example, in Kansas three sites on Little Mill Creek were assessed using Rapid Bioassessment Protocols (Plafkin et al. 1989; see Fig. 4). Based on the results of a comparative analysis, habitats at the three sites were comparable and of high quality. Biological impairment, however, was identified at two of the three sites and directly related to proximity to a point source discharge from a sewage treatment plant. The severely impaired Site (STA 2) was located approximately 100 meters downstream from the plant. The slightly impaired Site (STA 3) was located between one and two miles downstream from the plant. However, the unimpaired Site (STA 1(R)) was approximately 150 meters upstream from the plant (Plafkin et al. 1989). This simple example illustrates the basic principles of diagnosis. In this case the treatment plant appears responsible for impairment of the resident biota and the discharge needs to be evaluated.

Based on the biological survey the results are clear. However, impairment in resident populations of macroinvertebrates probably would not have been recognized using more traditional methods.

In Maine, a more complex problem arose when effluents from a textile plant met chemical-specific and effluent toxicity criteria, yet a biological survey of downstream biota revealed up to 80 percent reduction in invertebrate richness below plant outfalls. Although the source of impairment seemed clear, the cause of impairment was more difficult to determine. By engaging in a diagnostic evaluation, Maine was able to determine that the discharge contained chemicals not regulated under current programs and that part of the toxicity effect was due to the sequential discharge of unique effluents (tested individually these effluents were not toxic; when exposure was in a particular sequence, toxicity occurred). Use of biological criteria resulted in the detection and diagnosis of this toxicity problem, which allowed Maine to develop workable alternative operating procedures for the textile industry to correct the problem (Courtemanch 1989, and pers. comm.).

During diagnosis it is important to consider and discriminate among multiple sources of impairment. In a North Carolina stream (see Figure 5) four sites were evaluated using rapid bioassessment techni-
Distinguishing between point and nonpoint sources of impairment requires an evaluation of the nature and magnitude of different sites in a surface water. (Plafkin, et al. 1989)

An ecocregional reference site (R) established the highest level of biological integrity for that stream type. Site (1), well upstream from a local town, was used as the upstream reference condition. Degraded conditions at Site (2) suggested nonpoint source problems and habitat degradation because of proximity to residential areas on the upstream edge of town. At Site (3) habitat alterations, nonpoint runoff, and point source discharges combined to severely degrade resident biota. At this site, sedimentation and toxicity from municipal sewage treatment effluent appeared responsible for a major portion of this degradation. Site (4), although several miles downstream from town, was still impaired despite significant improvement in habitat quality. This suggests that toxicity from upstream discharges may still be occurring (Barbour, 1990 pers. comm.). Using these kinds of comparisons, through a diagnostic procedure and by using available chemical and biological assessment tools, the relative effects of impacts can be determined so that solutions can be formulated to improve water quality.

When point and nonpoint impact and physical habitat degradation occur simultaneously, diagnosis may require the combined use of biological, physical, and chemical evaluations to discriminate between these impacts. For example, sedimentation of a stream caused by logging practices is likely to result in a decrease in species that require loose gravel for spawning but increase species naturally adapted to fine sediments. This shift in community components correlates well with the observed impact. However, if the impact is a point source discharge or nonpoint runoff of toxicants, both species types are likely to be impaired whether sedimentation occurs or not (although gravel breeding species can be expected to show greater impairment if sedimentation occurs). Part of the diagnostic process is derived from an understanding of organism sensitivities to different kinds of impacts and their habitat requirements. When habitat is good but water quality is poor, aquatic community components sensitive to toxicity will be impaired. However, if both habitat and water quality degrade, the resident community is likely to be composed of tolerant and opportunistic species.

When an impaired use cannot be easily related to an obvious cause, the diagnostic process becomes investigative and iterative. The iterative diagnostic process as shown in Figure 6 may require additional time and resources to verify cause and source. Initially, potential sources of impact are identified and mapped to determine location relative
Chapter 7: Hypothesis Testing

Figure 6.—Diagnostic Process

Establish Biological Criteria

Conduct Field Assessment to Determine Impairment

Yes

Evaluate Data to Determine Probable Cause

No

No Further Action

Generate Testable Hypotheses for Probable Cause

Collect Data and Evaluate Results

No Apparent Cause

Obvious Cause

Propose New Alternative Hypotheses and Collect New Data

Formulate Remedial Action

to the area suffering from biological impairment. An analysis of the physical, chemical, and biological characteristics of the study area will help identify the most likely sources and determine which data will be most valuable. Hypotheses that distinguish between possible causes of impairment should be generated. Study design and appropriate data collection procedures need to be developed to test the hypotheses. The severity of the impairment, the difficulty of diagnosis, and the costs involved will determine how many iterative loops will be completed in the diagnostic process.

Normally, diagnoses of biological impairment are relatively straightforward. States may use biological criteria as a method to confirm impairment from a known source of impact. However, the diagnostic process provides an effective way to identify unknown impacts and diagnose their cause so that corrective action can be devised and implemented.
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Appendix A

Common Questions and Their Answers

Q. How will implementing biological criteria benefit State water quality programs?

A. State water quality programs will benefit from biological criteria because they:
   a) directly assess impairments in ambient biota from adverse impacts on the environment;
   b) are defensible and quantifiable;
   c) document improvements in water quality resulting from agency action;
   d) reduce the likelihood of false positives (i.e., a conclusion that attainment is achieved when it is not);
   e) provide information on the integrity of biological systems that is compelling to the public.

Q. How will biological criteria be used in a permit program?

A. When permits are renewed, records from chemical analyses and biological assessments are used to determine if the permit has effectively prevented degradation and led to improvement. The purpose for this evaluation is to determine whether applicable water quality standards were achieved under the expiring permit and to decide if changes are needed. Biological surveys and criteria are particularly effective for determining the quality of waters subject to permitted discharges. Since biosurveys provide ongoing integrative evaluations of the biological integrity of resident biota, permit writers can make informed decisions on whether to maintain or restrict permit limits.

Q. What expertise and staff will be needed to implement a biological criteria program?

A. Staff with sound knowledge of State aquatic biology and scientific protocol are needed to coordinate a biological criteria program. Actual field monitoring could be accomplished by summer-hire biologists led by permanent staff aquatic biologists. Most States employ aquatic biologists for monitoring trends or issuing site-specific permits.

Q. Which management personnel should be involved in a biologically-based approach?

A. Management personnel from each area within the standards and monitoring programs should be involved in this approach, including permit engineers, resource managers, and field personnel.

Q. How much will this approach cost?

A. The cost of developing biological criteria is a State-specific question depending upon many variables. However, States that have implemented a biological criteria program have found it to be cost effective (e.g., Ohio). Biological criteria provide an integrative assessment over time. Biota reflect multiple impacts. Testing for impairment of resident aquatic communities can actually require less monitoring than would be required to detect many impacts using more traditional methods (e.g., chemical testing for episodic events).
Q. What are some concerns of dischargers?

A. Dischargers are concerned that biological criteria will identify impairments that may be erroneously attributed to a discharger who is not responsible. This is a legitimate concern that the discharger and State must address with careful evaluations and diagnosis of cause of impairment. However, it is particularly important to ensure that waters used for the reference condition are not already impaired as may occur when conducting site-specific upstream-downstream evaluations. Although a discharger may be contributing to surface water degradation, it may be hard to detect using biosurvey methods if the waterbody is also impaired from other sources. This can be evaluated by testing the possible toxicity of effluent-free reference waters on sensitive organisms.

Dischargers are also concerned that current permit limits may become more stringent if it is determined that meeting chemical and whole-effluent permit limits are not sufficient to protect aquatic life from discharger activities. Alternative forms of regulation may be needed; these are not necessarily financially burdensome but could involve additional expense.

Burdensome monitoring requirements are additional concerns. With new rapid bioassessment protocols available for streams, and under development for other surface waters, monitoring resident biota is becoming more straightforward. Since resident biota provide an integrative measure of environmental impacts over time, the need for continual biomonitoring is actually lower than chemical analyses and generally less expensive. Guidance is being developed to establish acceptable research protocols, quality assurance/quality control programs and training opportunities to ensure that adequate guidance is available.

Q. What are the concerns of environmentalists?

A. Environmentalists are concerned that biological criteria could be used to alter restrictions on dischargers if biosurvey data indicate attainment of a designated use even though chemical criteria and/or whole-effluent toxicity evaluations predict impairment. Evidence suggests that this occurs infrequently (e.g., in Ohio, 6 percent of 431 sites evaluated using chemical-specific criteria and biosurveys resulted in this disagreement). In those cases where evidence suggests more than one conclusion, independent application applies. If biological criteria suggest impairment but chemical-specific and/or whole-effluent toxicity implies attainment of the use, the cause for impairment of the biota is to be evaluated and, where appropriate, regulated. If whole effluent and/or chemical-specific criteria imply impairment but no impairment is found in resident biota, the whole-effluent and/or chemical-specific criteria provide the basis for regulation.

Q. Do biological criteria have to be codified in State regulations?

A. State water quality standards require three components: (1) designated uses, (2) protective criteria, and (3) an antidegradation clause. For criteria to be enforceable they must be codified in regulations. Codification could involve general narrative statements of biological criteria, numeric criteria, and/or criteria accompanied by specific testing procedures. Codifying general narratives provides the most flexibility—specific methods for data collection the least flexibility—for incorporating new data and improving data gathering methods as the biological criteria program develops. States should carefully consider how to codify these criteria.

Q. How will biocriteria fit into the agency's method of implementing standards?

A. Resident biota integrate multiple impacts over time and can detect impairment from known and unknown causes. Biocriteria can be used to verify improvement in water quality in response to regulatory efforts and detect continuing degradation of waters. They provide a framework for developing improved best management practices for nonpoint source impacts. Numeric criteria can provide effective monitoring criteria for inclusion in permits.

Q. Who determines the values for biological criteria and decides whether a waterbody meets the criteria?

The process of developing biological criteria, including refined use classes, narrative criteria, and numeric criteria, must include agency managers, staff biologists, and the public through public hearings and comment. Once criteria are established, determining attainment/nonattainment of a use re-
quires biological and statistical evaluation based on established protocols. Changes in the criteria would require the same steps as the initial criteria: technical modifications by biologists, goal clarification by agency managers, and public hearings. The key to criteria development and revision is a clear statement of measurable objectives.

Q. What additional information is available on developing and using biological criteria?

A. This program guidance document will be supplemented by the document *Biological Criteria Development by States* that includes case histories of State implementation of biological criteria as narratives, numerics, and some data procedures. The purpose for the document is to expand on material presented in Part I. The document will be available in October 1990.

A general *Biological Criteria Technical Reference Guide* will also be available for distribution during FY 1991. This document outlines basic approaches for developing biological criteria in all surface waters (streams, rivers, lakes, wetlands, estuaries). The primary focus of the document is to provide a reference guide to scientific literature that describes approaches and methods used to determine biological integrity of specific surface water types.

Over the next triennium more detailed guidance will be produced that focuses on each surface water type (e.g., technical guidance for streams will be produced during FY 91). Comparisons of different biosurvey approaches will be included for accuracy, efficacy, and cost effectiveness.
Appendix B

Biological Criteria Technical Reference Guide

Table of Contents (tentative)

SECTION 1. INTRODUCTION
- Purpose of the Technical Support Document
- Organization of the Support Document

SECTION 2. CONCEPTUAL FRAMEWORK FOR BIOLOGICAL CRITERIA
- Definitions
- Biocriteria and the Scientific Method
- Hypothesis Formulation and Testing
- Predictions
- Data Collection and Evaluation

SECTION 3. QUALITY ASSURANCE/QUALITY CONTROL
- Data Quality Objectives
- Quality Assurance Program Plans and Project Plans
- Importance of QA/QC for Bioassessment
- Training
- Standard Procedures
- Documentation
- Calibration of Instruments

SECTION 4. PROCESS FOR THE DEVELOPMENT OF BIOCRITERIA
- Designated Uses
- Reference Site or Condition
- Biosurvey
- Biological Criteria
SECTION 5. BIOASSESSMENT STRATEGIES TO DETERMINE BIOLOGICAL INTEGRITY
- Detailed Ecological Reconnaissance
- Biosurveys of Targeted Community Segments
- Rapid Bioassessment Protocols
- Bioindicators

SECTION 6. ESTABLISHING THE REFERENCE CONDITION
- Reference Conditions Based on Site-Specific Comparisons
- Reference Conditions Based on Regions of Ecological Similarity
- Reference Conditions Based on Habitat Assessment

SECTION 7. THE REFERENCE CATALOG

SECTION 8. THE INFLUENCE OF HABITAT ON BIOLOGICAL INTEGRITY
- Habitat Assessment for Streams and Rivers
- Habitat Assessment for Lakes and Reservoirs
- Habitat Assessment for Estuaries and Near-Coastal Areas
- Habitat Assessment for Wetlands

SECTION 9. BIOSURVEY METHODS TO ASSESS BIOLOGICAL INTEGRITY
- Biotic Assessment in Freshwater
- Biotic Assessment in Estuaries and Near-Coastal Areas
- Biotic Assessment in Wetlands

SECTION 10. DATA ANALYSIS
- Sampling Strategy and Statistical Approaches
- Diversity Indices
- Biological Indices
- Composite Community Indices

APPENDIX A. Freshwater Environments
APPENDIX B. Estuarine and Near-Coastal Environments
APPENDIX C. Wetlands Environments
APPENDIX D. Alphabetical Author/Reference Cross Number Index for the Reference Catalog
APPENDIX E. Reference Catalog Entries

LIST OF FIGURES
- Figure 1 Bioassessment decision matrix
- Figure 2 Specimen of a reference citation in the Reference Catalog
Appendix C

Biological Criteria
Development by States

Table of Contents (tentative)

I. Introduction
II. Key Concepts
III. Biological Criteria Across the 50 States
IV. Case Study of Ohio
   A. Introduction
      1. Derivation of Biological Criteria
      2. Application of Biological Criteria
   B. History
      1. Development of Biological Criteria
      2. Current Status of Biological Criteria
   C. Discussion
      1. Program Resources
      2. Program Evaluation
V. Case Study of Maine
   A. Introduction
      1. Derivation of Biological Criteria
      2. Application of Biological Criteria
   B. History
      1. Development of Biological Criteria
      2. Program Rationale
   C. Discussion
      1. Program Resources
      2. Program Evaluation
VI. Case Study of North Carolina
   A. Introduction
      1. Derivation of Biological Criteria
      2. Application of Biological Criteria
   B. History
      1. Development of Biological Criteria
      2. Current Status of Biological Criteria
   C. Discussion
      1. Program Resources
      2. Program Evaluation
VII. Case Study of Arkansas
   A. Introduction
      1. Derivation of Biological Criteria
      2. Application of Biological Criteria
   B. History
      1. Development of Biological Criteria
      2. Current Status of Biological Criteria
   C. Discussion
      1. Program Resources
      2. Program Evaluation
VIII. Case Study of Florida
   A. Introduction
      1. Derivation of Biological Criteria
      2. Application of Biological Criteria
   B. History
   C. Discussion
IX. Case Summaries of Six States
   A. Connecticut
   B. Delaware
   C. Minnesota
   D. Nebraska
   E. New York
   F. Vermont
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