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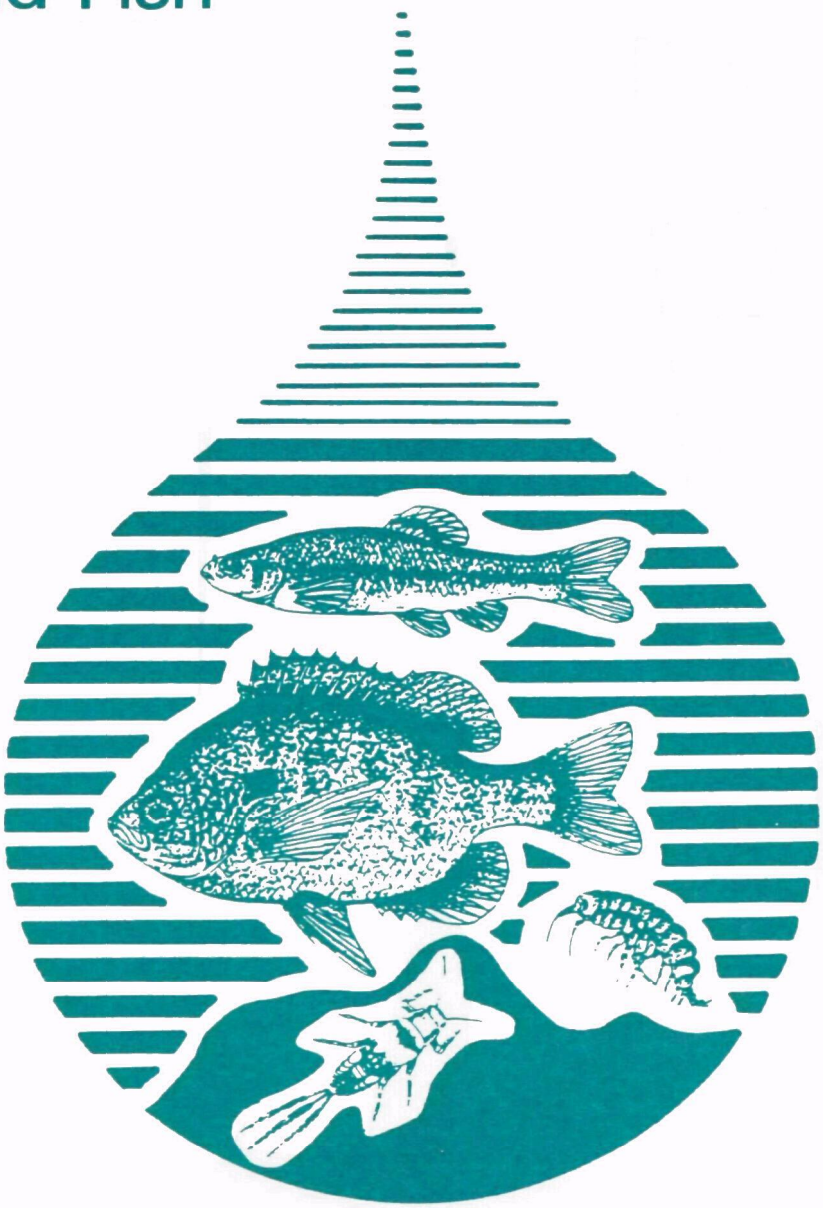
Rapid Bioassessment Protocols For Use In Streams And Rivers

Benthic Macroinvertebrates And Fish



RAPID BIOASSESSMENT PROTOCOLS FOR USE IN STREAMS AND RIVERS: BENTHIC MACROINVERTEBRATES AND FISH

by

James L. Plafkin[a]
Michael T. Barbour
Kimberly D. Porter
Sharon K. Gross
Robert M. Hughes

[a]U.S. Environmental Protection Agency
Assessment and Watershed Protection Division
401 M Street, S.W.
Washington, D.C. 20460

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FOREWORD

In December 1986, U.S. EPA's Assistant Administrator for Water initiated a major study of the Agency's surface water monitoring activities. The resulting report, entitled "Surface Water Monitoring: A Framework for Change" (U.S. EPA 1987), emphasizes the restructuring of existing monitoring programs to better address the Agency's current priorities, e.g., toxics, nonpoint source impacts, and documentation of "environmental results." The study also provides specific recommendations on effecting the necessary changes. Principal among these are:

1. To issue guidance on cost-effective approaches to problem identification and trend assessment.

2. To accelerate the development and application of promising biological monitoring techniques.

In response to these recommendations, the Assessment and Watershed Protection Division has developed rapid bioassessment protocols designed to provide basic aquatic life data for planning and management purposes such as screening, site ranking, and trend monitoring. All of the protocols utilize fundamental assessment techniques to generate basic information on ambient physical, chemical, and biological conditions. Level of assessment and level of effort vary with successive protocols, and choice of a given protocol should depend on the specific objective of the monitoring activity and available resources. Although none of the protocols are meant to provide the rigor of fully comprehensive studies, each is designed to supply pertinent, cost-effective information when applied in the appropriate context.

Martha G. Prothro
Director, Office of Water Regulations and Standards
U.S. EPA, Washington, D.C.

ACKNOWLEDGMENTS

Dr. James L. Plafkin of the Assessment and Watershed Protection Division (AWPD) served as principal editor and coauthor of this document. Other coauthors were consultants Michael T. Barbour, Kimberly D. Porter, and Sharon Gross working for AWPD and Dr. Robert M. Hughes working for EPA's Corvallis Research Laboratory.

Many others also contributed to the development of this document and deserve special thanks. First and foremost, the Rapid Bioassessment Workgroup. The Workgroup, composed of both State and EPA Regional biologists (listed in Chapter 1), was instrumental in providing a framework for the basic approach and served as primary reviewers of various drafts. Dr. Kenneth Cummins and Dr. William Hilsenhoff provided invaluable advice on formulating certain assessment metrics, and Dr. Anthony Maciorowski and Paul Leonard supplied helpful editorial comments on the final drafts. Special thanks go to the biologists in the field (well over a hundred) who contributed their valuable time to review the document and provide constructive input. Their help in this endeavor is sincerely appreciated.

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1. INTRODUCTION

1.1 PURPOSE OF THE DOCUMENT

The primary purpose of this document is to provide States with a practical technical reference for conducting cost-effective biological assessments of lotic systems. The protocols presented are not necessarily intended to replace those already in use by State agencies. Instead, they provide options for agencies that wish to implement rapid biological assessment techniques. Three macroinvertebrate and two fish protocols are presented: Benthic Rapid Bioassessment Protocol I (RBP I) and fish Rapid Bioassessment Protocol IV (RBP IV) are cost-effective screening procedures that provide some supporting data; benthic Rapid Bioassessment Protocol II (RBP II) can help set priorities for more intensive evaluations; and benthic Rapid Bioassessment Protocol III (RBP III) and fish Rapid Bioassessment Protocol V (RBP V) are progressively more rigorous and provide more confirmational data, but also require more resources. The choice of a particular protocol should depend on the purpose of the bioassessment, the need to document conclusions with confirmational data, the degree of discrimination desired, and available resources. Although the benthic protocols were designed and tested in wadable freshwater streams rather than large rivers (or lakes, estuaries, or marine systems), the fundamental approach should be applicable to large freshwater rivers as well. The fish protocols were validated in freshwater streams and large rivers and are applicable to both.

The original rapid bioassessment protocols were designed as inexpensive screening tools for determining if a stream is supporting or not supporting a designated aquatic life use. The basic information generated would enhance the coverage of broad geographical assessments, such as State and National 305(b) Water Quality Inventories. However, members of a 1986 benthic Rapid Bioassessment Workgroup and reviewers of this document indicated that the rapid bioassessment protocols can also be applied to other program areas, for example:

- Characterizing the existence and severity of use impairment
- Helping to identify sources and causes of use impairment
- Evaluating the effectiveness of control actions
- Supporting use attainability studies
- Characterizing regional biotic components

Therefore, the scope of this guidance might now be considered applicable to a wider range of planning and management purposes than originally envisioned, i.e., they may be appropriate for priority setting, point and nonpoint-source evaluations, use attainability analyses, and trend monitoring, as well as initial screening.

1.2 DEVELOPMENT OF THIS DOCUMENT

This document was developed in two phases. The first phase centered on the development and refinement of the benthic rapid bioassessment protocols. The second phase involved the addition of analogous protocols pertinent to the assessment of fish communities.

The benthic protocols were developed by consolidating procedures in use by various State water quality agencies. In 1985, a survey was conducted to identify States that routinely perform screening-level bioassessments and believe that such efforts are important to their monitoring programs. Guidance documents and field methods in common use were evaluated in an effort to identify successful bioassessment methods that use different levels of effort. Original survey materials and information obtained from direct personal contacts were used to develop the draft protocols.

Missouri Department of Natural Resources and Michigan Department of Natural Resources both use the "stream walk" approach upon which the screening protocol (RBP I) in this document is based. The second protocol (RBP II) is more time and labor intensive, incorporating field sampling and family-level taxonomy, and is a less intense version of RBP III. The concept of family-level taxonomy is based on the approach used by the Virginia State Water Control Board. The third protocol (RBP III) incorporates certain aspects of the methods used by the North Carolina Division of Environmental Management and the New York Department of Environmental Conservation and is the most rigorous of the three approaches.

A workgroup of State and U.S. EPA Regional biologists (listed below) was formed to review and refine the draft benthic protocols. The Rapid Bioassessment Workgroup included biologists using the State methods described above and biologists from other regions where pollution sources and aquatic systems differed from those areas for which the draft protocols were initially developed.

EPA

James Plafkin, AWPD
Michael Bilger, Region I
Michael Bastian, Region VI
William Wuerthele, Region VIII
Evan Hornig, Region X

STATES

Brenda Sayles, Michigan DNR
John Howland, Missouri DNR
Robert Bode, New York DEC
David Lenat, North Carolina DEM
Michael Shelor, Virginia SWCB
Joseph Ball, Wisconsin DNR

The rapid bioassessment protocols for benthos presented here include modifications discussed in the workgroup's first meeting held in 1986, as well as comments on a subsequent draft. This document also includes results of a field validation study (Section 6.4) conducted with the North Carolina Department of Environmental Management to examine certain methodological issues highlighted in the workgroup's review (Chapter 2). In addition, these protocols and the concept of "rapid" bioassessment have been discussed at the 1986, 1987, and 1988 annual meetings of the North American Benthological Society and in personal communications with Dr. Kenneth Cummins, Dr. William Hilsenhoff, Dr. James Karr, and Dr. Vincent Resh.

In response to a number of comments received from State and EPA personnel on an earlier version of the rapid bioassessment protocols, a set of fish protocols was also developed. Fish protocol V is based on Karr's work (1981) with the Index of Biological Integrity (IBI), Gammon's Index of Well Being (1980), and standard fish population assessment models, coupled with certain modifications for implementation in different geographical regions. Ohio EPA has developed biological criteria using the IBI and Index of Well Being (IWB), and a substantial database on their use for site-specific fish assessments exists.

1.3 A FRAMEWORK FOR IMPLEMENTING THE RAPID BIOASSESSMENT PROTOCOLS

The rapid bioassessment protocols advocate an integrated assessment, comparing habitat (e.g., physical structure, flow regime) and biological measures with empirically defined reference conditions (Figure 1.3-1). Reference conditions are established through systematic monitoring of actual sites that represent the natural range of variation in "least disturbed" water chemistry, habitat, and biological condition. Of these three components of ecological integrity, ambient water quality may be the most difficult to characterize because of the complex array of chemical constituents (natural and otherwise) that affect it. Therefore, the implementation framework presented below first describes the development of an empirical relationship between habitat quality and biological condition, then refines this relationship for a given region. As additional information is obtained from systematic monitoring of potentially impacted and site-specific control sites, the predictive power of the empirical relationship is enhanced. Once the relationship between habitat and biological potential is understood, water quality impacts can be objectively discriminated from habitat effects and control efforts can be focused on the most important source of impairment.

Explanatory Notes—
Bioassessment Implementation Framework

The following notes describe the implementation framework of the RBPs presented in Figure 1.3-1; each note corresponds to a similarly numbered element in the flow diagram. The reader should examine the figure first, then refer back to the numbered notes for explanations.

1. The "reference site" (RS) should represent a database consisting of the best attainable physical habitat, water chemistry, and biological parameters for specific environmental conditions. Acceptable ranges for the habitat and biological parameters of concern are based on this reference database.

   In the RBP assessment scheme, selected parameters are integrated to define generic habitat categories and bioclassifications. The integrated characterizations describe important attributes of the designated use and represent criteria for attainment/non-attainment of the designated use. Figure 1.3-1 also illustrates how designated uses and criteria may be established or refined as ambient monitoring activities proceed, and how new data are incorporated into the reference database.

   Considerable effort may be required initially to identify reference sites and the habitat and biological characteristics of a specified aquatic life use. Alternatively, data required to define new or refined use characterizations and assessment criteria could be collected through implementation of an effective ambient monitoring program. However, when the *initial* reference database includes a spectrum of "least disturbed" habitats and concomitant biotic conditions, the need for site-specific controls may be greatly reduced. The value of a comprehensive reference database becomes more evident with progression through the implementation framework.
2. The purpose of the habitat assessment is to determine whether "IS" (impaired site) has the *potential* to support a biological community comparable to that of the reference (see note 6).
3. Generally applicable ranges for several important habitat characteristics are incorporated into the habitat assessment field sheets (Figure 5.2-1) and the habitat evaluation can be made quickly onsite. However, preliminary reconnaissance is especially helpful when impaired site habitat (HIS) proves to be much lower in quality than reference habitat (HRS) and an evaluation of reversible habitat alterations ("attainability") may also be necessary. Reconnaissance information allows planning for the additional work needed to characterize more appropriate reference sites.
4. In the early stages of developing assessment criteria for a given aquatic life use, HIS may often appear degraded relative to the HRS database. The likelihood of such an outcome is proportional to the richness of the initial HRS database. As more potentially impacted stations are assessed, however, certain stations will be shown to support:
   —Biological communities equivalent to the reference sites despite apparent habitat deficiencies. Information from such sites will enrich the reference database and broaden the applicability of the use designation.
   —A relatively degraded community that is limited by intrinsic or irreversible habitat constraints. In this case, the original use is not attainable, and data collected from such a site should be used to revise the use designation.
5. The robustness of the comparison between the biological condition at the impaired site (BIS) and that at the reference (BRS) is limited by the rigor of the assessment procedure used (e.g., many versus few replicates) and the scope of the overall assessment (i.e., the number of biological community segments actually evaluated). The comparison of BIS and BRS is useful for detecting or confirming *appreciable* impact to the biotic community and may be insensitive to certain subtle and/or threshold effects.
6. If BIS = BRS, there is no detectable impairment. This conclusion assumes no overriding limitations on the biological potential of "IS" relative to "RS that are not accounted for by the previous habitat comparison (see Note 2). Factors that could uniquely affect "IS" are discussed in Section 2.3. For example, stations "RS" and "IS" may be located on a first order stream with primary organic inputs from a coniferous forest. In this situation, certain characteristics of the benthic community, such as taxa richness, may actually increase with organic enrichment from point source discharges rather than decrease as otherwise expected. This atypical situation should be assessed as if HIS + (Reversible Habitat Alterations) < HRS (Step 7).
7. The HIS + (Reversible Habitat Alterations) versus HRS comparison amounts to a simplistic use attainability analysis (UAA) that only considers habitat. The comparison involves scaling up the observed habitat parameter values to the extent that they might be feasibly improved. For example, bank stability, bank vegetation, and streamside cover could be greatly enhanced by fencing a pasture and planting trees, whereas other parameters may be unalterable. This "mini-UAA" can help to assess site-specific potential in the determination of actual impairment. If HIS and HRS are *potentially* equivalent, then use impairment can be appropriately assessed in terms of the resident biota. If HIS and HRS are not equivalent even when reversible habitat alterations are considered, biological effects may not be independent of habitat constraints. These potential scenarios are discussed in more detail in Chapter 8, Integration of Habitat, Water Quality, and Biosurvey Data. The approach to conducting a habitat assessment and bioassessment is discussed in Chapters 5, 6, and 7.

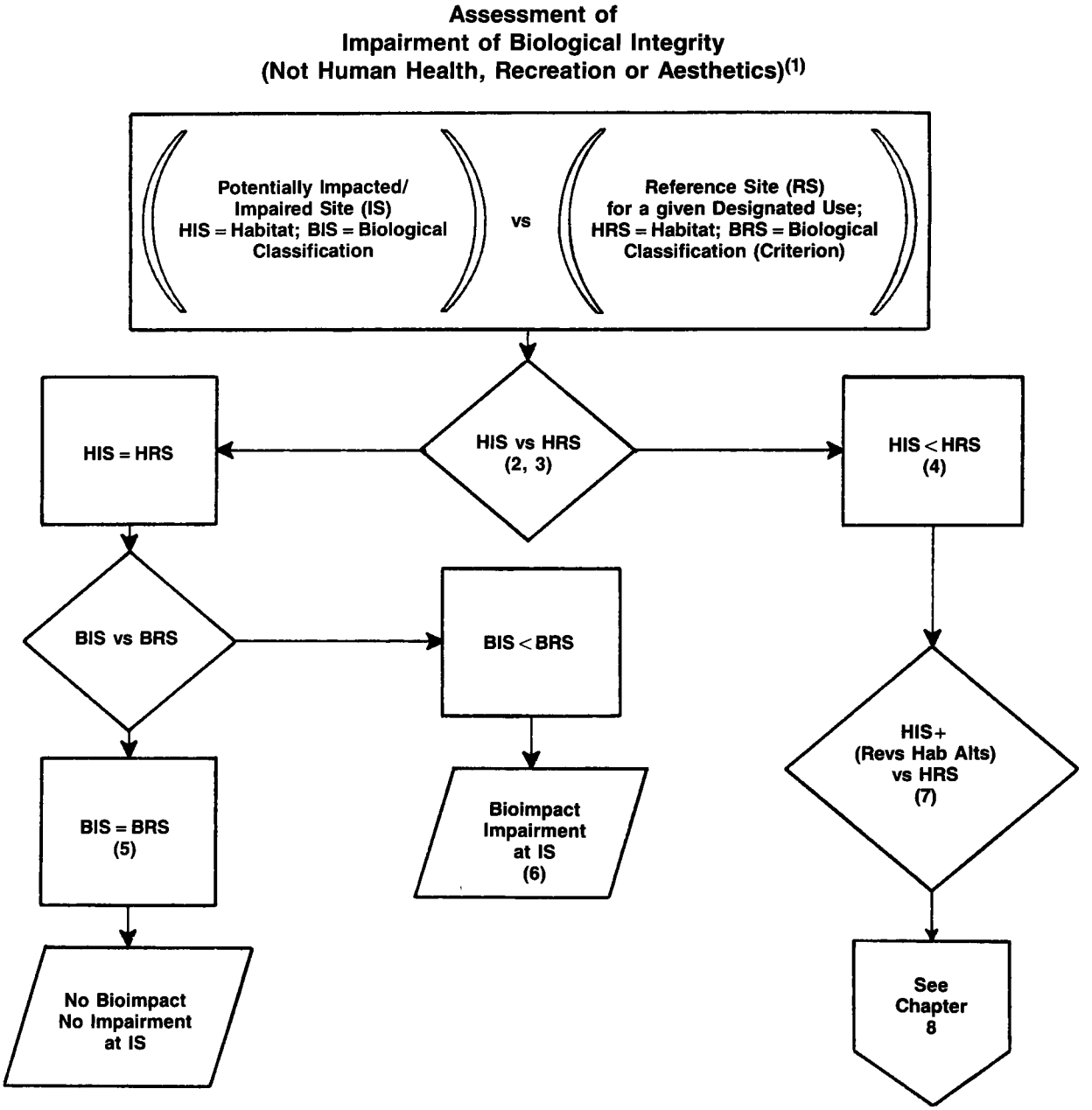


Figure 1.3-1. Bioassessment decision matrix. (Numbers in parentheses refer to points of discussion in text).

2. THE CONCEPT OF BIOMONITORING

2.1 BIOSURVEYS, BIOASSAYS, AND CHEMICAL MONITORING

The water quality-based approach to pollution assessment requires various types of data. Biosurvey techniques, such as the rapid bioassessment protocols, are best used for detecting aquatic life impairments and assessing their relative severity. Once an impairment is detected, however, additional chemical and biological (toxicity) testing is usually necessary to identify the causative agent and its source and to implement appropriate mitigation (U.S. EPA 1985). Following mitigation, biosurveys are important for evaluating the effectiveness of such control measures.

Biosurveys may be used within a planning and management framework to prioritize water quality problems for more stringent assessments and to document "environmental recovery" following control action. Some of the advantages of using biosurveys for this type of monitoring are:

1. Biological communities reflect overall ecological integrity (i.e., chemical, physical, and biological integrity). Therefore, biosurvey results directly assess the status of a waterbody relative to the primary goal of the Clean Water Act.
2. Biological communities integrate the effects of different pollutant stressors and thus provide a holistic measure of their aggregate impact. Communities also integrate the stresses over time and provide an ecological measure of fluctuating environmental conditions. Assessing the integrated response of biological communities to highly variable pollutant inputs offers a particularly useful approach for monitoring nonpoint-source impacts and the effectiveness of certain Best Management Practices.
3. Routine monitoring of biological communities can be relatively inexpensive, particularly when compared to the cost of assessing toxic pollutants, either chemically or with toxicity tests (Ohio EPA 1987a).
4. The status of biological communities is of direct interest to the public as a measure of a pollution free environment, while reductions in chemical pollutant loadings are not as readily understood by the layman as positive environmental results.
5. Where criteria for specific ambient impacts do not exist (e.g., nonpoint-source impacts that degrade habitat), biological communities may be the only practical means of evaluation.

Biosurvey methods have a long-standing history of use for "before and after" monitoring. However, the intermediate steps in pollution control, identifying causes and limiting sources, require information of a different type—chemical, physical, and/or additional biological data. These data are needed to:

1. *Identify the specific stress agents causing impact.* This may be a relatively simple task; but, given the array of potentially important pollutants (and their possible combinations), it is likely to be both difficult and costly. In situations where specific chemical stress agents are either poorly understood or too varied to assess individually, toxicity tests can be used to focus specific chemical investigations or to characterize generic stress agents (e.g., whole effluent toxicity).
2. *Identify and limit the specific sources of these agents.* Although biosurveys can be used to help locate the likely origins of impact, chemical analyses and/or toxicity tests are usually necessary to confirm the responsible sources and develop appropriate discharge limits.
3. *Design appropriate treatment to meet the prescribed limits and monitor compliance.* Treatment facilities are designed to remove identified chemical constituents with a specific efficiency. Chemical data are therefore required to construct such facilities and evaluate treatment effectiveness. To some degree, a biological endpoint resulting from toxicity testing can also be used to evaluate the effectiveness of prototype treatment schemes and can serve as a design parameter. In most cases, these same parameters are limited in discharge permits and, after controls are in place, are used to monitor for compliance. Where discharges are not controlled through a permit system (e.g., nonpoint-source runoff, combined sewer outfalls, and dams) compliance must be assessed in terms of ambient standards.

Effective implementation of the water quality-based approach requires that various monitoring techniques be considered within a larger context of water resource management. Both biological and chemical

methods play critical roles in a successful pollution control program. They should be considered complementary rather than mutually exclusive approaches that will enhance overall program effectiveness when used appropriately.

2.2 USE OF DIFFERENT TAXONOMIC GROUPS IN BIOSURVEYS

The bioassessment techniques presented in this document focus on the evaluation of water quality, habitat, and benthic macroinvertebrate and fish community parameters. Many State water quality agencies employ trained and experienced benthic biologists, have accumulated considerable background data on macroinvertebrates, and consider benthic surveys a useful assessment tool. However, water quality standards, legislative mandate, and public opinion are more directly related to the status of a waterbody as a fishery resource. For this reason, separate protocols were developed for fish and were incorporated as Chapter 7 in this document. The fish survey protocol is based largely on James Karr's IBI (Karr 1981; Karr et al. 1986; Miller et al. 1988a), which uses fish community structure to evaluate water quality. The integration of functional and structural/compositional metrics, which forms the basis for the IBI is a common element to the fish and benthic rapid bioassessment approaches.

Although no methods are presented here for conducting algal assessments, algal communities are also useful for water quality monitoring. They represent another trophic level, exhibit a different range of sensitivities, and will often indicate effects only indirectly observed in the benthic and fish communities. As in the benthic macroinvertebrate and fish communities, integration of structural/compositional and functional characteristics provides the best means of assessing impairment (Rodgers et al. 1979).

Algal community structural/compositional analyses may be taxonomic or non-taxonomic. Taxonomic analyses (e.g., diversity indices, taxa richness, indicator species) are commonly used, and are described in studies by Rodgers et al. (1979), Weitzel (1979), Palmer (1977), and Patrick (1973). Non-taxonomic measures, such as biomass and chlorophyll, can also be useful for detecting effects not indicated by taxonomic analysis. For example, toxic pollutants may cause sublethal (i.e., reproductive) effects which would not immediately be detected by taxonomic analyses such as taxa richness, but would be indicated by low biomass (Patrick 1973). A summary of non-taxonomic measurements is presented in Weitzel (1979).

Functional aspects of algal communities, such as primary productivity rates, can also be assessed. These analyses, which are described in Rodgers et al. 1979, can be performed at the taxonomic level (e.g., determination of species colonization rate) or at the non-taxonomic level (e.g., community respiration) to evaluate effects of toxicants or nutrient enrichment.

In determining the taxonomic group or groups appropriate for a particular biomonitoring situation, the advantages of using each taxonomic group must be considered along with the objectives of the program. Some of the advantages of using macroinvertebrates, fish, and algae in a biomonitoring program are presented in this section. References for this list are Cairns and Dickson 1971; Karr 1981; U.S. EPA 1983; Hughes et al. 1982; American Public Health Association et al. 1971; Patrick 1973; Rodgers et al. 1979; and Weitzel 1979.

Advantages of Using Benthic Macroinvertebrates

1. Macroinvertebrate communities are good indicators of localized conditions.
   - Because many benthic macroinvertebrates have limited migration patterns or a sessile mode of life, they are particularly well suited for assessing site-specific impacts (upstream-downstream studies).
2. Macroinvertebrate communities integrate the effects of short-term environmental variations.
   - Most species have a complex life cycle of approximately 1 year or more. Sensitive life stages will respond quickly to stress; the overall community will respond more slowly.
3. Degraded conditions can often be detected by an experienced biologist with only a cursory examination of the macroinvertebrate community.
   - Macroinvertebrates are relatively easy to identify to family; many "intolerant" taxa can be identified to lower taxonomic levels with ease.
4. Sampling is relatively easy, requires few people and inexpensive gear, and has no detrimental effect on the resident biota.
5. Benthic macroinvertebrates serve as a primary food source for many recreationally and commercially important fish.
6. Benthic macroinvertebrates are abundant in most streams.
   - Many small streams (1st and 2nd order), which *naturally* support a diverse macroinvertebrate fauna, only support a limited fish fauna.
7. Most State water quality agencies that routinely

collect biosurvey data focus on macroinvertebrates. (This may be due to the emphasis placed on macroinvertebrates for community-level evaluations in the 1976 Basic Monitoring Programs Guidance.)
- Many States already have background macroinvertebrate data.
- Most State water quality agencies have more expertise in aquatic entomology than in ichthyology.

Advantages of Using Fish

1. Fish are good indicators of long-term (several years) effects and broad habitat conditions because they are relatively long-lived and mobile (Karr et al. 1986).
2. Fish communities generally include a range of species that represent a variety of trophic levels (omnivores, herbivores, insectivores, planktivores, piscivores). They tend to integrate effects of lower trophic levels; thus, fish community structure is reflective of integrated environmental health.
3. Fish are at the top of the aquatic food chain and are consumed by humans, making them important subjects in assessing contamination.
4. Fish are relatively easy to collect and identify to the species level. Most specimens can be sorted and identified in the field and released unharmed.
   - Environmental requirements of common fish are comparatively well known.
   - Life history information is extensive for most species.
   - Information on fish distributions is commonly available.
5. Aquatic life uses (water quality standards) are typically characterized in terms of fisheries (coldwater, coolwater, warmwater, sport, forage).
   - Monitoring fish communities provides direct evaluation of "fishability", which emphasizes the importance of fish to anglers and commercial fishermen.
6. Fish account for nearly half of the endangered vertebrate species and subspecies in the United States.

Advantages of Using Algae

1. Algae generally have rapid reproduction rates and very short life cycles, making them valuable indicators of short-term impacts.
2. As primary producers, algae are most directly affected by physical and chemical factors.
3. Sampling is easy, inexpensive, requires few people, and creates minimal impact to resident biota.
4. Relatively standard methods exist for evaluation of functional and non-taxonomic structural (biomass, chlorophyll measurements) characteristics of algal communities.
5. Algal communities are sensitive to some pollutants which may not visibly affect other aquatic communities, or may only affect other communities at higher concentrations (i.e., herbicides).

2.3 STATION SITING

RBP I, RBP II, RBP III, and RBP V include the collection of biological samples to assess the biotic integrity of a given site. To meaningfully evaluate biological condition, sampling locations must be carefully selected to ensure generally comparable habitat at each station. Unless basically comparable physical habitat is sampled at all stations, community differences attributable to a degraded habitat will be difficult to separate from those resulting from water quality degradation. Availability of habitats at each sampling location can be established during preliminary reconnaissance (such as RBP I). In evaluations where several stations on a waterbody will be compared, the station with the greatest habitat constraints (in terms of productive habitat availability) should be noted. The station with the least number of productive habitats available will often determine the type of habitat to be sampled at all stations of comparison.

Locally modified sites, such as small impoundments and bridge areas, should be avoided unless data are needed to assess their effects. Sampling near the mouths of tributaries entering large waterbodies should also be avoided since these areas will have habitat more typical of the larger waterbody (Karr et al. 1986).

Although the specific bioassessment objective is an important consideration in locating sampling stations, all assessments require a site-specific control station or reference data from comparable sites within the same region. A site-specific control is generally thought to be most representative of "best attainable" conditions for a particular waterbody. However, regional reference stations may also be desirable to allow evaluation of conditions on a larger scale. Where feasible, effects should be bracketed by establishing a series or network of sampling stations at points of increasing distance from the impact source(s). These stations will provide a basis for delineating impact and recovery zones.

2.4 IMPORTANCE OF HABITAT ASSESSMENT

The procedure for assessing habitat quality presented in this document (Section 5.2) is an integral component of the final evaluation of impairment. The matrix used to assess habitat quality is based on key physical characteristics of the waterbody and surrounding land. All of the habitat parameters evaluated are related to overall aquatic life use and are a potential source of limitation to the aquatic biota.

Habitat, as affected by instream and surrounding topographical features, is a major determinant of aquatic community potential. Both the quality and quantity of available habitat affect the structure and composition of resident biological communities. Effects of such features can be minimized by sampling similar habitats at all stations being compared. However, when all stations are not physically comparable, habitat characterization is particularly important for proper interpretation of biosurvey results.

Where habitat quality is similar, detected impacts can be attributed to water quality factors. However, where habitat quality differs substantially from reference conditions, the question of use attainability and physical habitat alteration/restoration must be addressed. Final conclusions regarding the presence and degree of biological impairment should thus include an evaluation of habitat quality to determine the extent that habitat may be a limiting factor. The habitat characterization matrix included in the rapid bioassessment protocols provides an effective means of evaluating and documenting habitat quality at each biosurvey station.

2.5 THE ECOREGION CONCEPT

Innate regional differences exist in forests, agricultural potential, wetlands, and waterbodies. These regional differences have been mapped by Bailey (1976); USDA Soil Conservation Service (1981), Energy, Mines and Resources Canada (1986), and Omernik (1987). All four maps were developed from examination of several mapped land variables. It is assumed that waterbodies reflect the lands they drain and that similar lands should produce similar waterbodies. This ecoregional approach provides much more robust and ecologically-meaningful regional maps than could be attained by mapping a single variable. For example, hydrologic unit maps are useful for mapping drainage patterns, but have limited value for explaining the substantial changes that occur in water quality and biota independent of stream size and river basin. Recognition of these changes stimulated Warren's (1979) work, and Ohio's and Arkansas' development of ecoregional standards.

Omernik (1987) provides an ecoregional framework for interpreting spatial patterns in state and national data. The geographical framework is based on regional patterns in land-surface form, soil, potential natural vegetation, and land use, which vary across the country. Two major applications grew out of the regional approach. The first was the use of a relatively small number of minimally-impacted regional reference sites to assess feasible but protective biological goals for an entire region (Hughes et al. 1986). The second was the use of regions as a statistical framework for stratified random sampling of lakes in a national survey of the effects of acid deposition (Linthurst et al. 1986, Landers et al. 1987). These two site selection methods offer ecologically and statistically valid means to establish baseline conditions and assess water quality in entire regions by monitoring a relatively small number of sites.

Geographic patterns of similarity among ecosystems can be grouped into ecoregions. Naturally occurring biotic assemblages, as components of the ecosystem, would be *expected* to differ among ecoregions but be relatively similar within a given ecoregion. The ecoregion concept thus provides a geographic framework for more efficient management of aquatic ecosystems and their components (Hughes et al. 1986, Hughes 1985, and Hughes and Larsen 1988). For example, studies in Ohio (Larsen et al. 1986), Arkansas (Rohm et al. 1987), and Oregon (Hughes et al. 1987, Whittier et al. 1988) have shown that distributional patterns of fish communities coincide with the States' ecoregions as defined *a priori* by Omernik (1987). This, in turn, implies that similar water quality standards, criteria, and monitoring strategies are likely to be valid throughout a given ecoregion, but should be tailored to accommodate the innate differences among ecoregions (Ohio EPA 1987b). Figure 2.5-1 shows the 76 ecoregions developed by Omernik (1987) for the conterminous United States.

Macroinvertebrate communities reflect habitat differences on a smaller scale than fish, and may be better suited for site-specific assessments. Within an ecoregion (Omernik 1987), additional qualifiers such as stream size, hydrologic regime, and riparian vegetation need to be considered. In addition, streams or stream segments may represent characteristics atypical for that particular ecoregion. For instance, a given

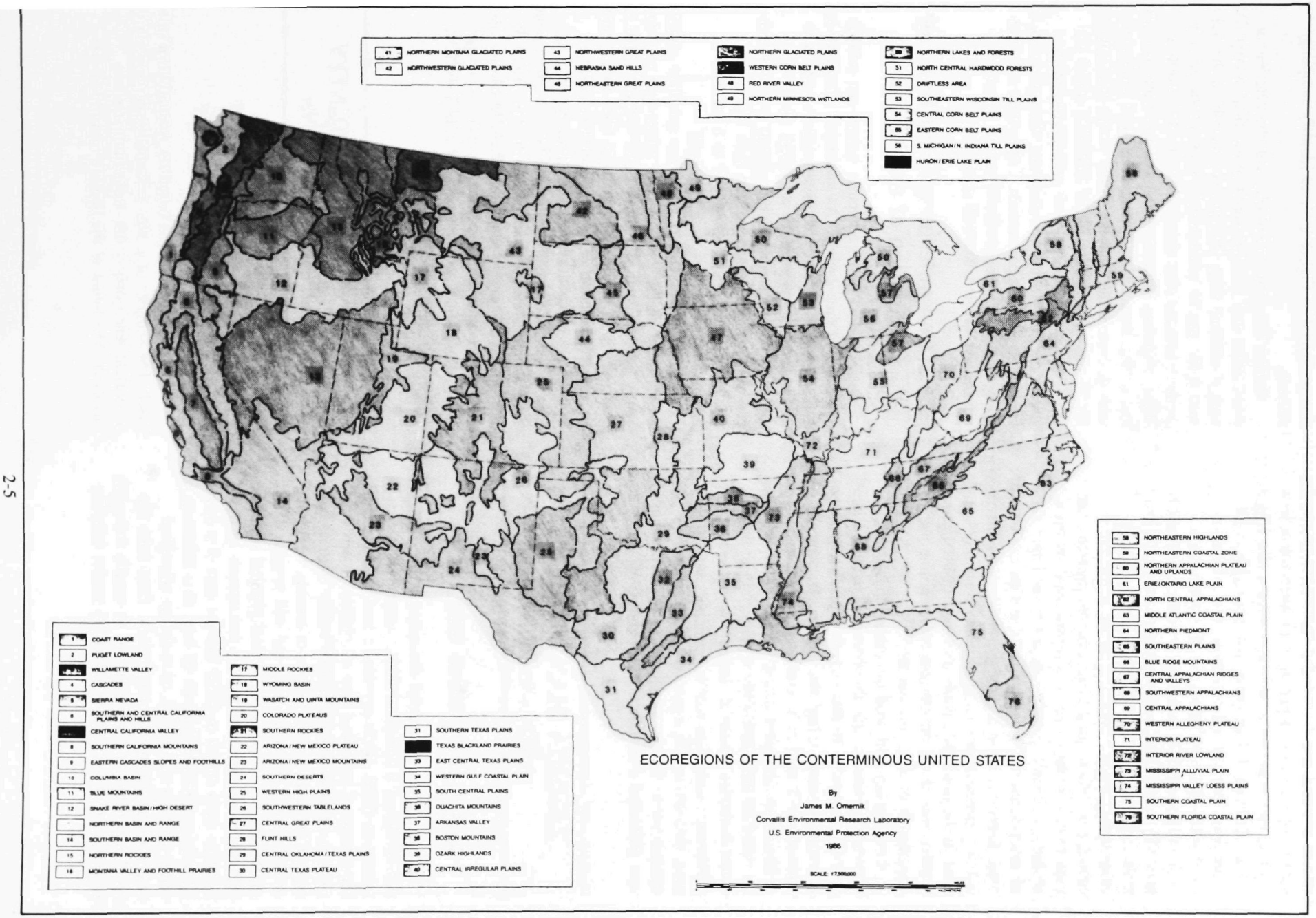


Figure 2.5-1. Ecoregions of the conterminous United States (after Omernik 1986).

stream segment may be wooded (deciduous or coniferous) or open, within a perennial or intermittent flow regime, and represent a particular stream size (Figure 2.5-2). Individual descriptors may not apply to all ecoregions, nor will all conditions (i.e., deciduous, coniferous, open) be present in all stream sizes.

The final rapid bioassessment guidance should be generally applicable to all ecoregions of the United States, although specific elements and evaluation criteria may require modification for particular ecoregions. When rapid bioassessment protocols are used to assess impact sources (upstream-downstream studies), reference criteria may not be as important if an unimpacted site-specific control station can be sampled. However, when a synoptic ("snapshot") survey is being conducted or an appropriate control does not exist in the immediate study area, use of idealized criteria may be the only means of discerning use impairment or assessing impact.

Each agency will need to evaluate the generic criteria suggested in this document for inclusion into specific programs. To this end, the application of the ecoregion concept versus the site-specific control approach will need to be evaluated by each agency. It is likely that additional investigation will be needed to: delineate areas that differ significantly in their innate biological potential; locate reference sites within each ecoregion that fully support aquatic life uses; and develop biological criteria (e.g., define optimal values for the metrics recommended) using data generated from one of the higher level protocols.

2.6 DATA MANAGEMENT AND ANALYSIS

2.6.1 Integration into BIOS

The U.S. Environmental Protection Agency (EPA) has developed a biological data management system known as BIOS. BIOS provides a centralized system for storage of biological data in addition to analytical tools for data analysis. The field survey file component of BIOS provides a means of storing, retrieving, and analyzing biosurvey data, and will process data on the distribution, abundance, and physical condition of aquatic organisms, as well as descriptions of their habitats. Data stored in BIOS become part of a comprehensive database that can be used as a reference, to refine analysis techniques, or to define ecological requirements for aquatic populations. Data from the rapid bioassessment protocols can be readily managed with the BIOS field survey file using header information presented in Figure 2.6-1 to identify sampling stations.

Habitat information and physical characterization information may also be stored in the field survey file with abundance data. Parameters available in the field survey file can be used to store some of the environmental characteristics associated with the sampling event, including physical characteristics, water quality, and habitat assessment. Physical/chemical parameters with discrete values may be stored in the field survey file or the water quality file of STORET, under the same station description. Such parameters include stream depth, velocity, and substrate characteristics, as well as many other parameters. The system will also allow storage of other pertinent station or sample information in the comments section.

2.6.2 Computerizing Field Data for Calculation of the Metrics

Entering data into a computer system can provide a substantial time savings. An additional advantage to computerization is analysis documentation, which is an important component for a QA/QC plan. An agency conducting rapid bioassessment programs can choose an existing system within their agency or utilize the BIOS system developed as a national database system.

The field survey file of BIOS can calculate several metrics used in the RBPs. Metric values that may currently be calculated in a BIOS PGM = TAXATABLE retrieval report include taxa richness, EPT Index, and percent contribution of the dominant taxon. Other metrics are planned as future additions to the field survey file. Additional metrics can be calculated using SAS, which is easily accessible to the file. BIOS may also be used to create a machine-readable file for use as input to either a user-written program or to an external analytical software package (SPSS, BMDP, dBase III).

2.7 BENTHIC COMMUNITY CONSIDERATIONS

2.7.1 Seasonality for Benthic Collections

Rapid bioassessment is based on evaluation of relatively few samples at a site. Seasonality is particularly important when only a few collection sites are involved. The intent of the benthic rapid bioassess-

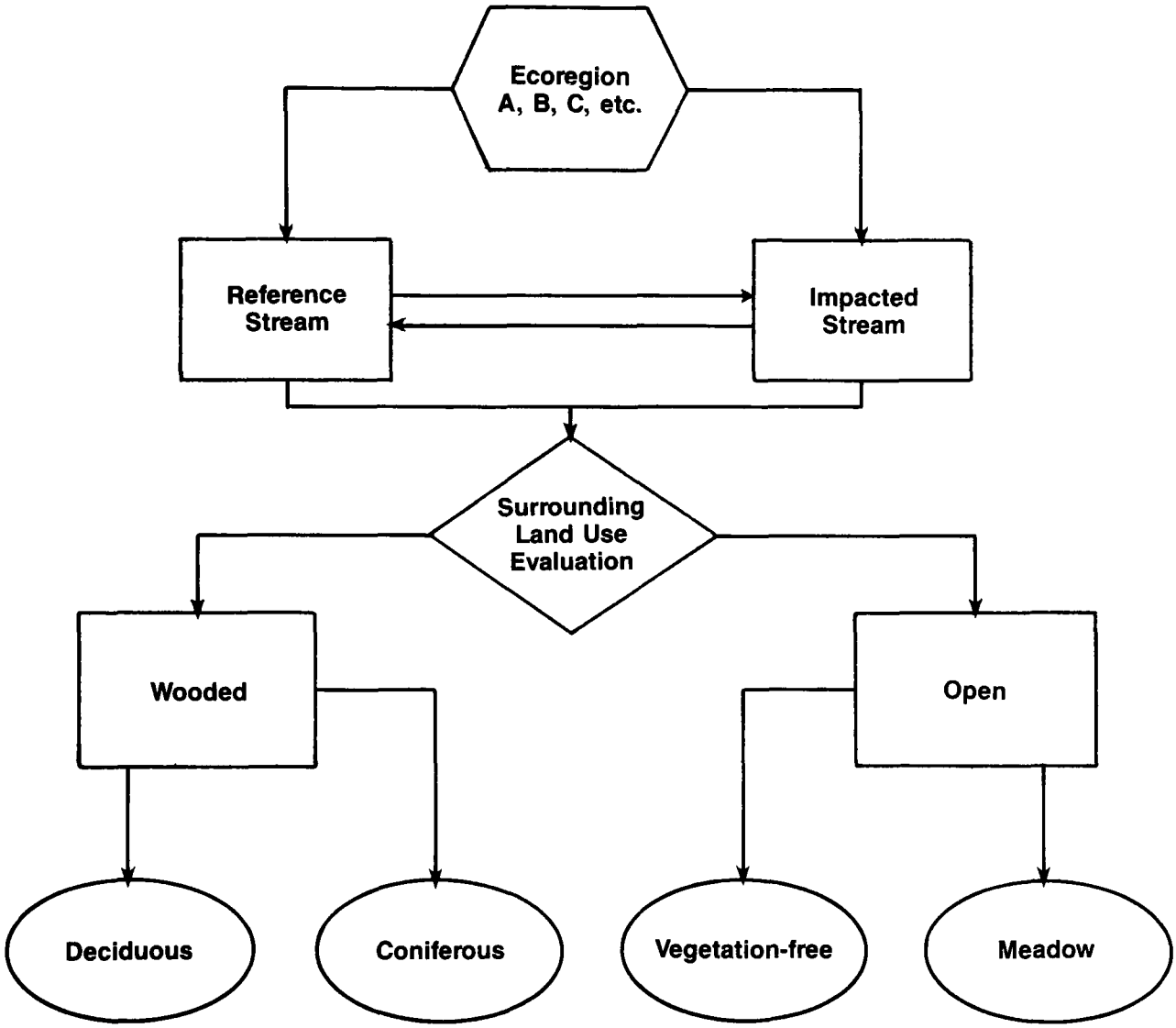


Figure 2.5-2. Flowchart illustrating potential delineation of reference sites within an ecoregion.

Waterbody Name _____ Location _____

Reach/Milepoint _____ Latitude/Longitude _____

County _____ State _____ Aquatic Ecoregion _____

Station Number _____ Investigators _____

Date _____ Time _____ Agency _____

Hydrologic Unit Code _____ Form Completed by _____

Reason for Survey _____

_____

_____

_____

Figure 2.6-1. Header information used for documentation and identification for sampling stations.

ment is to evaluate overall biological condition, optimizing the use of the benthic community's capacity to reflect integrated environmental effects over time. Ideally, the optimal biological sampling season will correspond to recruitment cycles of the invertebrates. Maximum information for a benthic community is obtained when most benthic macroinvertebrates are within a size range (later instars) retained during standard sieving and sorting, and can be identified with the most confidence.

Reproductive periods and different life stages of aquatic insects are related to the abundance of particular food supplies (Cummins and Klug 1979). Peak emergence and reproduction typically occur in the spring and fall, although onset and duration vary somewhat across the United States. During peak reproduction, approximately 80 percent of the macroinvertebrates will be too small to be captured in sufficient numbers to accurately characterize the community. Additionally, food source requirements for early instars are different from those for later instars. Therefore, the biologically optimal sampling season would occur when the habitat is utilized most heavily by later instars and the food resource has stabilized to support a balanced indigenous community.

Field collections scheduled to correspond to seasonal recruitment cycles of invertebrates will provide the optimal biological sampling period. However, sampling during these optimal biological periods may not be logistically feasible due to adverse weather conditions, manpower availability, scheduling constraints, or other factors. Additionally, an agency may be required to perform biological sampling during periods of greatest environmental stress such as low flow/high temperature periods for point-source discharges or high flow/runoff periods for nonpoint-source discharges. Although an estimate of benthic community structure during optimal biological conditions is expected to reflect effects of, or recovery from, environmental stress periods (Ohio EPA 1987a), assessment of worst-case conditions may be needed under certain permitting regulations, or as a follow-up to sampling during biologically optimal periods, where impairment is detected.

Optimal biological conditions for sampling vary with climate. Seven major climatological regions are represented within the United States (Figure 2.7-1). Temperature and/or rainfall are the principal factors influencing optimal biological conditions in each climatological region. Several ecoregions are represented within each of these climatological regions. Some scaling of the optimal collection period will be necessary, depending on the elevation of the site and the habitat type.

2.7.2 Benthic Sampling Methodology

2.7.2.1 Natural and Artificial Substrates

The benthic RBPs employ direct sampling of natural substrates. Because routine evaluation of a large number of sites is a primary objective of the RBPs, artificial substrates were eliminated from consideration due to time required for both placement and retrieval, and the amount of exposure time required for colonization. However, where conditions are inappropriate for the collection of natural substrate samples, artificial substrates may be an option. Artificial substrates may be useful in situations such as large rivers, where an impact is attributable to physical alteration and channelization or chemical effects. Artificial substrates may be used to separate the two impact sources.

Advantages and disadvantages of artificial substrates (Cairns 1982) relative to the use of natural substrates are presented below.

Advantages of Sampling With Artificial Substrates

1. Artificial substrates allow sample collection in locations that are typically difficult to sample effectively (e.g., bedrock, boulder, or shifting substrates; deep or high velocity water).
2. As a "passive" sample collection device, artificial substrates permit standardized sampling by eliminating subjectivity in sample collection technique. Direct sampling of natural substrate requires similar effort and degree of efficiency for the collection of each sample. Use of artificial substrates requires standardization of setting and retrieval; however, colonization provides the actual sampling mechanism.
3. Confounding effects of habitat differences are minimized by providing a standardized microhabitat. Microhabitat standardization may promote selectivity for specific organisms if the artificial substrate provides a different microhabitat than that naturally available at a site (see Disadvantage 2). Most artificial substrates, by design, select for the Scraper and Filtering Collector communities, which are the macroinvertebrate communities emphasized in this document. However, in some situations, accumulation of debris may cause a predominance of Collector-Gatherers (Hilsenhoff, personal communication).
4. Sampling variability is decreased due to a reduction in microhabitat patchiness, improving the potential for spatial and temporal similarity among samples.
5. Sample collection using artificial substrates may

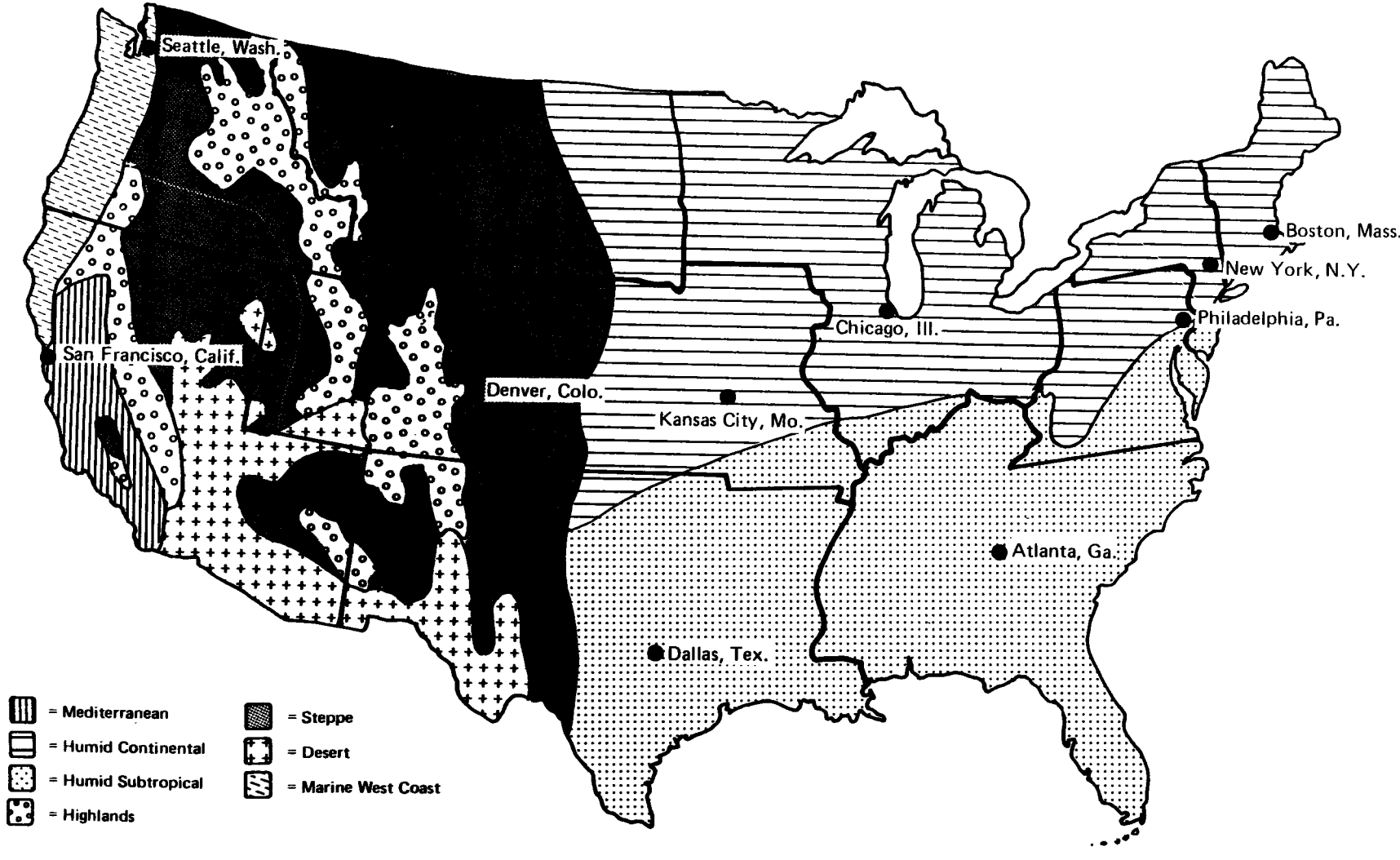


Figure 2.7-1. Classification of U.S. climatological regions. (Taken from Gabler et al. 1976.)

require less skill and training than direct sampling of natural substrates. Depending on the type of artificial substrate used, properly trained technicians could place and retrieve the substrates. However, an experienced specialist should be responsible for the selection of habitats and sample sites.

Disadvantages of Sampling With Artificial Substrates

1. Two trips (one to set and one to retrieve) are required for each artificial substrate sample; only one trip is necessary for direct sampling of the natural substrate. Artificial substrates require a long (8-week average) exposure period for colonization. This decreases their utility for certain rapid biological assessments.
2. Samples may not be fully representative of the benthic community at a station if the artificial substrate offers different microhabitats than those available in the natural substrate. Artificial substrates often selectively sample certain taxa, misrepresenting relative abundances of these taxa in the natural substrate. Artificial substrate samples would thus indicate colonization potential rather than the resident community structure. This could be advantageous if a study is designed to isolate water quality effects from substrate and other microhabitat effects. Where habitat quality is a limiting factor, artificial substrates could be used to discriminate between physical and chemical effects and assess a site's potential to support aquatic life on the basis of water quality alone.
3. Sampler loss or perturbation commonly occurs due to sedimentation, extremely high or low flows, or vandalism during the relatively long (at least several weeks) exposure period required for colonization.
4. Depending on the configuration of the artificial substrate used, transport and storage can be difficult. The number of artificial substrate samplers required for sample collection increases such inconvenience.

2.7.2.2 Single and Multiple Habitat Sampling

A central issue in the development of the rapid bioassessment protocols has been whether sampling all available habitats is necessary to evaluate biological integrity at a site, or if sampling only selected habitats will provide a sufficient characterization. The pilot study in North Carolina (see Section 6.4) addressed this issue and indicated that the riffle community and the multihabitat assemblage responded similarly to differences among stations. For example, under stress, taxa richness was reduced by the same proportion in both the riffle community and the multihabitat assemblage at a given station. These responses suggest that either the riffle community or the multihabitat assemblage will give a good assessment of biotic integrity but assessing both may be redundant.

The sampling of a single habitat type (e.g., riffle/run) is intended to limit the variability inherent in sampling natural substrates. Kicknet samples are used in the RBPs because they have been shown to provide good statistical replication (Pollard 1981). However, some streams lack the cobble substrate (riffle/run) to support the periphyton-based benthic community emphasized in the RBPs. In this case, an alternate habitat(s) will need to be sampled. Some State agencies, such as North Carolina DEM, have been successful in using a multihabitat sampling approach, and advocate this technique as being more appropriate in North Carolina than simply sampling the riffle/run habitat.

Discussions at the 1987 Biocriteria Workshop (U.S. EPA 1988) indicated strong support for multihabitat sampling where time and resources permit and the particular region or specific study emphasizes non-Scraper communities. It was generally agreed, however, that samples from various habitats should be processed and analyzed separately. Data can always be aggregated after individual samples are analyzed and tabulated, but potentially important comparisons among habitats are lost if samples are composited.

2.7.2.3 Sampling Coarse Particulate Organic Material (CPOM)

In addition to sampling the riffle habitat, the benthic RBPs recommend that a Coarse Particulate Organic Material (CPOM) sample also be collected (Sections 6.2.1.1 and 6.3.1.1). In lotic systems, CPOM generally exists in the form of plant debris (leaves, needles, twigs, bark) which accumulates in depositional areas. The rationale for collecting the CPOM samples is that, as a group, Shredders should be particularly affected by toxic pollutants that often adsorb to CPOM (Cummins 1987, personal communication). Toxicants adsorbed to CPOM affect Shredders directly through ingestion and indirectly by killing attached microbes that "prepare" CPOM for Shredder consumption. In a study by Newman et al. (1987), amphipod Shredders colonizing litter bags were significantly reduced in numbers by 230 5g/L total residual chlorine.

The CPOM sample is processed separately and

the organisms are identified as Shredders or Non-Shredders (Sections 6.2.1.1 and 6.3.1.1). Taxonomic identifications are not necessary in that most aquatic insects can be classified to Functional Feeding Group on the basis of morphological and behavioral features using the procedures in Cummins and Wilzbach (1985). These counts are used to calculate the ratio of Shredders to the total number of individuals collected, one of the eight metrics used in the final biosurvey analysis.

2.7.3 Benthic Sample Processing and Enumeration

One of the underlying goals of this guidance is to promote consistency in the conduct of rapid bioassessments. In RBP II, consistent sample handling is very important because relatively detailed comparisons are made among stations and sites. The RBP II 100-count subsampling procedure is adapted from Hilsenhoff (1987b) and is performed in the field. The level of effort and subsampling process used for RBP III is similar to RBP II, except subsampling is conducted in the laboratory. This laboratory subsampling procedure provides a more standard unit of effort.

Much of the useful information regarding assessment of biological condition can be obtained from a relatively small subsample, such as the 100-count subsample recommended in this document (see Section 6.4). However, an agency may be willing to expend additional time to attain a higher degree of resolution. In this case, a 200- or 300-count subsample may be selected. Some agencies may wish to process the entire sample for analysis.

2.7.4 Benthic Environmental Tolerance Characterizations

Assessment of biological condition using the benthic protocols presented in this document is based on the calculation of several metrics. Certain metrics rely on classification of benthic taxa according to their relative sensitivity to pollution. This approach reflects the longstanding indicator species concept, with sensitivity primarily related to responses of species to organic pollution. Responses to toxicants are also incorporated into the tolerance characterizations, but to a lesser extent. Evaluation of toxic effects is addressed primarily at the Functional Feeding Group level.

The specific tolerance characterizations used in the RBPs were obtained from Hilsenhoff (1987b, 1988). Hilsenhoff's species level tolerance characterization system was selected due to its extensive use across the country. A more recently developed family level system (Hilsenhoff 1988) has not been used extensively, but was based on Hilsenhoff's original widely accepted index. Several other general tolerance classification systems are fairly well established, generally applicable, and may also be used as guidelines. Some of these are listed in Appendix C. Additional biotic indices are also listed in U.S. EPA 1983. However, types of pollution and causes of impairment will differ regionally, and the meaning of "pollution tolerance" may vary among regions. Therefore, optimal implementation of the tolerance characterization approach requires that each State agency refine established tolerance classification systems for their own use. Winget and Mangum (1979) have developed a tolerance classification based on nonpoint-source effects (Biotic Condition Index). This classification may prove useful as a substitute for Hilsenhoff's when evaluating nonpoint-source problems.

2.8 FISH COMMUNITY CONSIDERATIONS

2.8.1 Seasonality for Fish Collections

Seasonal changes in the relative abundances of the fish community primarily occur during reproductive periods and (for some species) the spring and fall migratory periods. However, because larval fish sampling is not recommended in this protocol, reproductive period changes in relative abundance are not of primary importance.

Generally, the preferred sampling season is mid to late summer, when stream and river flows are moderate to low, and less variable than during other seasons. Although some fish species are capable of extensive migration, fish populations and individual fish tend to remain in the same area during summer (Funk 1957; Gerking 1959; Cairns and Kaesler 1971). The Ohio Environmental Protection Agency (Rankin 1987, personal communication) confirmed that few fishes in perennial streams migrate long distances. Hill and Grossman (1987) found that the three dominant fish species in a North Carolina stream had home ranges of 13 to 19 m over a period of 18 months. Ross et al. (1985) and Matthews (1986) found that stream fish assemblages were stable and persistent for 10 years, recovering rapidly from droughts and floods indicating that large population fluctuations are unlikely to occur in response to purely natural environmental phenomena. However, comparison of data collected during different seasons is discouraged, as is data collected during or immediately after major flow changes.

2.8.2 Fish Sampling Methodology

2.8.2.1 Use of Electrofishing, Seining, and Rotenoning

Although various gear types are routinely used to sample fish, electrofishers, seines, and rotenone are the most commonly used collection methods in freshwater habitats. Each method has advantages and disadvantages (Nielsen and Johnson 1983; Hendricks et al. 1980). However, electrofishing is recommended for most fish field surveys because of its greater applicability and efficiency. Local conditions may require consideration of seining and rotenoning as optional collection methods. Advantages and disadvantages of each gear type are presented below.

Advantages of Electrofishing

1. Electrofishing allows greater standardization of catch per unit of effort.
2. Electrofishing requires less time and manpower than some sampling methods (e.g., use of ichthyocides) (Hendricks et al. 1980).
3. Electrofishing is less selective than seining (although it is selective towards size and species) (Hendricks et al., 1980). (See disadvantage number 2).
4. If properly used, adverse effects on fish are minimized.
5. Electrofishing is appropriate in a variety of habitats.

Disadvantages of Electrofishing

1. Sampling efficiency is affected by turbidity and conductivity.
2. Although less selective than seining, electrofishing is size and species selective. Effects of electrofishing increase with body size. Species specific behavioral and anatomical differences also determine vulnerability to electroshocking (Reynolds 1983).
3. Electrofishing is a hazardous operation that can injure field personnel if proper safety procedures are ignored.

Advantages of Seining

1. Seines are relatively inexpensive.
2. Seines are lightweight and are easily transported and stored.
3. Seine repair and maintenance are minimal and can be accomplished onsite.
4. Seine use is not restricted by water quality parameters.
5. Effects on the fish population are minimal because fish are collected alive and are generally unharmed.

Disadvantages of Seining

1. Previous experience and skill, knowledge of fish habitats and behavior, and sampling effort are probably more important in seining than in the use of any other gear (Hendricks et al. 1980).
2. Seining sample effort and results are more variable than sampling with electrofishing or rotenoning.
3. Seine use is generally restricted to slower water with smooth bottoms, and is most effective in small streams or pools with little cover.
4. Standardization of unit of effort to ensure data comparability is difficult.

Advantages of Rotenoning

1. The effective use of rotenone is independent of habitat complexity.
2. Rotenoning provides greater standardization of unit of effort than seining.
3. Rotenoning has the potential, if used effectively, to provide more complete censusing of the fish population than seining or electrofishing.

Disadvantages of Rotenoning

1. Use of rotenone is prohibited in many States.
2. Application and detoxification can be time and manpower intensive.
3. Effective use of rotenone is affected by temperature, light, dissolved oxygen, alkalinity, and turbidity (Hendricks et al. 1980).
4. Rotenoning typically has a high environmental impact; concentration miscalculations can produce substantial fish kills downstream of the study site.

2.8.2.2 Sampling Representative Habitat

The sampling approach advocated in fish RBP V optimizes the conservation of manpower and resources by sampling areas of representative habitat. The fish survey provides a representative estimate of the fish community at all habitats within a site, and a realistic sample of fish likely to be encountered in the waterbody. When sampling large streams, rivers, or waterbodies with complex habitats, a complete inventory of the entire reach is not necessary for the level of assessment used in RBP V. The sampling area should be representative of the reach, incorporating riffles, runs, and pools if these habitats are typical of the stream in question. Although a sampling site with two riffles, two runs, and two pools is preferable, at least

one of each habitat type should be evaluated. Mid-channel and wetland areas of large rivers, which are difficult to sample effectively, may be avoided. Sampling effort may be concentrated in near-shore habitats where most species will be collected. In doing so, some deep water or wetland species may be under-sampled, however, the data should be adequate for the objective of RBP V.

2.8.3 Fish Sample Processing and Enumeration

To ensure data comparability for assessing biological condition with fish RBP V, sample processing and species enumeration must be standardized.

Processing of the fish biosurvey sample includes identification of all individuals to species, weighing (if biomass data are desired), and recording incidence of external anomalies. It is recommended that each fish be identified and counted. Subsamples of abundant species may be weighed if live wells are unavailable. (This is especially important for warmwater sites, where handling mortality is highly probable.) The data from the counted and weighed subsample is extrapolated for the total. Ohio EPA (1987a) found that subsampling reduced potential error and made the extra time required for weighing insignificant. Procedural details for subsampling are presented in Ohio EPA 1987c. Determination of trophic guild designation is also necessary for some IBI metrics.

2.8.4 Fish Environmental Tolerance Characterizations

Use of the IBI in fish RBP V requires classification of fish species in terms of environmental tolerance. Responses of individual species to pollution will vary regionally and according to the type of pollutant. Tolerance characterizations of selected midwestern and northwestern fish species are presented in Appendix D. Effective use of the tolerance characterization approach requires an appropriate regional tolerance characterization system. Regional modifications or substitutions may be based upon regional fish references, historical distribution records, objective assessment of a large statewide database, and toxicological test data. IBI tests in the southeastern and southwestern United States, and its widespread use by water resource agencies may result in additional modifications. Past modifications have occurred (Section 7.2.2.1, Miller et al. 1988a) without changing the IBI's basic theoretical foundations.

3. OVERVIEW OF PROTOCOLS AND SUMMARY OF COMPONENTS

The bioassessment protocols presented in this document provide guidance to those agencies not presently using biosurveys as investigative tools or who are seeking alternatives to their present methods. Agencies with successful biological monitoring programs are encouraged to continue their programs and provide further leadership and guidance in the use of bioassessment. The five separate protocols presented below reflect different levels of effort and expertise and focus on different objectives.

3.1 SUMMARY OF THE PROTOCOLS

A summary of the key features of the five protocols is presented in Table 3.1-1. The first and fourth protocols are subjective because an investigator or agency may conduct any level of investigation deemed necessary. The presence or absence of impairment using Rapid Bioassessment Protocols (RBPs) I and IV is supported by a limited analysis of the biological communities. Benthic RBP I and fish RBP IV are used as screening or reconnaissance techniques for discerning biological impairment. Benthic RBPs II and III, and fish RBP V are progressively more rigorous and are intended to provide more objective and reproducible evaluations than RBPs I and IV. RBPs II, III, and V are designed to be semi-quantitative and utilize an integrated analysis technique to provide continuity in the evaluation of impairment among sites and seasons. The primary difference between RBPs II, III and V is the level of taxonomic resolution (i.e., family level vs. genus/species level identification) necessary to perform an assessment. RBPs III and V require more time and expertise than RBP II, but are better able to discriminate degrees of impairment.

3.2 OBJECTIVES OF THE PROTOCOLS

As presented in Figure 3.2-1, selection of the appropriate bioassessment approach depends on the objectives of the study. RBPs I and IV provide a screening mechanism for identification of biological impairment; they are not intended to quantify the degree of impairment nor provide definitive data that would be used to establish a cause-and-effect. RBPs I and IV allow a cursory assessment incorporating the cost and time efficiencies necessary to evaluate a large number of sites. RBPs I and IV are used primarily to identify major water quality problems as an aid in planning and developing management strategies.

The information derived from benthic RBP II provides a basis for ranking sites as severely or moderately impaired. This classification can then be used to focus additional study or regulatory action. RBP II can also be used as a screening technique in lieu of benthic RBP I. Like RBP I, RBP II is designed to enable agencies to evaluate a large number of sites with relatively limited time and effort. However, the concept of a documented procedure for collections, inherent in RBP II and intended to promote a consistent level of effort, allows for better comparison among sites.

The primary objective of benthic RBP III and fish RBP V is to provide a consistent, well-documented biological assessment. Repeatable results provide a basis for comparison among sites over time (trend monitoring). The ability to discriminate the level of impairment among sites is enhanced by performing taxonomic identifications to the lowest practical level, thereby providing information on population as well as community level effects. RBPs III and V can be used to rank sites according to impairment in lieu of RBP II, but will also establish a basis for trend monitoring over a period of time. RBPs III and V place still greater emphasis on consistency in unit effort and documentation.

3.3 LEVEL OF EFFORT AND INVESTIGATOR EXPERTISE

The level of effort required for RBP I is estimated to be 1 to 2 hours per station, excluding travel time. The effort consists of habitat assessment, physicochemical measurements, and biological collections and observations. All of this effort is expended in the field. An additional 0.5 to 1 hour of data analysis and

TABLE 3.1-1 COMPARISON OF RAPID BIOASSESSMENT PROTOCOLS

	Protocol I	Protocol II	Protocol III	Protocol IV	Protocol V
Objectives	. Determine whether biological impairment exists . Determine if further investigation is needed	. Assess biological impairment . Provide information for ranking sites . Prioritize sites for further assessment and/or testing (toxicity, chemical)	. Assess biological impairment . Establish basis for trend monitoring . Prioritize for further assessment and/or testing (toxicity, chemical)	. Determine whether biological impairment exists . Determine if further investigation is needed	. Assess biological impairment . Establish basis for trend monitoring . Provide information for ranking sites . Prioritize for further assessment and/or testing (toxicity, chemical)
Level of Effort (per station)	. Field--1 to 2 hours/person (1 person) . Lab--None	. Field--1.5 to 2.5 hours/person (2 persons) . Lab--None	. Field--1 to 2 hours/person (2 persons) . Lab--2 to 3 hours (1 person)	. Field--None . Lab--None	. Field--1-5 hours/person (2 persons minimum) . Lab--None
Experience Required	. Data--0.5 to 1 hour (1 person) . Budget--Total of 1.5 to 3 work hours	. Data--2 to 4 hours (1 person) . Budget--Total of 5 to 9 work hours	. Data--1 to 3 hours (1 person) . Budget--Total of 5 to 10 work hours	. Data--3 hours (1 person) . Budget--Total- of 3 work hours	. Data--1-2 hours (1 person) . Budget--Total of 3 to 17 work hours
	. High level of professional impact assessment experience . Knowledge of benthic invertebrate ecology	. Professional impact assessment experience . Knowledge of benthic ecology and taxonomy	. Professional impact assessment experience . Knowledge of benthic ecology and taxonomy	. Survey design experience . Knowledge in broad patterns of fish species distribution and abundance	. Professional impact assessment experience . Knowledge in the use of the IBI and IWB
Minimal Skill Mix	. Biologist	. Biologist and technician	. Biologist and technician	. Biologist	. Biologist and technician(s)
Habitat Assessment	. Characterize and rate substrate/instream cover, channel morphology, and riparian/bank structure	. Characterize and rate substrate/instream cover, channel morphology, and riparian/bank structure	. Characterize and rate substrate/instream cover, channel morphology, and riparian/bank structure	. Characterize and rate substrate/instream cover, channel morphology, and riparian/bank structure	. Characterize and rate substrate/instream cover, channel morphology, and riparian/bank structure
Water Quality and Phys/Chem	. Measure conventional water quality parameters . Examine physical characteristics	. Measure conventional water quality parameters . Examine physical characteristics	. Measure conventional water quality parameters . Examine physical characteristics	. Measure conventional water quality parameters . Examine physical characteristics	. Measure conventional water quality parameters . Examine physical characteristics

TABLE 3.1-1 (Cont.)

	Protocol I	Protocol II	Protocol III	Protocol IV	Protocol V
Biosurvey	. Cursory examination . Determine relative abundance of macrobenthos; field IDs	. Examination focusing on the riffle/run community, supplemented with a CPOM sample . 100-organism subsample IDed in field to family or order level . Functional Feeding Group analysis of riffle/run and CPOM sample in the field	. Examination focusing on the riffle/run community, supplemented with a CPOM sample . Collect riffle/run benthos; collect CPOM sample, determine Shredder abundance . Preserve riffle/run sample, return to lab, do 100-organism subsample, IDs to species level and Functional Feeding Group analysis	. Questionnaire survey . Survey ecoregional reference reaches and randomly selected reaches	. Examination with sampling of all major habitats and cover types . Collect fish, note condition, ID to species level in the field . Preserve voucher collection for deposit in museum
Analysis	. Minimal; determine presence or absence of impairment	. Integrated assessment of metrics measuring various components of family level community structure	. Integrated assessment of metrics measuring various components of genus/species level community structure	. Summarize survey responses to determine degree and probable cause of impairment	. Integrated assessment of metrics measuring various components of species, family, and trophic level community structure
Conclusion	. Determine if impairment exists . Indicate generic cause of impairment (habitat, organic enrichment, toxicity)	. Characterize conditions as no impairment, moderate impairment, severe impairment . Indicate generic cause of impairment (habitat, organic enrichment, toxicity)	. Evaluate site as no impairment, slight impairment, moderate impairment, severe impairment . Indicate generic cause of impairment (habitat, organic enrichment, toxicity)	. Determine if impairment exists . Indicate generic cause of impairment (habitat, water quality)	. Evaluate biological integrity as excellent, good, fair, poor, very poor . Indicate generic cause of impairment (habitat, organic enrichment, toxicity)

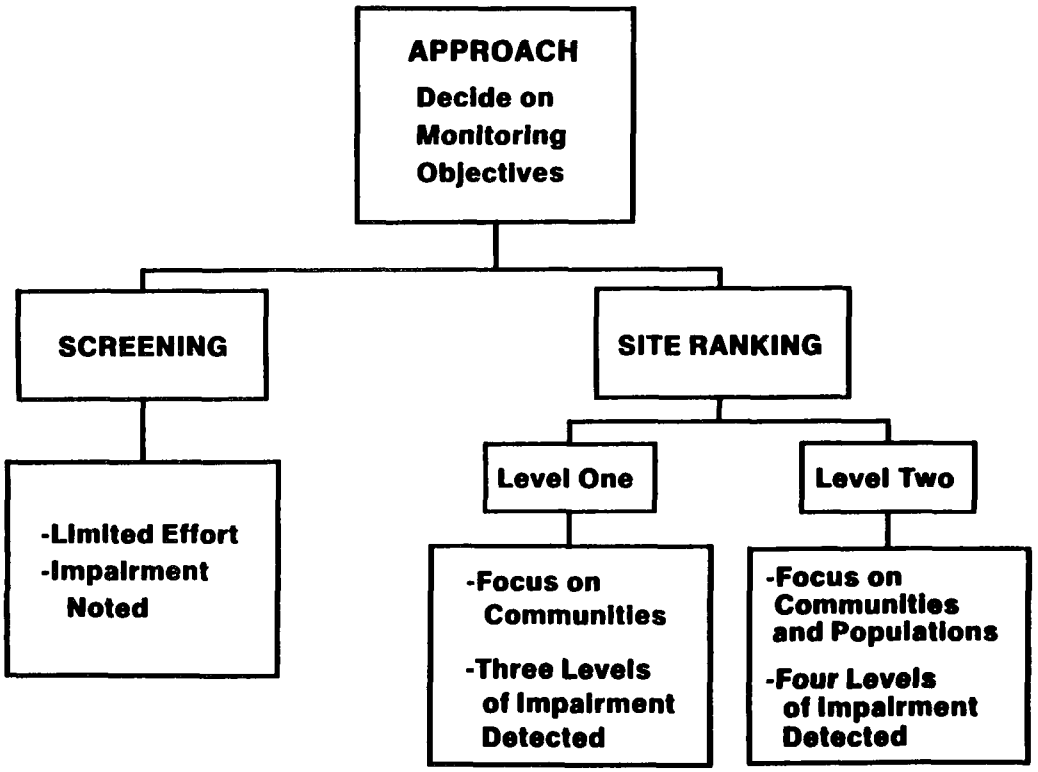


Figure 3.2-1. Overview of the five bioassessment approaches and their primary objectives.

evaluation might be needed. No laboratory analysis is anticipated for RBP I. Only a single experienced biologist is needed to conduct RBP I. However, a second person, for reasons of safety, quality control, or training, may be assigned by the agency.

The biologist conducting RBP I should have professional impact assessment experience with knowledge of benthic invertebrate ecology. A HIGHLY EXPERIENCED INDIVIDUAL IS REQUIRED. The accuracy of the assessment depends upon the biologist's professional integrity.

The field survey for RBP II can be completed by two persons in 1.5 to 2.5 workhours per person for each station, excluding travel time. The necessity for a reconnaissance survey (similar to RBP I) is somewhat dependent on agency familiarity with the site and purpose of the investigation. Habitat assessment, physicochemical measurements, and biological collections/ observations are also required in this protocol. All of the sorting and identifications (family level) are done in the field to minimize laboratory time. RBP II requires one field investigator experienced in impact assessment, benthic ecology, and taxonomy. The second field person can be a trained technician with a biological background. Data analysis may take from 1 to 2 hours per station and should be performed by an experienced biologist. Use of computers for data entry and calculation of results provides optimal time efficiency for data analysis. Hand calculation of results may require an additional 1 to 2 hours per station for data analysis.

The field effort for RBP III can be done by two biologists in 1 to 2 workhours per biologist for each station. RBP III is the most detailed of the benthic protocols and provides a means of obtaining repeatable results; the sorting and identification tasks are conducted in the laboratory. Laboratory processing is estimated to take 2 to 3 hours per station for one biologist. Data analysis is expected to take an additional 1 to 3 hours per station for one biologist. One of the field investigators must be experienced in impact assessment of benthic communities. This investigator should also perform the data analysis to provide continuity throughout the assessment process. The person performing the laboratory analysis needs to be experienced in taxonomy, but does not necessarily need to perform the final assessment. Data analysis can be expedited by computerized data entry for calculation of results. An additional 1 to 2 hours per station may be required if results are hand calculated. As field and analysis procedures are mastered, time efficiencies can be expected.

The level of field effort for RBP IV requires 1 to 3 work hours per reach. This includes reach selec-

tion, respondent identification, mailing, questionnaire completion, and response tabulation. No additional field or laboratory work is required and no travel or equipment are necessary. All effort is expended in the office and is independent of season or weather conditions. The designer of the survey must be familiar with survey design. Respondents should also be familiar with broad patterns in species distribution and abundance. The accuracy of statements depends on the respondent's professional knowledge and integrity. Only one respondent is needed per reach but some confirmation from additional sources and methods is advisable for quality assurance.

The level of effort for RBP V includes 1 to 5 hours for fish collection and identification (depending upon habitat complexity and gear used), and 1 to 2 hours for data analysis. Data analysis can be performed by one biologist. The field effort requires a minimum of 2 persons; up to 5 people may be needed in wide shallow rivers and rivers with complex habitats. Typically, using this protocol, stream sites can be sampled and data analyzed within a total of 5 hours. At least one biologist should have professional impact assessment experience, with specific knowledge in the use of the Index of Biotic Integrity (IBI) and the Index of Well Being (IWB). This biologist should be involved in all phases of the protocol to improve continuity and to provide greater insight. The remaining crew members can be experienced technicians. Sample quality can be assessed by replicate sampling and inter-crew sampling.

4. QUALITY ASSURANCE/QUALITY CONTROL

Effective quality assurance and quality control (QA/QC) procedures and a clear delineation of QA/QC responsibilities are essential to ensure the utility of environmental monitoring data. The term "quality control" refers to the routine application of procedures for obtaining prescribed standards of performance in the monitoring and measurement process. The term "quality assurance" includes the quality control functions and involves a totally integrated program for ensuring the reliability of monitoring and measurement data.

4.1 PROGRAM DESCRIPTION

The U.S. EPA QA/QC program requires that all EPA national program offices, EPA regional offices, and EPA laboratories participate in a centrally planned, directed, and coordinated Agency-wide QA/QC program. This requirement also applies to efforts carried out by the States and interstate agencies that are supported by EPA through grants, contracts, or other formalized agreements. The EPA QA program is based upon EPA order 5360.1, "Policy and Program Requirements to Implement the Quality Assurance Program" (U.S. EPA 1984a), which describes the policy, objectives, and responsibilities of all EPA Program and Regional offices.

Each office or laboratory that generates data under EPA's QA/QC program must implement, at a minimum, the prescribed procedures to ensure that precision, accuracy, completeness, comparability, and representativeness of data are known and documented.

4.2 DATA QUALITY OBJECTIVES

A full assessment of the data quality needed to meet the intended use should be made prior to specification of QA/QC controls. The determination of data quality is accomplished through the development of data quality objectives (DQOs). DQOs are qualitative and quantitative statements developed by data users to specify the quality of data needed to support specific decisions or regulatory actions. Establishment of DQOs involves interaction of decision-makers and the technical staff.

The process for developing DQOs includes a first stage involving input by the decision-maker regarding the information needed, reasons for the need, how the information will be used, and specification of any time and resource constraints. The second stage in developing DQOs involves clarification of the specific problem. Here, the technical staff and decision-maker interact to establish a detailed specification of the problem and any constraints imposed on data collection. The third stage involves developing alternative approaches to data collection, selecting the approach to be used, and establishing the final data quality objectives. Once the data collection approach and data quality objectives have been established, a clear understanding of data quality will help ensure successful study completion. U.S. EPA (1984b) describes the process for developing DQOs in more detail.

4.3 QUALITY ASSURANCE PROGRAM PLANS AND PROJECT PLANS

To provide adequate control and guidance, the Agency's QA program relies on the development and implementation of two QA documents: the QA Program Plan and the QA Project Plan. These plans are required of all recipients of EPA grants and assistance programs. Grant regulations, 40 CFR Part 30, require submission of QA Program Plans to EPA as a prior condition of receiving an EPA grant. QA Project Plans also must be developed according to an acceptable schedule within the QA Program Plan. The QA Program Plan (U.S. EPA 1980a) describes management policies, organization, objectives, principles, and general procedures that establish how data of known and acceptable quality will be produced. The QA Project Plan describes and defines specific objectives, network design, procedures, methods, and controls that will be applied to a specific project to ensure the production of data of known and acceptable quality. Two guidance documents are available to assist in preparation of the QA Project Plan: a general guidance document (U.S. EPA 1980b) and a more detailed guidance document that combines a work plan with the QA Project Plan (U.S. EPA 1984c). These documents also provide guidance on the use of a short form for limited surveys.

4.4 EPA RESPONSIBILITIES

EPA Headquarters is responsible for providing guidance for developing Quality Assurance Program Plans and Quality Assurance Project Plans. This includes updates necessitated by new Agency requirements and additional technical guidance for the Regional offices and States to develop sound plans. In addition, Headquarters is responsible for developing guidance for inclusion of Data Quality Objectives in Quality Assurance Project Plans and providing guidance to the Regions on application of the DQO development process. In order to provide on-going guidance, the Office of Water has formed a Water Monitoring Data Quality Objective Advisory Group.

EPA Regional offices are responsible for developing Quality Assurance Program Plans and Quality Assurance Project Plans for the activities that they conduct. In addition, they are responsible for ensuring that States prepare QA Program Plans and Project Plans in conformance with grant requirements specified in 40 CFR Part 30. The Regions are responsible for developing DQO requirements compatible with Headquarter's requirements and meeting the Regions' specific needs. The Regions are also responsible for assisting the States in developing DQO requirements that meet State needs.

4.5 IMPORTANCE OF QA/QC FOR RAPID BIOASSESSMENTS

Quality assurance and control (QA/QC) should be a continuous process implemented throughout the entire bioassessment program. All aspects of the study, including field collection, habitat assessment, lab processing, and data analysis are subject to QA/QC procedures. As with any scientific study, quality must be assured before the results can be accepted. As described below, quality assurance is accomplished through establishment of thorough investigator training, protocol guidelines, comprehensive field and lab data documentation and management, verification of data reproducibility, and instrument calibration.

The protocols for rapid bioassessment presented in this document can be modified to achieve specific objectives. A different habitat assessment approach, replicate sampling, more intensive sample enumeration, or modified analytical metrics may be preferred by a particular State over the methods suggested in the RBPs. Such refinements can be accommodated, provided they are clearly documented in an EPA approved Quality Assurance Program and/or Project Plan.

Training

All personnel conducting assessments must be trained in a consistent manner (preferably by the same person) to ensure that the assessments are conducted properly and to ensure standardization. At least one investigator for each site should be a professional biologist trained and skilled in field aquatic sampling methods and organism identification. Additionally, the investigator should be familiar with the objectives of each site investigation. Each agency should have a designated QA/QC officer (or a person in charge of the program) responsible for maintaining consistency among investigators. At regularly scheduled intervals, the QA/QC officer should visit selected overlap sites and perform assessment techniques to use as a replicate of a previous assessment. Results from two separate assessments conducted by two different teams can determine if reproducible results are being attained. Quality control of taxonomic identifications can also be evaluated in this way.

Standard Procedures

It is the responsibility of each agency to define precise methods and review inconsistencies before the assessment begins. Because RBPs I and IV are primarily subjective investigations, a specific level of effort should be established prior to the actual field investigation. Taxonomic identification is required at various levels for RBP I (order level), RBP II (family level), RBP III (genus and species level), and RBP V (species level). Field experience and taxonomic expertise requirements for the particular level of assessment performed must be met. Any deviations from the protocol should be documented as to the reason for deviation, and corrective actions taken.

Field validation, conducted at a frequency to be determined by each agency, should involve two procedures: (1) collection of replicate samples at various stations to check on the accuracy of the collection effort, and (2) repeat field collections and analyses performed by separate field crews to provide support for the bioassessment. In addition, field crews should occasionally alternate personnel to maintain objectivity in the bioassessment.

Documentation

The field data sheets should be filled out as completely and as accurately as possible to provide a record in support of the survey and analysis conclusions. Abbreviations commonly used in documentation (e.g.,

scientific names) should be standardized to decrease data manipulation errors. Field and laboratory data sheets and final reports should be filed.

Habitat Assessment

Because the habitat characterization step of the protocols is primarily a subjective evaluation, final conclusions are potentially subject to variability among investigators. This limitation can be minimized, however, by ensuring that each investigator is appropriately trained in the evaluation technique and periodic cross-checks are conducted among investigators to promote consistency.

Benthic Collections

The data developed during the benthic collection effort is directly comparable to data developed at other sites because: (1) only similar habitats are sampled at each site, and (2) a uniform method (consistent unit of effort, 100-organism count) is used for benthic data acquisition. To ensure that sampling methods are applicable to a specific region, results may be evaluated by comparing results obtained using other sampling methods. To ensure reproducible data, well characterized sites should be periodically resampled by a variety of investigators. To document consistency among field teams, selected sites should be sampled simultaneously by several field teams.

Fish Collections

To ensure fish field survey data is representative of the fish assemblage at a particular site requires careful regional analysis and station siting. Data comparability is maintained by using similar collection methods and sampling effort in waterbodies of similar size. Also, where possible, major habitats (riffle, run, pool) are sampled at each site, and the proportion of each habitat type sampled, should be comparable.

Precision, accuracy, and completeness should be evaluated in pilot studies along with sampling methods and site size. Variability among replicates from the same site or similar sites, should not produce differences exceeding 10 percent at minimally-impacted sites and 15 percent at highly-impacted sites. IBI differences at the same site should not exceed 4 (Karr et al. 1986).

Data reproducibility may be ensured by having a variety of investigators periodically resample well characterized sites. Investigator accuracy for use of the IBI and the IWB may be determined by having investigators evaluate a standard series of data sets or preserved field collections.

Calibration of Instruments

Instruments used for measuring water quality, current velocity, or any other measurable parameters should be calibrated with known standards. All field measurements should be accompanied by documentation of the type of instrument and the identification number of the instrument used.

5. HABITAT ASSESSMENT AND PHYSICOCHEMICAL PARAMETERS

An evaluation of habitat quality is critical to any assessment of ecological integrity. The habitat quality evaluation can be accomplished by characterizing selected physicochemical parameters and systematic habitat assessment. Through this approach, key parameters can be identified to provide a consistent assessment of habitat quality. This evaluation of habitat quality is relevant to all levels of rapid bioassessment.

5.1 PHYSICAL CHARACTERISTICS AND WATER QUALITY

Both physical characteristics and water quality parameters are pertinent to characterization of the stream habitat. An example of the data sheet used to characterize the physical characteristics and water quality of a site is shown in Figure 5.1-1. The information requested includes measurements made routinely during biological surveys. This phase of the survey is broken into two sections: Physical Characterization and Water Quality (Figure 5.1-1). These sections are discussed separately below.

5.1.1 Physical Characterization

Physical characterization parameters include estimations of general land use and physical stream characteristics such as width, depth, flow, and substrate. The evaluation begins with the riparian zone (stream bank and drainage area) and proceeds instream to sediment/substrate descriptions. Such information will provide insight as to what organisms may be present or are expected to be present, and to presence of stream impacts. The information requested in the Physical Characterization section of the Field Data Sheet (Figure 5.1-1) is briefly discussed below.

Predominant Surrounding Land Use: Observe the prevalent land-use type in the vicinity (noting any other land uses in the area which, although not predominant, may potentially affect water quality).

Local Watershed Erosion—The existing or potential detachment of soil within the local watershed (the portion of the watershed that drains directly into the stream) and its movement into a stream is noted. Erosion can be rated through visual observation of watershed and stream characteristics. (Note any turbidity observed during water quality assessment below.)

Local Watershed Nonpoint-Source Pollution—This item refers to problems and potential problems other than siltation. Nonpoint-source pollution is defined as diffuse agricultural and urban runoff. Other compromising factors in a watershed that may affect water quality are feedlots, wetlands, septic systems, dams and impoundments, and/or mine seepage.

Estimated Stream Width (m): Estimate the distance from shore to shore at a transect representative of the stream width in the area.

Estimated Stream Depth (m): Riffle, run, and pool. Estimate the vertical distance from water surface to stream bottom at a representative depth at each of the three habitat types.

High Water Mark (m): Estimate the vertical distance from the stream bank to the peak overflow level, as indicated by debris hanging in bank or floodplain vegetation, and deposition of silt or soil. In instances where bank overflow is rare, a high water mark may not be evident.

Velocity: Record an estimate of stream velocity in a representative run area.

Dam Present: Indicate the presence or absence of a dam upstream or downstream of the sampling station. If a dam is present, include specific information relating to alteration of flow.

Channelized: Indicate whether or not the area around the sampling station is channelized.

Canopy Cover: Note the general proportion of open to shaded area which best describes the amount of cover at the sampling station.

Sediment Odors: Disturb sediment and note any odors described (or include any other odors not listed)

PHYSICAL CHARACTERIZATION/WATER QUALITY
FIELD DATA SHEET

PHYSICAL CHARACTERIZATION

RIPARIAN ZONE/INSTREAM FEATURES

Predominant Surrounding Land Use:

Forest Field/Pasture Agricultural Residential Commercial Industrial Other _____

Local Watershed Erosion: None Moderate Heavy

Local Watershed NPS Pollution: No evidence Some Potential Sources Obvious Sources

Estimated Stream Width _____ m Estimated Stream Depth: Riffle _____ m Run _____ m Pool _____ m

High Water Mark _____ m Velocity _____ Dam Present: Yes ___ No ___ Channelized: Yes ___ No ___

Canopy Cover: Open Partly Open Partly Shaded Shaded

SEDIMENT/SUBSTRATE:

Sediment Odors: Normal Sewage Petroleum Chemical Anaerobic None Other _____

Sediment Oils: Absent Slight Moderate Profuse

Sediment Deposits: Sludge Sawdust Paper Fiber Sand Relict Shells Other _____

Are the undersides of stones which are not deeply embedded black? Yes No

Inorganic Substrate Components

Substrate Type	Diameter	Percent Composition in Sampling Area
Bedrock		
Boulder	>256-mm (10 in.)	
Cobble	64-256-mm (2.5-10 in.)	
Gravel	2-64-mm (0.1-2.5 in.)	
Sand	0.06-2.00-mm (gritty)	
Silt	.004-.06-mm	
Clay	<.004-mm (slick)	

Organic Substrate Components

Substrate Type	Characteristic	Percent Composition in Sampling Area
Detritus	Sticks, Wood, Coarse Plant Materials (CPOM)	
Muck-Mud	Black, Very Fine Organic (FPOM)	
Marl	Grey, Shell Fragments	

WATER QUALITY

Temperature _____ C Dissolved Oxygen _____ pH _____ Conductivity _____ Other _____

Instrument(s) Used _____

Stream Type: Coldwater Warmwater

Water Odors: Normal Sewage Petroleum Chemical None Other _____

Water Surface Oils: Slick Sheen Globs Flecks None

Turbidity: Clear Slightly Turbid Turbid Opaque Water Color _____

WEATHER CONDITIONS

PHOTOGRAPH NUMBER

OBSERVATIONS AND/OR SKETCH

Figure 5.1-1. Physical Characterization/Water Quality Field Data Sheet for use with all Rapid Bioassessment Protocols.

which are associated with sediment in the area of the sampling station.

Sediment Oils: Note the term which best describes the relative amount of any sediment oils observed in the sampling area.

Sediment Deposits: Note those deposits described (or include any other deposits not listed) which are present in the sampling area. Also indicate whether the undersides of rocks not deeply embedded are black (which generally indicates low dissolved oxygen or anaerobic conditions).

Inorganic Substrate Components: Visually estimate the relative proportion of each of the seven substrate/particle types listed that are present in the sampling area.

Organic Substrate Components: Indicate relative abundance of each of the three substrate types listed.

5.1.2 Water Quality

Information requested in this section (Figure 5.1-1) is standard to many aquatic studies and allows for some comparison between sites. Additionally, conditions that may significantly affect aquatic biota are documented. Documentation of recent and current weather conditions is important because of the potential impact that weather may have on water quality. To complete this phase of the bioassessment, a photograph may be helpful in identifying station location and documenting habitat conditions. Any observations or data not requested but deemed important by the field observer should be recorded. This section is identical for all protocols and the specific data requested are described below.

Temperature (C), Dissolved Oxygen, pH, Conductivity: Measure and record values for each of the water quality parameters indicated, using the appropriate calibrated water quality instrument(s). Note the type of instrument and unit number used.

Stream Type: Note the appropriate stream designation according to State water quality standards.

Water Odors: Note those odors described (or include any other odors not listed) that are associated with the water in the sampling area.

Water Surface Oils: Note the term that best describes the relative amount of any oils present on the water surface.

Turbidity: Note the term which, based upon visual observation, best describes the amount of material suspended in the water column.

5.2 HABITAT ASSESSMENT

The habitat assessment matrix (Figure 5.2-1) is based on the Stream Classification Guidelines for Wisconsin developed by Ball (1982) and Methods of Evaluating Stream, Riparian, and Biotic Conditions developed by Platts et al. (1983). Because this habitat assessment approach is intended to support biosurvey analysis, the various habitat parameters are weighted to emphasize the most biologically significant parameters. All parameters are evaluated for each station studied. The ratings are then totaled and compared to a reference to provide a final habitat ranking. Scores increase as habitat quality increases. To ensure consistency in the evaluation procedure, descriptions of the physical parameters and relative criteria are included in the rating form.

Reference conditions are used to normalize the assessment to the "best attainable" situation. This approach is critical to the assessment because stream characteristics will vary dramatically across different regions. Other habitat assessment approaches may be used; or a more rigorously quantitative approach to measuring the habitat parameters may be used. However, the importance of a holistic habitat assessment to enhance the interpretation of biological data cannot be overemphasized. A more detailed discussion of the relationship between habitat quality and biological condition is presented in Chapter 8.

Habitat parameters pertinent to the assessment of habitat quality are separated into three principal categories: primary, secondary, and tertiary parameters. Primary parameters are those that characterize the stream "microscale" habitat and have the greatest direct influence on the structure of the indigenous communities. The primary parameters, which include characterization of the bottom substrate and available cover, estimation of embeddedness, and estimation of the flow or velocity and depth regime, have the widest score range (0–20) to reflect their contribution to habitat quality. The secondary parameters measure the "macroscale" habitat such as channel morphology characteristics. These parameters evaluate: channel alteration, bottom scouring and deposition, and stream sinuosity. The secondary parameters have a score range of 0–15. Tertiary parameters evaluate riparian and bank structure and comprise three parameters: bank stability, bank vegetation, and streamside cover. These tertiary parameters include those that are most often ignored in biosurveys. The tertiary parameters have a score range of 0–10.

Condition/Parameter	Condition			
	Excellent	Good	Fair	Poor
PRIMARY—SUBSTRATE AND INSTREAM COVER				
1. Bottom substrate and available cover	16–20	11–15	6–10	0–5
2. Embeddedness	16–20	11–15	6–10	0–5
3. Flow/velocity	16–20	11–15	6–10	0–5
SECONDARY—CHANNEL MORPHOLOGY				
4. Channel alteration	12–15	8–11	4–7	0–3
5. Bottom scouring and deposition	12–15	8–11	4–7	0–3
6. Pool/riffle, run/bend ratio	12–15	8–11	4–7	0–3
TERTIARY—RIPARIAN AND BANK STRUCTURE				
7. Bank stability	9–10	6–8	3–5	0–2
8. Bank vegetation	9–10	6–8	3–5	0–2
9. Streamside cover	9–10	6–8	3–5	0–2

Habitat evaluations are first made on instream habitat, followed by channel morphology, and finally on structural features of the bank and riparian vegetation. Stream segment length or area assessed will vary with each site. Generally, primary parameters are evaluated within the first riffle/pool sequence, or the immediate sampling area, such as in the case of fish sampling. Secondary and tertiary parameters are evaluated over a larger stream area, primarily in an upstream direction where conditions will have the greatest impact on the community being studied. The actual habitat assessment process involves rating the nine parameters as excellent, good, fair, or poor based on the criteria included on the Habitat Assessment Field Data Sheet (Figure 5.2-1).

A total score is obtained for each biological station and compared to a site-specific control or regional reference station. The ratio between the score for the station of interest and the score for the control or regional reference provides a percent comparability measure for each station. The station is then classified on the basis of its similarity to expected conditions (as represented by the control or reference station), and its apparent potential to support an acceptable level of biological health.

Use of a percent comparability evaluation allows for regional and stream-size differences which affect flow or velocity, substrate, and channel morphology. Some regions are characterized by streams having a low channel gradient. Such streams are typically shallower, have a greater pool/riffle or run/bend ratio, and less stable substrate than streams with a steep channel gradient. Although some low gradient streams do not provide the diversity of habitat or fauna afforded by steeper gradient streams, they are characteristic of certain regions. Using the approach presented here, these streams may be evaluated relative to other low gradient streams.

Assessment Category	Percent of Comparability
Comparable to Reference	≥90%
Supporting	75–88%
Partially Supporting	60–73%
Non-Supporting	≤58%

Listed below is a general explanation for each of the nine habitat parameters to be evaluated.

5.2.1 Primary Parameters—Substrate and Instream Cover

The primary instream habitat characteristics directly pertinent to the support of aquatic communities consists of substrate type and stability, availability of refugia, and migration/passage potential. These primary habitat parameters are weighted the highest to reflect their degree of importance to biological communities.

1. **Bottom Substrate**—This refers to the availability of habitat for support of aquatic organisms. A variety of substrate materials and habitat types is

HABITAT ASSESSMENT FIELD DATA SHEET

Habitat Parameter	Category			
	Excellent	Good	Fair	Poor
1. *Bottom substrate/ available cover[a]	Greater than 50% rubble, gravel, submerged logs, undercut banks, or other stable habitat. 16-20	30-50% rubble, gravel or other stable habitat. Adequate habitat. 11-15	10-30% rubble, gravel or other stable habitat. Habitat availability less than desirable. 6-10	Less than 10% rubble gravel or other stable habitat. Lack of habitat is obvious. 0-5
2. Embeddedness[b]	Gravel, cobble, and boulder particles are between 0 and 25 % surrounded by fine sediment 16-20	Gravel, cobble, and boulder particles are between 25 and 50 % surrounded by fine sediment 11-15	Gravel, cobble, and boulder particles are between 50 and 75 % surrounded by fine sediment 6-10	Gravel, cobble, and boulder particles are over 75 % surrounded by fine sediment 0-5
3. ≤0.15 cms (5cfs) → *Flow at rep. low flow[a]	Cold >0.05 cms (2 cfs) Warm >0.15 cms (5 cfs) 10-20	0.03-0.05 cms (1-2 cfs) 0.05-0.15 cms (2-5 cfs) 11-15	0.01-0.03 cms (.5-1 cfs) 0.03-0.05 cms (1-2 cfs) 6-10	<0.01 cms (.5 cfs) <0.03 cms (1 cfs) 0-5
or >0.15 cms (5cfs) → Velocity/depth	Slow (<0.3 m/s), deep (>0.5 m); slow, shallow (<0.5 m); fast (>0.3 m/s), deep; fast, shallow habitats all present. 16-20	Only 3 of the 4 habitat categories present (missing riffles or runs receive lower score than missing pools). 11-15	Only 2 of the 4 habitat categories present (missing riffles/runs receive lower score). 6-10	Dominated by one velocity/depth category (usually pool). 0-5
4. * Channel alteration[a]	Little or no enlargement of islands or point bars, and/or no channelization. 12-15	Some new increase in bar formation, mostly from coarse gravel; and/or some channelization present. 8-11	Moderate deposition of new gravel, coarse sand on old and new bars; pools partially filled w/silt; and/or embankments on both banks. 4-7	Heavy deposits of fine material, increased bar development; most pools filled w/silt; and/or extensive channelization. 0-3
5. Bottom scouring and deposition[a]	Less than 5% of the bottom affected by scouring and deposition. 12-15	5-30% affected. Scour at constrictions and where grades steepen. Some deposition in pools. 8-11	30-50% affected. Deposits and scour at obstructions, constrictions and bends. Some filling of pools. 4-7	More than 50% of the bottom changing nearly year long. Pools almost absent due to deposition. Only large rocks in riffle exposed. 0-3

(a) From Ball 1982.
(b) From Platts et al. 1983.
Note: * = Habitat parameters not currently incorporated into BIOS

Figure 5.2-1. Habitat Assessment Field Data Sheet for use with all Rapid Bioassessment Protocols.

HABITAT ASSESSMENT FIELD DATA SHEET (cont.)

Habitat Parameter	Category			
	Excellent	Good	Fair	Poor
6. Pool/riffle, run/bend ratio[a] (distance between riffles divided by stream width)	5-7. Variety of habitat. Deep riffles and pools. 12-15	7-15. Adequate depth in pools and riffles. Bends provide habitat. 8-11	15-25. Occassional riffle or bend. Bottom contours provide some habitat. 4-7	>25. Essentially a straight stream. Generally all flat water or shallow riffle. Poor habitat. 0-3
7. Bank stability[a]	Stable. No evidence of erosion or bank failure. Side slopes generally <30%. Little potential for future problem. 9-10	Moderately stable. Infrequent, small areas of erosion mostly healed over. Side slopes up to 40% on one bank. Slight potential in extreme floods. 6-8	Moderately unstable. Moderate frequency and size of erosional areas. Side slopes up to 60% on some banks. High erosion potential during extreme high flow. 3-5	Unstable. Many eroded areas. Side slopes >60% common. "Raw" areas frequent along straight sections and bends. 0-2
8. Bank vegetative stability[b]	Over 80% of the streambank surfaces covered by vegetation or boulders and cobble. 9-10	50-79% of the streambank surfaces covered by vegetation, gravel or larger material. 6-8	25-49% of the streambank surfaces covered by vegetation, gravel, or larger material. 3-5	Less than 25% of the streambank surfaces covered by vegetation, gravel, or larger material. 0-2
9. Streamside cover[b]	Dominant vegetation is shrub. 9-10	Dominant vegetation is of tree form. 6-8	Dominant vegetation is grass or forbes. 3-5	Over 50% of the streambank has no vegetation and dominant material is soil, rock, bridge materials, culverts, or mine tailings. 0-2
Column Totals	____	____	____	____

Score _____

Figure 5.2-1. (Cont.).

desirable. The presence of rock and gravel in flowing streams is generally considered the most desirable habitat. However, other forms of habitat may provide the niches required for community support. For example, logs, tree roots, submerged or emergent vegetation, undercut banks, etc., will provide excellent habitat for a variety of organisms, particularly fish. Bottom substrate is evaluated and rated by observation.

2. **Embeddedness**—The degree to which boulders, rubble, or gravel are surrounded by fine sediment indicates suitability of the stream substrate as habitat for benthic macroinvertebrates and for fish spawning and egg incubation. Embeddedness is evaluated by visual observation of the degree to which larger particles are surrounded by sediment. In some western areas of the United States, embeddedness is regarded as the stability of cobble substrate by measuring the depth of burial of large particles (cobble, boulders).
3. **Stream Flow and/or Stream Velocity**—Stream flow relates to the ability of a stream to provide and maintain a stable aquatic environment. Stream flow (water quantity) is most critical to the support of aquatic communities when the representative low flow is ≤ 0.15 cms (5 cfs). In these small streams, flow should be estimated in a straight stretch of run area where banks are parallel and bottom contour is relatively flat. Even where a few stations may have flows in excess of 0.15 cms, flow may still be the predominating constraint. Therefore, the evaluation is based on flow rather than velocity.

   In larger streams and rivers (>0.15 cms), velocity, in conjunction with depth, has a more direct influence than flow on the structure of benthic communities (Osborne and Hendricks 1983) and fish communities (Oswood and Barber 1982). The quality of the aquatic habitat can therefore be evaluated in terms of a velocity and depth relationship. As patterned after Oswood and Barber (1982), four general categories of velocity and depth are optimal for benthic and fish communities: (1) slow (<0.3 m/s), shallow (<0.5 m); (2) slow (<0.3 m/s), deep (>0.5 m); (3) fast (>0.3 m/s), deep (>0.5 m); and (4) fast (>0.3 m/s), shallow (<0.5 m). Habitat quality is reduced in the absence of one or more of these four categories.

5.2.2 Secondary Parameters—Channel Morphology

Channel morphology is determined by the flow regime of the stream, local geology, land surface form, soil, and human activities (Platts et al. 1983). The sediment movement along the channel, as influenced by the tractive forces of flowing water and the sinuosity of the channel, also affects habitat conditions.

4. **Channel Alteration**—The character of sediment deposits from upstream is an indication of the severity of watershed and bank erosion and stability of the stream system. The growth or appearance of sediment bars tends to increase in depth and length with continued watershed disturbance. Channel alteration also results in deposition, which may occur on the inside of bends, below channel constrictions, and where stream gradient flattens out. Channelization (e.g., straightening, construction of concrete embankments) decreases stream sinuosity, thereby increasing stream velocity and the potential for scouring.
5. **Bottom Scouring and Deposition**—These parameters relate to the destruction of instream habitat resulting from the problems described above. Characteristics to observe are scoured substrate and degree of siltation in pools and riffles. Scouring results from high velocity flows. The potential for scouring is increased by channelization. Deposition and scouring result from the transport of sediment or other particulates and may be an indication of large scale watershed erosion. Deposition and scouring is rated by estimating the percentage of an evaluated reach that is scoured or silted (i.e., 50-ft silted in a 100-ft stream length equals 50 percent).
6. **Pool/Riffle or Run/Bend Ratio**—These parameters assume that a stream with riffles or bends provides more diverse habitat than a straight (run) or uniform depth stream. Bends are included because low gradient streams may not have riffle areas, but excellent habitat can be provided by the cutting action of water at bends. The ratio is calculated by dividing the average distance between riffles or bends by the average stream width. If a stream contains riffles and bends, the dominant feature with the best habitat should be used.

5.2.3 Tertiary Parameters—Riparian and Bank Structure

Well-vegetated banks are usually stable regardless of bank undercutting; undercutting actually provides excellent cover for fish (Platts et al. 1983). The ability of vegetation and other materials on the streambanks to prevent or inhibit erosion is an important determinant of the stability of the stream channel and instream habitat for indigenous organisms. Because riparian and bank structure indirectly affect the instream habitat features, they are weighted less than the primary or secondary parameters.

Stream channel alteration downstream of WWTP.

Tertiary parameters are evaluated by observation of both upper and lower bank characteristics. The upper bank is the land area from the break in the general slope of the surrounding land to the normal high water line. The upper bank is normally vegetated and covered by water only during extreme high water conditions. Land forms vary from wide, flat floodplains to narrow, steep slopes. The lower bank is the intermittently submerged portion of the stream cross section from the normal high water line to the lower water line. The lower channel defines the stream width.

7. **Bank Stability**—Bank stability is rated by observing existing or potential detachment of soil from the upper and lower stream bank and its potential movement into the stream. Steeper banks are generally more subject to erosion and failure, and may not support stable vegetation. Streams with poor banks will often have poor instream habitat. Adjustments should be made in areas with clay banks where steep, raw areas may not be as susceptible to erosion as other soil types.
8. **Bank Vegetative Stability**—Bank soil is generally held in place by plant root systems. Erosional protection may also be provided by boulder, cobble, or gravel material. An estimate of the density of bank vegetation (or proportion of boulder, cobble, or gravel material) covering the bank provides an indication of bank stability and potential instream sedimentation.
9. **Streamside Cover**—Streamside cover vegetation is evaluated in terms of provision of stream-shading and escape cover or refuge for fish. A rating is obtained by visually determining the dominant vegetation type covering the exposed stream bottom, bank, and top of bank. Platts (1974) found that streamside cover consisting primarily of shrub had a higher fish standing crop than similar-size streams having tree or grass streamside cover. Riparian vegetation dominated by shrubs and trees provides the CPOM source in allochthonous systems.

Poor bank stability with high erosional potential.

Stream banks stabilized by dense vegetation.

6. BENTHIC MACROINVERTEBRATE BIOSURVEY AND DATA ANALYSIS

The biosurvey and data analysis components of the three benthic bioassessment protocols are presented below. All three protocols have common biosurvey and data analysis elements. Common elements and discussions are repeated in each protocol to maintain discrete protocol integrity.

Examples of field and laboratory data sheets referred to in this chapter are presented for guidance. The example data sheets do not include headers for documenting identifier information, and may be modified for the needs of different agencies. Descriptive guidance for use with each data sheet is found in Appendix A.

The three protocols consist of three basic components: water quality/physical characteristics (Figure 5.1-1), habitat assessment (Figure 5.2-1), and a biosurvey (Figures 6.1-1, 6.2-1, and 6.3-1). The overall habitat assessment evaluates habitat quality using the key environmental parameters described in Chapter 5. If a degraded community is found from the results of the biosurvey, habitat information will aid interpretation of effects relative to the biotic potential of a site. The water quality and physical characterizations provide data on stream habitat quality as well as potential sources and/or causes of impairment.

6.1 RAPID BIOASSESSMENT PROTOCOL I—Benthic Macroinvertebrates

Rapid Bioassessment Protocol I (RBP I) is a screening or reconnaissance assessment that involves systematic documentation of specific visual observations made in the field by a trained professional. RBP I is used to discriminate obviously impacted and non-impacted areas from potentially affected areas requiring further investigation. Use of RBP I allows rapid screening of a large number of sites. Areas identified for further study can then be rigorously evaluated using RBPs II, III, and V; quantitative fish or benthic surveys; or ambient toxicity studies.

Because RBP I involves limited data generation, its effectiveness depends largely on the experience ("best professional judgment") of the professional biologist performing the assessment. The biologist conducting RBP I should have professional impact assessment experience with a knowledge of aquatic ecology and basic expertise in benthic macroinvertebrate taxonomy.

6.1.1 Field Methods

The biosurvey component of RBP I focuses on qualitative sampling of benthic macroinvertebrates, supplemented by a preliminary field examination of other aquatic biota (periphyton, macrophytes, slimes, and fish). Qualitative benthic samples are collected from all available habitats using a dip net or kick net, or by hand. Benthic macroinvertebrate orders/families (e.g., families for Megaloptera and Diptera) collected are listed on the Biosurvey Field Data Sheet (Figure 6.1-1), with an estimate of their relative abundance in the sampling area. Each State agency should develop its own definitions for abundance categories. Lower levels of identification, if they are easily determined, can enhance the assessment. Any observations on the relative abundance of other aquatic biota are also noted; these observations provide additional information on the presence or absence of impact.

6.1.2 Data Analysis Techniques

Impairment may be indicated by the absence of generally pollution-sensitive benthic macroinvertebrate taxa such as Ephemeroptera, Plecoptera, and Trichoptera (EPT); dominance of generally pollution-tolerant groups such as Oligochaeta or Chironomidae; or overall low benthic abundance or taxa richness. Benthic abundance or taxa richness indicative of impairment is variable and must be evaluated with respect to the waterbody being evaluated. Some headwater streams are naturally unproductive and will be characterized by low benthic abundance and taxa richness in their pristine state. Impairment may also be indicated by an overabundance of slimes or filamentous algae in the area or an absence of expected fish populations.

On the basis of the observations made on habitat, water quality, physical characteristics, and the qualitative biosurvey, the investigator determines whether impairment is detected. The determination of impairment requires the judgment of an *experienced professional.* If impairment is detected, the investigator provides an estimation of the probable cause and source on the Impairment Assessment Sheet (Figure 6.1-2). The aquatic biota that indicated an impair-

Rapid Bioassessment Protocol I

Biosurvey Field Data Sheet

RELATIVE ABUNDANCE OF AQUATIC BIOTA

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

0 = Absent/Not Observed 1 = Rare 2 = Common 3 = Abundant 4 = Dominant

MACROBENTHOS QUALITATIVE SAMPLE LIST (Indicate Relative Abundance R = Rare, C = Common, A = Abundant, D = Dominant)

Porifera	Anisoptera	Chironomidae
Hydrozoa	Zygoptera	Plecoptera
Platyhelminthes	Hemiptera	Ephemeroptera
Turbellaria	Coleoptera	Trichoptera
Hirudinea	Lepidoptera	Other
Oligochaeta	Sialidae	
Isopoda	Corydalidae	
Amphipoda	Tipulidae	
Decapoda	Empididae	
Gastropoda	Simuliidae	
Bivalvia	Tabanidae	
	Culicidae	

Rare < 3 Common 3–9 Abundant > 10 Dominant > 50 (Estimate)

Observations

Figure 6.1-1. Biosurvey Field Data Sheet for use with Rapid Bioassessment Protocol I.

IMPAIRMENT ASSESSMENT SHEET

1. Detection of impairment: Impairment detected (Complete items 2-6) No impairment detected (Stop here)

2. Biological impairment indicator:

Benthic macroinvertebrates	Other aquatic communities
____ absence of EPT taxa	____ Periphyton
____ dominance of tolerant groups	____ filamentous
____ low benthic abundance	____ other
____ low taxa richness	____ Macrophytes
____ other	____ Slimes
	____ Fish

3. Brief description of problem: _____

Year and date of previous surveys: _____

Survey data available in: _____

4. Cause: (indicate major cause) organic enrichment toxicants flow habitat limitations other _____

5. Estimated areal extent of problem (m^2) and length of stream reach affected (m), where applicable: _____

6. Suspected source(s) of problem:

____ point source discharge (name, type of facility, location)
____ construction site runoff
____ combined sewer outfall
____ silviculture runoff
____ animal feedlot
____ agricultural runoff
____ urban runoff
____ ground water
____ other
____ unknown

Briefly explain:

Figure 6.1-2. Impairment Assessment Sheet for use with macroinvertebrate Rapid Bioassessment Protocols.

ment are noted, as are potential sources of pollutants. The downstream extent of impact is estimated, and multiplied by either the approximate stream width at the estimated fully mixed zone or the width of the discharge plume. This calculation provides an estimate of the area impacted at the site.

6.2 RAPID BIOASSESSMENT PROTOCOL II—Benthic Macroinvertebrates

Rapid Bioassessment Protocol II (RBP II) utilizes the systematic field collection and analysis of major benthic taxa. RBP II provides a more intense assessment than RBP I and can detect sites of intermediate impairment with relatively little additional time and effort. The protocol can be used to prioritize sites for more intensive evaluation (i.e., RBP III, replicate sampling, ambient toxicity testing, chemical characterization) or can be used in lieu of RBP I as a screening technique. RBP II is based on RBP III at a reduced level of effort. RBP II incorporates the concept of benthic analysis at the family taxonomic level, as advocated by some States (e.g., Virginia, Illinois), and utilizes field sorting and identification. This level of effort involves minimal taxonomic identification and is sufficient to address the objectives of RBP II. Furse et al. (1984) stated that family-level classifications are valuable in developing local site inventories of organisms and in the evaluation of pollution monitoring programs. The strength of RBP II is a result of systematic data collection procedures and the use of recently developed data analysis techniques.

6.2.1 Field Methods

The biosurvey component of RBP II focuses on standardized sampling of benthic macroinvertebrates, supplemented by a cursory field observation of other aquatic biota (periphyton, macrophytes, slimes, and fish) (Figure 6.2-1). Although RBP II emphasizes the benthic community, the observation of effects on other aquatic biota will support the final evaluation. (This approach is adapted from Michigan DNR's protocol.)

6.2.1.1 Sample Collection

The collection procedure provides representative samples of the macroinvertebrate fauna from comparable habitat types at all stations constituting a site evaluation, and is supplemented with separate Coarse Particulate Organic Matter (CPOM) samples. RBP II focuses on the riffle/run habitat because it is the most productive habitat available in stream systems and includes many pollution-sensitive taxa of the Scraper and Filtering Collector Functional Feeding Groups. The CPOM sample provides a measure of effects (particularly toxicity effects), on a third trophic component of the benthic community, the Shredders.

In sampling situations where a riffle/run habitat with a rock substrate is not available, any submerged fixed structure will provide a substrate for the Scraper and Filtering Collector Functional Groups emphasized here. This allows for the same approach to be used in non-wadable streams and large rivers and wadable streams and rivers with unstable substrates.

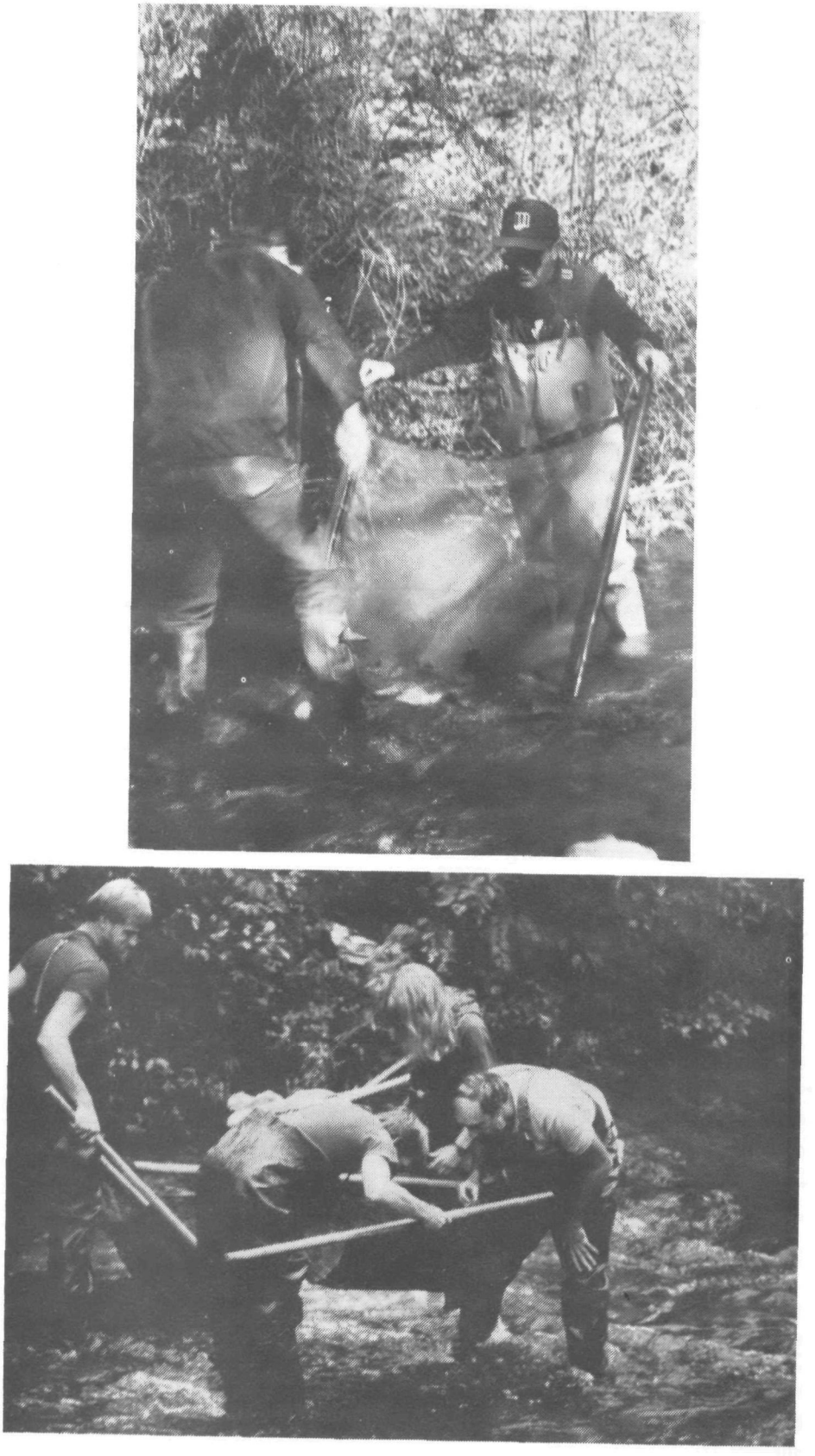
Riffle/Run Sample

Riffle areas with relatively fast currents and cobble and gravel substrates generally provide the most diverse community. Riffles should be sampled using a kick net to collect from an approximately 1 m^2 area. Two 1 m^2 riffle samples should be collected at each station: one from an area of fast current velocity and one from an area of slower current velocity. The two samples are composited for processing. In streams lacking riffles, run areas with cobble or gravel substrate are also appropriate for kick net sampling.

Where riffle/run communities with a rock substrate are not available, other submerged fixed structures (e.g. submerged boulders, logs, bridge abutments, pier pilings) should be sampled by hand picking. These structures provide suitable habitat for the Scrapers and Filtering Collectors and will allow use of RBP II for a wider range of regions and stream orders. Benke et al. (1984) determined that although submerged wood substrates, or snags, accounted for only a small portion of the available substrate in a blackwater river in Georgia, this habitat provided the greatest taxa richness and more than half of all benthic biomass.

CPOM Sample

In addition to the riffle/run sample collected for evaluation of the Scraper and Filtering Collector Functional Feeding Groups, a CPOM sample should also be collected to provide data on the abundance of Shredders at the site. Large particulate Shredders are important in forested areas of stream ecosystems ranging from stream orders 1 through 4 (Minshall et al. 1985). The absence of Shredders of large particulate material is characteristic of unstable, poorly retentive headwater streams in disturbed watersheds or in dry areas where leaf material processing is accomplished by terrestrial detritivores (Minshall et al. 1985). McArthur et al. (1988) reported that very few Shredders were found in summer leaf packs in South Carolina because processing was so rapid.



Kick net sampling in riffle area.

Rapid Bioassessment Protocol II

Biosurvey Field Data Sheet

RELATIVE ABUNDANCE OF AQUATIC BIOTA

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

0 = Absent/Not Observed 1 = Rare 2 = Common 3 = Abundant 4 = Dominant

MACROBENTHOS QUALITATIVE SAMPLE LIST		List Families Present/Indicate Abundance
Oligochaeta		
	Anisoptera	
Gastropoda		Coleoptera
	Zygoptera	
Bivalvia		Diptera
	Plecoptera	
Ephemeroptera		
	Trichoptera	

Other

RIFFLE SAMPLE
FUNCTIONAL FEEDING GROUPS (Indicate No. of Individuals Representing Group)

Scrapers	Filtering Collectors

CPOM SAMPLE FUNCTIONAL FEEDING GROUPS (Indicate No. of Individuals Representing Group)

Shredders	Total Org. in Sample

Observations

Figure 6.2-1. Biosurvey Field Data Sheet for use with Rapid Bioassessment Protocol II.

The CPOM sample is processed separately from the riffle/run sample and used only for characterizing the Functional Feeding Group representation. Sampling the CPOM component requires a composite collection of various plant parts such as leaves, needles, twigs, bark, or their fragments. Potential sample sources include leaf packs, shorezones, and other depositional areas where CPOM may accumulate. Only the upper surface of litter accumulation in depositional areas should be sampled to ensure that they are from the aerobic zone. For the Shredder community analysis, several handfuls of material should be adequate. A variety of CPOM forms should be collected if available. CPOM collected may be washed in a dip net or a sieve bucket.

Shredder abundance is maximum when the CPOM is about 50 percent decomposed (Cummins et al. 1989). Care must be taken to *avoid* collecting recent or fully decomposed leaf litter to optimize collection of the Shredder community. For this CPOM collection technique, seasonality may have an important influence on Shredder abundance data. For instance, fast-processing litter (e.g., basswood, alder, maples, birch) would have the highest Shredder representation in the winter (Cummins et al. 1989). The slow-processing litter (e.g., oaks, rhododendrons, beech, conifers) would have the highest Shredder representation in the summer.

6.2.1.2 Sample Sorting and Identification

Riffle/Run Sample

Sorting and enumeration in the field to obtain a 100-count organism subsample is recommended for the riffle/run sample. After processing in the field, the organisms and sample residue should be preserved for archiving. Thus, a re-analysis (quality control) or more thorough processing (e.g., larger counts, more detailed taxonomy) would be possible. The subsampling method described in this protocol is based on Hilsenhoff's Improved Biotic Index (Hilsenhoff 1987b) and is similar to that used by New York DEC (Bode 1988). This subsampling technique provides for a consistent unit of effort and a representative estimate of the benthic fauna.

The subsampling procedure consists of evenly distributing the composite sample into a gridded pan with a light colored bottom. Grids are randomly selected and all organisms within those grids are removed until approximately 100 organisms are picked out. Because this subsampling technique is being applied to samples with live organisms, narcotization using club soda or tobacco is recommended. A more detailed description of this technique may be found in Appendix B.

An alternative method of subsampling live samples in the field is to simply sort 100 organisms in a random manner. Narcotization to slow the organisms is less important with this subsampling technique. To lessen sampling bias, the investigator should pick smaller, cryptic organisms, as well as the larger, more obvious organisms.

All organisms in the subsample should be classified according to Functional Feeding Group. Field classification is important because many families comprise genera and species representing a variety of functional groups. Knowing the family-level identification of the organisms will generally be insufficient for categorization by Functional Feeding Group. Functional Feeding Group classification can be done in the field, on the basis of morphological and behavioral features, using Cummins and Wilzbach (1985). Care should be taken in noting early instars, which may constitute different Functional Feeding Groups from the later instars.

The Scraper and Filtering Collector Functional Groups are the most important indicators in the riffle/run community. Numbers of individuals representing each of these two groups are recorded on the Biosurvey Field Data Sheet (Figure 6.2-1). All organisms in the subsample should be identified to family or order, enumerated, and recorded, along with any observations on abundance of other aquatic biota, on the Biosurvey Field Data Sheet. A summary of all benthic data to be used in the final analysis will be recorded on the Data Summary Sheet (Figure 6.2-2) upon return to the laboratory.

The use of family-level identification in this protocol is based on Hilsenhoff's Family Biotic Index which uses higher taxonomic levels of identification (Hilsenhoff 1988). Tolerance characterizations for the Family Biotic Index (FBI) and excerpts from Hilsenhoff's paper describing the index are included in Appendix C. Assessment based on family-level identifications has been used successfully by the States of Virginia and Illinois.

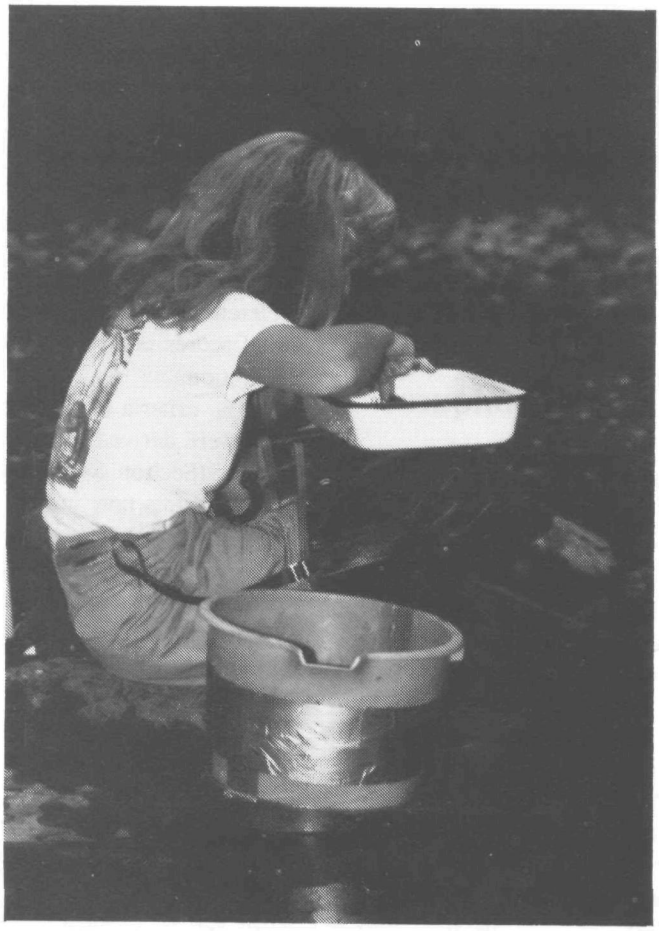
CPOM Sample

Organisms collected in the supplemental CPOM sample are classified as Shredders or Non-Shredders. Taxonomic identification is not necessary for this component. The composited CPOM sample may be field sorted in a small pan with a light colored bottom or in the net or sieve through which it was rinsed. (If a large number of benthic macroinvertebrates have been collected, a representative subsampling of 20–60 organisms may be removed for Functional Feeding Group classification.) Numbers of individuals representing the Shredder Functional Group, as well

DATA SUMMARY SHEET

Station No.								
Station Location								
Taxa Richness								
FBI (modified)								
Functional Feeding Groups								
Riffle Community								
Scrapers/Filt. Collect.								
CPOM Community								
Shredders/Total								
EPT/Chironomidae								
% Contribution (dom. family)								
EPT Index								
Community Similarity Index								
Comments:								

Figure 6.2-2. Data Summary Sheet suggested for use in recording benthic data utilized in Rapid Bioassessment Protocol II.



Field sorting of benthic macroinvertebrate samples for Rapid Bioassessment Protocol II.

as total number of macroinvertebrates collected in this sample, should be recorded on the Biosurvey Field Data Sheet (Figure 6.2-1) for later analysis. The Shredder/Non-Shredder metric may be deemed optional in rivers or in some regions where Shredder abundance is naturally low. However, the potential utility of such a metric for assessing toxicant effects warrants serious consideration in this bioassessment approach.

6.2.2 Data Analysis Techniques

Biological impairment of the benthic community may be indicated by the absence of generally pollution-sensitive macroinvertebrate taxa such as Ephemeroptera, Plecoptera, and Trichoptera (EPT); excess dominance by any particular taxon, especially pollution-tolerant forms such as some Chironomidae and Oligochaeta taxa; low overall taxa richness; or appreciable shifts in community composition relative to the reference condition. Impairment may also be indicated by an overabundance of fungal slimes or filamentous algae, or an absence of expected populations of fish. All of these indicators can be evaluated using the sampling data generated in RBP II.

On the basis of observations made in the assessment of habitat, water quality, physical characteristics, and the qualitative biosurvey, the investigator concludes whether impairment is detected. If impairment is detected, an estimation of the probable cause and source is provided on the Impairment Assessment Sheet (Figure 6.1-2). The aquatic biota that indicated an impairment are noted along with observed indications of potential problem sources. The downstream extent of impact is estimated and multiplied by appropriate stream width to provide an estimate of the areal extent of the problem.

The data analysis scheme used in RBP II integrates several community, population, and functional parameters into a single evaluation of biotic integrity (Table 6.2-1). Each parameter, or metric, measures a different component of community structure and has a different range of sensitivity to pollution stress (Figure 8.2-1). This integrated approach provides more assurance of a valid assessment because a variety of parameters are evaluated. Deficiency of any one metric in a particular situation should not invalidate the entire approach.

The eight metrics used in RBP II are the same as those in RBP III, but the scoring criteria used to evaluate the metrics have been modified to accommodate the less rigorous taxonomy (family-level identifications) of RBP II. The integrated data analysis (Figure 6.2-3) is performed as follows. Using the raw benthic data, a numerical value is calculated for each metric. Calculated values are then compared to values derived from either a reference site within the same region, a reference database applicable to the region, or a suitable control station on the same stream. Each metric is then assigned a score according to the comparability (percent similarity) of calculated and reference values. Scores for the eight metrics are then totaled and compared to the total metric score for the reference station. The percent comparison between the total scores provides a final evaluation of biological condition.

The criteria to be used for scoring the eight metrics were derived from an evaluation of pilot study results (Section 6.4); certain compliance monitoring requirements now in use (Vermont Department of Environmental Conservation 1987); and discussions with various aquatic biologists regarding the level of detection considered dependable for certain metrics. However, these *criteria may need to be adjusted for use in particular regions.*

Inherent variability in each metric was considered in establishing percent comparability criteria. The metrics based on taxa richness, FBI, and EPT Indices have low variability (Resh 1988). This variability is accounted for in the criteria for characterization of biological condition (Figure 6.2-3), based on existing data. For metrics based on standard taxa richness and FBI and EPT Indices, differences of 10–20 percent relative to the reference condition would be considered nominal, and the station being assessed would receive the maximum metric score. Because increasing FBI values denote worsening biological condition, percent difference for this metric is calculated by dividing the reference value by the value for the station of comparison.

Metrics that utilize ratios fluctuate more widely, however, and comparing percent differences between ratios (ratios of ratios) will compound the variability. Scoring increments are therefore set at broad intervals of 25 percent or greater. For metrics based on Functional Feeding Group ratios, Cummins (1987, personal communication) contends that differences as great as 50 percent from the reference may be acceptable, but differences in the range of 50–100 percent are not only important but discriminate degrees of impact more clearly.

The contribution of the dominant taxon to total abundance is a simple estimator of evenness. Scoring criteria are based on theoretical considerations rather than direct comparison with a reference.

The Community Loss Index (a representative similarity index) already incorporates a comparison with a reference. Therefore, actual index values are used in scoring.

The metrics used to evaluate the benthic data and their significance are explained below.

TABLE 6.2-1 CRITERIA[a] FOR CHARACTERIZATION OF BIOLOGICAL CONDITION FOR RAPID BIOASSESSMENT PROTOCOL II

Metric	Biological Condition		
	Non-Impaired	Moderately Impaired	Severely Impaired
1. Taxa Richness	Comparable to the best situation to be expected within an ecoregion. Balanced trophic structure. Optimum community structure (composition and dominance) for stream size and habitat quality.	Fewer taxa due to loss of most intolerant forms. Reduction in EPT index.	Few taxa present. If high densities of organisms, then dominated by one or two taxa. Only tolerant organisms present.
2. Family Biotic Index (modified)			
3. Ratio of Scrapers/Filtering Collectors[b]			
4. Ratio of EPT and Chironomid Abundances			
5. % Contribution of Dominant Family			
6. EPT Index			
7. Community Similarity Index[c]			
8. Ratio of Shredders/Total[b]			

(a) Scoring criteria are generally based on percent comparability to the reference station.
(b) Determination of Functional Feeding Group is independent of taxonomic grouping.
(c) Community Similarity Indices are used in comparison to a reference station.

Site-Specific Study

Sampling & Analysis

CRITERIA FOR CHARACTERIZATION OF BIOLOGICAL CONDITION FOR PROTOCOL II

Metric	Biological Condition Scoring Criteria 6	3	0
1. Taxa Richness[a]	>80%	40-80%	<40%
2. Family Biotic Index (modified)[b]	>85%	50-85%	<50%
3. Ratio of Scrapers/Filt. Collectors[a,c]	>50%	25-50%	<25%
4. Ratio of EPT and Chironomid Abundances[a]	>75%	25-75%	<25%
5. % Contribution of Dominant Family[a]	<30%	30-50%	>50%
6. EPT Index[a]	>90%	70-90%	<70%
7. Community Loss Index[e]	<0.5	0.5-4.0	>4.0
8. Ratio of Shredders/Total[a,c]	>50%	25-50%	<25%

(a) Score is a ratio of study site to reference site × 100.
(b) Score is a ratio of reference site to study site × 100.
(c) Determination of Functional Feeding Group is independent of taxonomic grouping.
(d) Scoring criteria evaluate actual percent contribution, not percent comparability to the reference station.
(e) Range of values obtained. A comparison to the reference station is incorporated in these indices.

BIOASSESSMENT

% Comp. to Ref. Score[a]	Biological Condition Category	Attributes
>79%	Non-impaired	Comparable to the best situation to be expected within an ecoregion. Balanced trophic structure. Optimum community structure (composition and dominance) for stream size and habitat quality.
29-72%	Moderately impaired	Fewer species due to loss of most intolerant forms. Reduction in EPT index.
<21%	Severely impaired.	Few species present. If high densities of organisms, then dominated by one or two taxa. Only tolerant organisms present.

(a) Percentage values obtained that are intermediate to the above ranges will require subjective judgement as to the correct placement. Use of the habitat assessment and physicochemical data may be necessary to aid in the decision process.

Recommendations

Figure 6.2-3. Flowchart of bioassessment approach advocated for Rapid Bioassessment Protocol II.

Riffle/Run Sample

Metric 1. Taxa Richness

Reflects health of the community through a measurement of the variety of taxa (total number of families) present. Generally increases with increasing water quality, habitat diversity, and habitat suitability. Sampling of highly similar habitats will reduce the variability in this metric attributable to factors such as current speed and substrate type. Some pristine headwater streams may be naturally unproductive, supporting only a very limited number of taxa. In these situations, organic enrichment may result in an increased number of taxa (including EPT taxa).

Metric 2. Modified Family Biotic Index

Tolerance values range from 0 to 10 for families and increase as water quality decreases. The index was developed by Hilsenhoff (Hilsenhoff 1988) to summarize the various tolerances of the benthic arthropod community with a single value. The Modified Family Biotic Index was developed to detect organic pollution and is based on the original species-level index (Hilsenhoff 1982). Tolerance values for each family were developed by weighting species according to their relative abundance in the State of Wisconsin.

The family-level index has been modified for this document to include organisms other than just arthropods using the genus and species-level biotic index developed by the State of New York (Bode 1988). The formula for calculating the Family Biotic Index is:

$$FBI = \Sigma \frac{x_i \, t_i}{n}$$

where

x_i = number of individuals within a taxon

t_i = tolerance value of a taxon

n = total number of organisms in the sample

Hilsenhoff's family-level *tolerance values may require modification for some regions.* Alternative tolerance classifications and biotic indices have been developed by some State agencies (Appendix C). Additional biotic indices are listed in U.S. EPA (1983).

Although the FBI may be applicable for toxic pollutants, it has only been evaluated for organic pollutants. The State of Wisconsin is conducting a study to evaluate the ability of Hilsenhoff's index to detect non-organic effects.

Metric 3. Ratio of Scraper and Filtering Collector Functional Feeding Groups

The Scraper and Filtering Collector metric reflects the riffle/run community food-base. When compared to a reference site, shifts in the dominance of a particular feeding type indicate a community responding to an overabundance of a particular food source. The predominant feeding strategy reflects the type of impact detected. Assignment of individuals to Functional Feeding Groups is independent of taxonomy, with some families representing several functional groups.

A description of the Functional Feeding Group concept can be found in Cummins (1973) and Merritt and Cummins (1984). Functional Feeding Group designations for most aquatic insect families may be found in Merritt and Cummins (1984). Most aquatic insects can also be classified to Functional Feeding Group in the field, on the basis of morphological and behavioral features, using Cummins and Wilzbach (1985).

The relative abundance of Scrapers and Filtering Collectors in the riffle/run habitat is an indication of the periphyton community composition, availability of suspended Fine Particulate Organic Material (FPOM), and availability of attachment sites for filtering. Scrapers increase with increased diatom abundance and decrease as filamentous algae and aquatic mosses (which scrapers cannot efficiently harvest) increase. However, filamentous algae and aquatic mosses provide good attachment sites for Filtering Collectors, and the organic enrichment often responsible for overabundance of filamentous algae can also provide FPOM that is utilized by the Filterers.

Filtering Collectors are also sensitive to toxicants bound to fine particles and should be the first group to decrease when exposed

to steady sources of such bound toxicants. This situation is often associated with point-source discharges where certain toxicants adsorb readily to dissolved organic matter (DOM) forming FPOM during flocculation. Toxicants thus become available to Filterers via FPOM. The Scraper to Filtering Collector ratio may not be a good indicator of organic enrichment if adsorbing toxicants are present. In these instances the FBI and EPT Index may provide additional insight. Qualitative field observations on periphyton abundance may also be helpful in interpreting results.

Metric 4. Ratio of EPT and Chironomidae Abundances

The EPT and Chironomidae abundance ratio uses relative abundance of these indicator groups (Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae) as a measure of community balance. Good biotic condition is reflected in communities with an even distribution among all four major groups and with substantial representation in the sensitive groups Ephemeroptera, Plecoptera, and Trichoptera. Skewed populations having a disproportionate number of the Chironomidae relative to the more sensitive insect groups may indicate environmental stress (Ferrington 1987, Shackleford 1988). Certain species of some genera such as *Cricotopus* are highly tolerant (Lenat 1983, Mount et al. 1984) and as opportunists may become numerically dominant in habitats exposed to metal discharges where EPT taxa are not abundant, thereby providing a good indicator of toxicant stress (Winner et al. 1980). Clements et al. (1988) found that mayflies were more sensitive than chironomids to exposure levels of 15 to 32 μg/L of copper. Chironomids tend to become increasingly dominant in terms of percent taxonomic composition and relative abundance along a gradient of increasing enrichment or heavy metals concentration (Ferrington 1987).

An alternative to the ratio of EPT and Chironomidae abundance metric is the Indicator Assemblage Index (IAI) developed by Shackleford (1988). The IAI integrates the relative abundances of the EPT taxonomic groups and the relative abundances of chironomids and annelids upstream and downstream of a pollutant source to evaluate impairment. The IAI may be a valuable metric in areas where the annelid community may fluctuate substantially in response to pollutant stress.

Metric 5. Percent Contribution of Dominant Family

The percent contribution of the dominant family to the total number of organisms uses abundance of the numerically dominant taxon relative to the rest of the population as an indication of community balance at the family-level. A community dominated by relatively few families would indicate environmental stress. (This metric may be redundant if the Pinkham and Pearson Similarity Index is used as a community similarity index for metric number 7.)

Metric 6. EPT Index

The EPT Index generally increases with increasing water quality. The EPT Index value is the total number of distinct taxa within the groups Ephemeroptera, Plecoptera, and Trichoptera. The EPT Index value summarizes the taxa richness within the insect groups that are generally considered pollution sensitive. This was developed for species-level identifications; however, the concept is valid for use at family-level identifications.

Headwater streams which are naturally unproductive may experience an increase in taxa (including EPT taxa) in response to organic enrichment.

Metric 7. Community Similarity Indices

Community Similarity Indices are used in situations where a reference community exists, either through sampling or through prediction for a region. Data sources or ecological data files may be available to predict a reference community to be used for comparison. The combined information provided through a regional analysis and EPA's ERAPT ecological database (Dawson and Hellenthal 1986) may be useful for this analysis. These indices are designed to be used with either species level identifications or higher taxonomic levels. Three of the many community similarity indices available are discussed below:

- Community Loss Index—Measures the loss of benthic taxa between a reference station and the station of comparison. The Community Loss Index was developed by Courtemanch and Davies (1987) and is an index of compositional dissimilarity, with values increasing as the degree of dissimilarity with the reference station increases. Values range from 0 to "infinity." Based on preliminary data analysis, this index provides greater discrimination than either of the following two community similarity indices.

- Jaccard Coefficient of Community Similarity—Measures the degree of similarity in taxonomic composition between two stations in terms of taxon presence or absence. The Jaccard Coefficient discriminates between highly similar collections. Coefficient values, ranging from 0 to 1.0, increase as the degree of similarity with the reference station increases. See Jaccard (1912), Boesch (1977), and U.S. EPA (1983) for more detail. The formulae for the Community Loss Index and the Jaccard Coefficient are

$$\text{Community Loss} = \frac{d-a}{e}$$

$$\text{Jaccard Coefficient} = \frac{a}{a+b+c}$$

where

a = number of taxa common to both samples
b = number of taxa present in Sample B but not A
c = number of taxa present in Sample A but not B
d = total number of taxa present in Sample A
e = total number of taxa present in Sample B

Sample A = reference station (or mean of reference database)
Sample B = station of comparison

- Pinkham and Pearson Community Similarity Index—Incorporates abundance and compositional information and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant taxa. See Pinkham and Pearson (1976) and U.S. EPA (1983) for more detail. The formula is

$$S.I._{ab} = \Sigma \frac{\min (x_{ia}, x_{ib})}{\max (x_{ia}, x_{ib})} \left[\frac{x_{ia}}{x_a} \cdot \frac{x_{ib}}{x_b} \right]^{\text{weighting factor}} / 2$$

where

x_{ia}, x_{ib} = number of individuals in the i^{th} taxon in Sample A or B

Other community similarity indices suggested by reviewers of this document include Spearman's Rank Correlation (Snedecor and Cochran 1967), Morisita's Index (Morisita 1959), Biotic Condition Index (Winget and Mangum 1979), and Bray-Curtis Index (Bray and Curtis 1957, Whittaker 1952). Calculation of a chi-square "goodness of fit" (Cochran 1952) may also be appropriate.

CPOM Sample

Metric 8. Ratio of Shredder Functional Feeding Group and Total Number of Individuals Collected

Also based on the Functional Feeding Group concept, the abundance of the Shredder Functional Group relative to the abundance of all other Functional Groups allows evaluation of potential impairment as indicated by the CPOM-based Shredder community. Shredders are sensitive to riparian zone impacts and are particularly good indicators of toxic effects when the toxicants involved are readily adsorbed to the CPOM and either affect microbial communities colonizing the CPOM or the Shredders directly (Cummins 1987, personal communication).

The degree of toxicant effects on Shredders versus Filterers depends on the nature of the toxicants and the organic particle adsorption efficiency. Generally, as the size of the particle decreases, the adsorption efficiency increases as a function of the increased surface to volume ratio (Hargrove 1972). Because water-borne toxicants are readily adsorbed to FPOM, toxicants of a

terrestrial source (e.g., pesticides, herbicides) accumulate on CPOM prior to leaf fall thus having a substantial effect on Shredders (Swift et al. 1988a and 1988b). The focus of this approach is on a comparison to the reference community which should have a reasonable representation of Shredders as dictated by seasonality, region, and climate. This allows for an examination of Shredder or Collector "relative" abundance as indicators of toxicity.

The data collected in the 100-organism riffle/run subsample and the CPOM sample are summarized according to the information required for each metric and entered on the Data Summary Sheet (Figure 6.2-2).

Each metric result is given a score based on percent comparability to a reference station. Scores are totaled and compared to the total metric score for the reference station. The percent comparison between the total scores provides a final evaluation of biological condition. Values obtained may sometimes be intermediate to established ranges and require some judgment as to assessment of biological condition. In these instances, habitat assessment, physical characterization, and water quality data may aid in the evaluation process.

6.3 RAPID BIOASSESSMENT PROTOCOL III—Benthic Macroinvertebrates

Rapid Bioassessment Protocol III (RBP III) is a more rigorous bioassessment technique than RBP II, involving systematic field collection and subsequent lab analysis in order to allow detection of more subtle degrees of impairment. Discrimination of four levels of impairment should be possible with this assessment. Although Protocol III requires more detailed taxonomy than can ordinarily be accomplished in the field, lab analysis procedures emphasize a minimal level of effort to ensure the protocol's time- and cost-effectiveness. Where differences among stations are subtle, however, more detailed sample analyses (e.g., enumeration of larger subsamples) or processing of a greater number of samples (to define replicability or assess more habitats) may be necessary to resolve such differences.

Data provided by RBP III can be used to prioritize sites for more intensive evaluation (e.g., quantitative biological surveys, ambient toxicity testing, chemical characterization). Besides providing a means of evaluating effects among stations, this protocol provides a basis for monitoring trends in benthic community structure that might be attributable to improvement or worsening of conditions over time.

6.3.1 Field Methods

The biosurvey component of RBP III focuses on the sampling of benthic macroinvertebrates supplemented by cursory field observation of the periphyton, macrophyton, slime, and fish communities. The information on observed effects upon other aquatic biota is recorded on the Biosurvey Field Data Sheet (Figure 6.3-1) and may be used to support or further evaluate benthic data.

The habitat assessment evaluates habitat quality on the basis of key parameters of the waterbody and surrounding land as described in Chapter 5. Habitat assessment is especially important in situations where benthos and other biological communities indicate an impairment. In these instances, an evaluation of habitat quality will aid in the interpretation of effects relative to a site's biotic potential. The water quality/physical characterization provides pertinent data on habitat quality as well as potential sources or causes of impairment.

6.3.1.1 Sample Collection

The purpose of the standardized collection procedure is to obtain representative samples of the macroinvertebrate fauna from comparably productive habitat types available at all stations constituting a site evaluation, supplemented with separate CPOM samples. This protocol focuses on the riffle/run habitat as the most productive habitat available in stream systems. The riffle/run benthic community includes many representatives of the Scraper and Filtering Collector Functional Feeding Groups. Riffle/run sampling is supplemented with collection of a CPOM sample. The CPOM sample provides a measure of effects on a third trophic component of the benthic community, the Shredders.

Where riffle/run habitat with a rock substrate is not available, other submerged fixed structures e.g., submerged boulders, logs, bridge abutments, pier pilings, will provide a substrate for the Scraper and Filtering Collector Functional Groups emphasized here. Sampling submerged fixed structures would also be appropriate in non-wadable streams and large rivers and wadable streams and rivers with unstable substrates.

Rapid Bioassessment Protocol III

Biosurvey Field Data Sheet

RELATIVE ABUNDANCE OF AQUATIC BIOTA

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

0 = Absent/Not Observed 1 = Rare 2 = Common 3 = Abundant 4 = Dominant

MACROBENTHOS QUALITATIVE SAMPLE LIST (Indicate Relative Abundance R = Rare, C = Common, A = Abundant, D = Dominant)

Porifera	*Anisoptera*	*Chironomidae*
Hydrozoa	Zygoptera	Plecoptera
Platyhelminthes	Hemiptera	Ephemeroptera
Turbellaria	Coleoptera	Trichoptera
Hirudinea	Lepidoptera	Other
Oligochaeta	Sialidae	
Isopoda	Corydalidae	
Amphipoda	Tipulidae	
Decapoda	Empididae	
Gastropoda	Simuliidae	
Bivalvia	Tabanidae	
	Culicidae	

Rare < 3 Common 3–9 Abundant > 10 Dominant > 50 (Estimate)

CPOM SAMPLE FUNCTIONAL FEEDING GROUPS (Indicate No. of Individuals Representing Group)

Shredders	Total Org. In Sample

Observations

Figure 6.3-1. Biosurvey Field Data Sheet for use with Rapid Bioassessment Protocol III.

Riffle/Run Sample

In most situations, a riffle area with relatively fast current and a cobble and gravel substrate provide the most diverse community. Riffles should be sampled using a kick net to collect from an approximately 1 m^2 area. Two 1 m^2 riffle samples should be collected at each station: one from an area of fast current velocity and one from an area of slower current velocity. The two samples are composited for processing. In streams lacking riffles, run areas with cobble or gravel substrate are also appropriate for kick net sampling.

Where a riffle/run community with a rock substrate is not available, other submerged fixed structures, e.g., submerged boulders, logs, bridge abutments, pier pilings, should be sampled by hand picking. These structures provide suitable habitat for the Scrapers and Filtering Collectors and will allow use of RBP III for a wider range of regions and stream orders. Evaluation of benthic production in a blackwater stream in Georgia by Benke et al. (1984) indicated that although submerged wood substrates (snags) comprised a minor portion of available substrate, the greatest taxa richness and more than half of all benthic biomass were associated with this habitat.

Field inspection of the sample is recommended to obtain a preliminary assessment of presence and relative abundance of major groups (to be indicated on the Biosurvey Field Data Sheet, Figure 6.3-1), and to determine if the sampling effort was adequate to obtain at least 100 organisms. In some samples from severely impaired areas, organism abundance may not total 100 organisms. The samples collected at the two current velocities from the same habitat are composited, preserved, labeled, and returned to the laboratory for processing.

CPOM Sample

In addition to the riffle/run sample collected for evaluation of the Scraper and Filtering Collector Functional Feeding Groups, a CPOM sample should also be collected to provide data on the relative abundance of the Shredders at the site. Shredders of large particulate material are important in forested areas of stream ecosystems ranging from stream orders 1 through 4 (Minshall et al. 1985). The absence of large particulate Shredders is characteristic of unstable, poorly retentive headwater streams in disturbed watersheds or in dry areas where leaf material processing is accomplished by terrestrial detritivores (Minshall et al. 1985). McArthur et al. (1988) reported that very few Shredders were found in summer leaf packs in South Carolina because processing was so rapid.

The CPOM sample is processed separately from the riffle/run sample and used for Functional Feeding Group characterization. Sampling of the CPOM component requires a composite collection of any of a variety of forms of CPOM (plant parts such as leaves, needles, twigs, bark, or fragments of these). Potential sample sources include leaf packs and shorezone areas where CPOM may accumulate. For the Shredder community analysis, collection of several handfuls of material should be adequate. A variety of CPOM forms should be collected if they are available. Material collected may be washed in a dip net or a sieve bucket.

Maximum Shredder abundance is obtained when the CPOM is about 50 percent decomposed (Cummins et al. 1989). Care must be taken to *avoid* collecting recent or fully decomposed leaf litter to optimize collection of the Shredder community. Seasonality may have an important influence on Shredder abundance data. For instance, fast-processing litter (e.g., basswood, alder, maples, birch) would have the highest Shredder representation in the winter (Cummins et al. 1989). The slow-processing litter (e.g., oaks, rhododendrons, beech, conifers) would have the highest Shredder representation in the summer.

6.3.1.2 Field Processing of the CPOM Sample

Organisms collected in the supplemental CPOM sample are classified as either Shredders or Non-Shredders. Taxonomic identification is not necessary for this component. The composited CPOM sample may be sorted in the field in a small pan with a light colored bottom. (If a large number of benthic macroinvertebrates has been collected, a representative subsampling of 20–60 organisms may be removed for Functional Feeding Group classification.) Numbers of individuals representing the Shredder Functional Group, as well as total number of macroinvertebrates collected in this sample, should be recorded on the Biosurvey Field Data Sheet (Figure 6.3-1) for later analysis.

6.3.2 Lab Methods

6.3.2.1 Sample Sorting and Identification

A 100-organism subsample is recommended as a time-saving sorting procedure for use with the riffle/run sample. The subsampling method described for use in this protocol is based on that used for Hilsenhoff's Biotic Index (Hilsenhoff 1987b) and is similar to that used by New York DEC (Bode 1988) and in Arkansas (Shackleford 1988). The subsampling proce-

dure consists of evenly distributing the composite sample in a gridded pan with a light-colored bottom. As grids are randomly selected, all organisms within those grids are removed, until at least 100 organisms have been selected from the sample. This method of subsampling provides a representative estimate of the benthic fauna as well as a consistent unit of effort. A more detailed description of this technique may be found in Appendix B. Although pilot study results (Section 6.4.6) indicated that a 100-organism subsample is sufficient, a 200- or 300-organism subsample may be preferred, depending on investigator preference, budget constraints, and individual sample characteristics. Some agencies may prefer to expend additional resources to process whole samples instead of subsampling.

All benthic macroinvertebrates in the subsample (or sample) should be identified to the lowest positively identified taxonomic level (generally genus or species), enumerated, and recorded on the Laboratory Bench Sheet (Figure 6.3-2). Based on the taxonomic identifications, Functional Feeding Group classifications can be assigned for most aquatic insects using a reference such as Merritt and Cummins (1984). Once a Functional Feeding Group classification list has been established, it can be incorporated into the computer analysis for computation of the metrics. Care should be taken to note the presence of early instars which may represent different Functional Feeding Groups from later instars. The Scraper and Filtering Collector Functional Groups are considered the important indicators in the riffle/run community; if this metric is not calculated using a computer program, numbers of individuals representing each of these two groups are recorded on the Laboratory Bench Sheet (Figure 6.3-2).

6.3.3 Data Analysis Techniques

Based on observations made in assessing habitat, water quality, physical characteristics, and the qualitative biosurvey, the investigator makes a preliminary judgment on the presence or absence of biological impairment and an estimation of probable cause and source on the Impairment Assessment Sheet (Figure 6.1-2).

The integrated benthic data analysis is performed as follows. Using the raw benthic data, a numerical value is calculated for each metric. Calculated values are then compared to values derived from either an unimpaired reference site within the same region or a suitable control station on the same stream. Each metric is then assigned a score according to the comparability (percent similarity) of calculated and reference values. Scores for the eight metrics are then totaled and compared to the total metric score for the reference station. The percent comparison between the total scores provides a final evaluation of biological condition.

Criteria to be used for scoring the eight metrics were derived from an evaluation of pilot study results (Section 6.4), certain project compliance monitoring requirements now in use (Vermont Department of Environmental Conservation 1987), and discussions with various aquatic biologists regarding the level of detection considered dependable for certain metrics. However, it is envisioned that these *criteria may need to be adjusted for use in particular regions.*

Inherent variability in each metric was considered in establishing percent comparability criteria. The metrics based on taxa richness, HBI, and EPT indices have low variability (Resh 1988). This variability is accounted for in the criteria for characterization of biological condition (Figure 6.2-3) based on existing data. For metrics based on standard taxa richness and HBI and EPT Indices, differences of 10–20 percent relative to the reference condition would be considered nominal, and the station being assessed would receive the maximum metric score. Because increasing HBI values denote worsening biological condition, percent difference for this metric is calculated by dividing the reference value by the value for the station of comparison.

Metrics that utilize ratios will fluctuate more widely, however, and comparing percent differences between ratios (ratios of ratios) will compound the variability. Scoring increments are therefore set at broad intervals of 25 percent or greater. For metrics based on Functional Feeding Group ratios, Cummins (1987, personal communication) contends that differences as great as 50 percent from the reference may be acceptable, but differences in the range of 50–100 percent are not only important but discriminate degrees of impact more clearly.

The percent contribution of the dominant taxon to total abundance is a simple estimator of evenness. Scoring criteria are based on theoretical considerations rather than direct comparison with a reference.

The Community Loss Index already incorporates comparison with a reference. Therefore, actual index values are used in scoring.

Analysis of the benthic data combines several community population and functional parameters. An integrated assessment is used, based on eight metrics (Table 6.3-1). Each metric has a different range of sensitivity measuring a slightly different component of community structure (Figure 8.2-1). The data collected in the 100-organism riffle/run subsample and the CPOM sample are summarized according to the information required for each metric and entered on the

LABORATORY BENCH SHEET

	Number of Organisms			
Station Number				
Station Location				
Species Name				
Total Organisms				
Number of Taxa				

Figure 6.3-2. Laboratory Bench Sheet suggested for use in recording benthic data utilized in Rapid Bioassessment Protocol III.

TABLE 6.3-1 CRITERIA[a] FOR CHARACTERIZATION OF BIOLOGICAL CONDITION FOR RAPID BIOASSESSMENT PROTOCOL III

Metric	Biological Condition			
	Non-Impaired	Slightly Impaired	Moderately Impaired	Severely Impaired
1. Taxa Richness	Comparable to the best situation to be expected within an ecoregion. Balanced trophic structure. Optimum community structure (composition and dominance) for stream size and habitat quality.	Community structure less than expected. Composition (species richness) lower than expected due to loss of some intolerant forms. Percent contribution of tolerant forms increases.	Fewer species due to loss of most intolerant forms. Reduction in EPT index.	Few species present. If high densities of organisms, then dominated by one or two taxa. Only tolerant organisms present.
2. Hilsenhoff Biotic Index (modified)				
3. Ratio of Scrapers/Filtering Collectors[b]				
4. Ratio of EPT and Chironomid Abundances				
5. % Contribution of Dominant Taxon				
6. EPT Index				
7. Community Similarity Index[c]				
8. Ratio of Shredders/Total[b]				

(a) Scoring criteria are generally based on percent comparability to the reference station.
(b) Determination of Functional Feeding Group is independent of taxonomic grouping.
(c) Community Similarity Indices are used in comparison to a reference station.

Data Summary Sheet (Figure 6.3-3). Each metric result is given a score based on percent comparability to a reference station. Evaluation of biological condition is based on comparison to the reference condition (site-specific or reference database) that is representative of the "best attainable" condition. Using this approach, metrics can be eliminated if found inapplicable, without altering the biological classification. However, this integrated assessment approach is intended to remain intact to avoid jeopardizing the integrity of the bioassessment concept.

Scores are totaled and a Biological Condition Category is assigned based on percent comparability with the reference station score. Values obtained may sometimes be intermediate to established ranges and require some subjective judgment as to assessment of biological condition. In these instances, habitat assessment, physical characterization, and water quality data may aid in the evaluation process. An explanation of the importance of interpreting biological data in the context of habitat quality is presented in Chapter 8.

The metrics used to evaluate the benthic data and their significance are described below.

Riffle/Run Sample

Metric 1. Species Richness

Reflects health of the community through a measurement of the variety of taxa (total number of genera and/or species) present. Generally increases with increasing water quality, habitat diversity, and/or habitat suitability. Sampling of highly similar habitats will reduce the variability in this metric attibutable to factors such as current speed and substrate type. Some pristine headwater streams may be naturally unproductive, supporting only a very limited number of taxa. In these situations, organic enrichment may result in an increase in number of taxa (including EPT taxa).

Metric 2. Modified Hilsenhoff Biotic Index

Tolerance values range from 0 to 10, increasing as water quality decreases. The index was developed by Hilsenhoff (1987b) to summarize overall pollution tolerance of the benthic arthropod community with a single value. This index was developed as a means of detecting organic pollution in communities inhabiting rock or gravel riffles, and has been modified for this document to include non-arthropod species as well, on the basis of the biotic index used by the State of New York (Bode 1988).

Although Hilsenhoff's biotic index was originally developed for use in Wisconsin, it is successfully used by several States and should prove reliable for extensive use, *requiring regional modification in some instances.* Alternative tolerance classifications and biotic indices have also been developed by some State agencies (Appendix C). The formula for calculating the Biotic Index is:

$$HBI = \Sigma \frac{x_i\, t_i}{n}$$

where

x_i = number of individuals within a species
t_i = tolerance value of a species
n = total number of organisms in the sample

Although it may be applicable for other types of pollutants, use of the HBI in detecting non-organic pollution effects has not been thoroughly evaluated. The State of Wisconsin is conducting a study to evaluate the ability of Hilsenhoff's index to detect non-organic effects. Winget and Mangum (1979) have developed a tolerance classification system applicable to the assessment of nonpoint source impact. Additional biotic indices are also listed in U.S. EPA (1983).

Metric 3. Ratio of Scraper and Filtering Collector Functional Feeding Groups

The Scraper and Filtering Collector Functional Group ratio reflects the riffle/run community foodbase and provides insight into the nature of potential disturbance factors. The proportion of the two feeding groups is important because predominance of a particular feeding type may indicate an unbalanced community responding to an overabundance of a particular food source. The predominant feeding strategy reflects the type of impact detected.

A description of the Functional Feeding Group concept can be found in Cummins (1973). Genus-level Functional Feeding Group designations for most aquatic insects can be found in Merritt and Cummins (1984).

DATA SUMMARY SHEET

Station No.								
Station Location								
Taxa Richness								
FBI (modified)								
Functional Feeding Groups								
Riffle Community								
Scrapers/Filt. Collect.								
CPOM Community								
Shredders/Total								
EPT/Chironomidae								
% Contribution (dom. family)								
EPT Index								
Community Similarity Index								
Comments:								

Figure 6.3-3. Data Summary Sheet suggested for use in recording benthic data utilized in Rapid Bioassessment Protocol III.

The relative abundance of Scrapers and Filtering Collectors in the riffle/run habitat provides an indication of the periphyton community composition and availability of suspended Fine Particulate Organic Material (FPOM) associated with organic enrichment. Scrapers increase with increased abundance of diatoms and decrease as filamentous algae and aquatic mosses (which cannot be efficiently harvested by Scrapers) increase. However, filamentous algae and aquatic mosses provide good attachment sites for Filtering Collectors, and the organic enrichment often responsible for overabundance of filamentous algae provides FPOM utilized by the Filterers.

Filtering Collectors are also sensitive to toxicants bound to fine particles and may decrease in abundance when exposed to sources of such bound toxicants (Cummins 1987). The Scraper to Filtering Collector ratio may not be a good indication of organic enrichment if adsorbing toxicants are present. This situation is often associated with point source discharges where certain toxicants adsorb readily to dissolved organic matter (DOM) forming FPOM during flocculation. Toxicants thus become available to Filterers via FPOM. In these instances the HBI and EPT Index may provide additional insight. Qualitative field observations on periphyton abundance may also be helpful in interpreting results.

Metric 4. Ratio of EPT and Chironomidae Abundances

The EPT and Chironomidae abundance ratio uses relative abundance of these indicator groups as a measure of community balance. Good biotic condition is reflected in communities having a fairly even distribution among all four major groups and with substantial representation in the sensitive groups Ephemeroptera, Plecoptera, and Trichoptera. Skewed populations having a disproportionate number of the generally tolerant Chironomidae relative to the more sensitive insect groups may indicate environmental stress (Ferrington 1987). Certain species of some genera such as *Cricotopus* are highly tolerant (Lenat 1983, Mount et al. 1984), opportunistic, and may become numerically dominant in habitats exposed to metal discharges where EPT taxa are not abundant, thereby providing a good indicator of toxicant stress (Winner et al. 1980). Clements et al. (1988) found that mayflies were more sensitive than chironomids when exposed to 15 to 32 μg/L of copper.

Chironomids tend to become increasingly dominant in terms of percent taxonomic composition and relative abundance along a gradient of increasing enrichment or heavy metals concentration (Ferrington 1987).

An alternative to the ratio of EPT and Chironomidae abundance metric is the Indicator Assemblage Index (IAI) developed by Shackleford (1988). The IAI integrates the relative abundances of the EPT taxonomic groups and the relative abundances of chironomids and annelids upstream and downstream of a pollutant source to evaluate impairment. The IAI may be a valuable metric in areas where the annelid community may fluctuate substantially in repsonse to pollutant stress.

Metric 5. Percent Contribution of Dominant Taxon

The percent contribution of the numerically dominant taxon to the total number of organisms is an indication of community balance at the lowest positive taxonomic level. (The lowest positive taxonomic level is assumed to be genus or species in most instances.) A community dominated by relatively few species would indicate environmental stress. (If the Pinkham and Pearson Similarity Index is used as a community similarity index for metric number 7, this metric may be redundant.) Shackleford (1988) has modified this metric to reflect "dominants in common" (DIC) utilizing the dominant five taxa at the stations of comparison.

This DIC approach is based on the original metric used in earlier drafts of this RBP document. The DIC will provide a measure of replacement or substitution between the reference community and the downstream station. The purpose of the modification to "percent contribution of dominant taxon" used in RBP III (and RBP II) is to focus on evenness/redundancy of the benthic community regardless of taxa composition. Compositional shifts are measured by other metrics such as the community similarity indices.

Metric 6. EPT Index

The EPT Index generally increases with increasing water quality. The EPT Index is the total number of distinct taxa within the orders Ephemeroptera, Plecoptera, and Trichoptera. This value summarizes taxa richness within the insect orders that are generally considered to be pollution sensitive.

Headwater streams which are naturally unproductive may experience an increase in taxa (including EPT taxa) in response to organic enrichment. In this situation, a "missing genera" approach may be more valuable. Shackleford (1988) uses a "missing genera" metric to evaluate the loss of EPT taxa from upstream to downstream to avoid the complication in data interpretation resulting from the addition or replacement of genera.

Metric 7. Community Similarity Indices

Community Similarity Indices are used in situations where reference communities exist. The reference community can be derived through sampling or prediction for a region using a reference database. Data sources or ecological data files may be available to establish a reference community for comparison. The combined information provided through a regional analysis and EPA's ERAPT ecological database (Dawson and Hellenthal 1986) may be useful for this analysis. Three of the many similarity indices available are discussed below:

- Community Loss Index—Measures the loss of benthic species between a reference station and the station of comparison. The Community Loss Index was developed by Courtemanch and Davies (1987) and is an index of dissimilarity with values increasing as the degree of dissimilarity from the reference station increases. Values range from 0 to "infinity." Based on preliminary data analysis, this index provides greater discrimination than the following two community similarity indices.
- Jaccard Coefficient of Community—Measures the degree of similarity in taxonomic composition between two stations in terms of taxon presence or absence. The Jaccard Coefficient discriminates between highly similar collections. Coefficient values, ranging from 0 to 1.0, increase as the degree of similarity with the reference station increases. See Jaccard (1912), Boesch (1977), and U.S. EPA (1983) for more detail. The formulae for the Community Loss Index and the Jaccard Coefficient are

$$\text{Community Loss} = \frac{d-a}{e}$$

$$\text{Jaccard Coefficient} = \frac{a}{a+b+c}$$

where

a = number of species common to both samples
b = number of species present in Sample B but not A
c = number of species present in Sample A but not B
d = total number of species present in Sample A
e = total number of species present in Sample B

Sample A = reference station
Sample B = station of comparison

- Pinkham and Pearson Community Similarity Index—Measures the degree of similarity in taxonomic composition in terms of taxon abundances and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant species. See Pinkham and Pearson (1976) and U.S. EPA (1983) for more detail. The formula is

$$S.I._{ab} = \Sigma \frac{\min (x_{ia}, x_{ib})}{\max (x_{ia}, x_{ib})} \left[\frac{x_{ia}}{x_a} \cdot \frac{x_{ib}}{x_b} / 2 \right]$$

weighting factor

where

x_{ia}, x_{ib} = number of individuals in the i^{th} species in Sample A or B

Other community similarity indices suggested by reviewers of this document include Spearman's Rank Correlation

(Snedecor and Cochran 1967), Morisita's Index (Morisita 1959), Biotic Condition Index (Winget and Mangum 1979), and Bray-Curtis Index (Bray and Curtis 1959, Whittaker 1952). Calculation of a chi-square "goodness of fit" (Cochran 1952) may also be appropriate.

CPOM Sample

Metric 8. Ratio of Shredder Functional Feeding Group and Total Number of Individuals Collected

Also based on the Functional Feeding Group concept, the abundance of the Shredder Functional Group relative to the abundance of all other Functional Groups allows evaluation of potential impairment as indicated by the CPOM-based Shredder community. Shredders are sensitive to riparian zone impacts and are particularly good indicators of toxic effects when the toxicants involved are readily adsorbed to the CPOM and either affect the microbial communities colonizing the CPOM or the Shredders directly (Cummins 1987).

The degree of toxicant effects on Shredders versus Filterers depends on the nature of the toxicants and the organic particle adsorption efficiency. Generally, as the size of the particle decreases, the adsorption efficiency increases as a function of the increased surface to volume ratio (Hargrove 1972). As stated in metric 3, water-borne toxicants are readily adsorbed to FPOM. Toxicants of a terrestrial source (e.g., pesticides, herbicides) accumulate on CPOM prior to leaf fall thus having a substantial effect on Shredders (Swift et al. 1988a and 1988b). The focus of this approach is on a comparison to the reference community, which should have an abundance and diversity of Shredders representative of the particular area under study. This allows for an examination of Shredder or Collector "relative" abundance as indicators of toxicity.

The data collected in the 100-organism riffle/run subsample and the CPOM sample are summarized according to the information required for each metric and entered on the Data Summary Sheet (Figure 6.3-3).

Each metric result is given a score based on percent comparability to a reference station. Scores are totaled and a Biological Condition Category is assigned based on percent comparability with the reference station score (Figure 6.3-3). Values obtained may sometimes be intermediate to established ranges and require some subjective judgment as to assessment of biological condition. In these instances, habitat assessment, physical characterization, and water quality data may aid in the evaluation process.

For RBP III, four categories of scores are established for the assessment of biological condition. The power to differentiate four categories for RBP III over three for RBP II is derived from additional effort necessary for the lowest possible taxonomic identifications. However, the rationale for metric percentage ranges is essentially the same as for RBP II. Figure 6.3-4 outlines the steps that would be taken in a biological assessment patterned after Protocol III.

6.4 RESULTS OF A PILOT STUDY CONDUCTED ON THE ARARAT AND MITCHELL RIVERS, NORTH CAROLINA

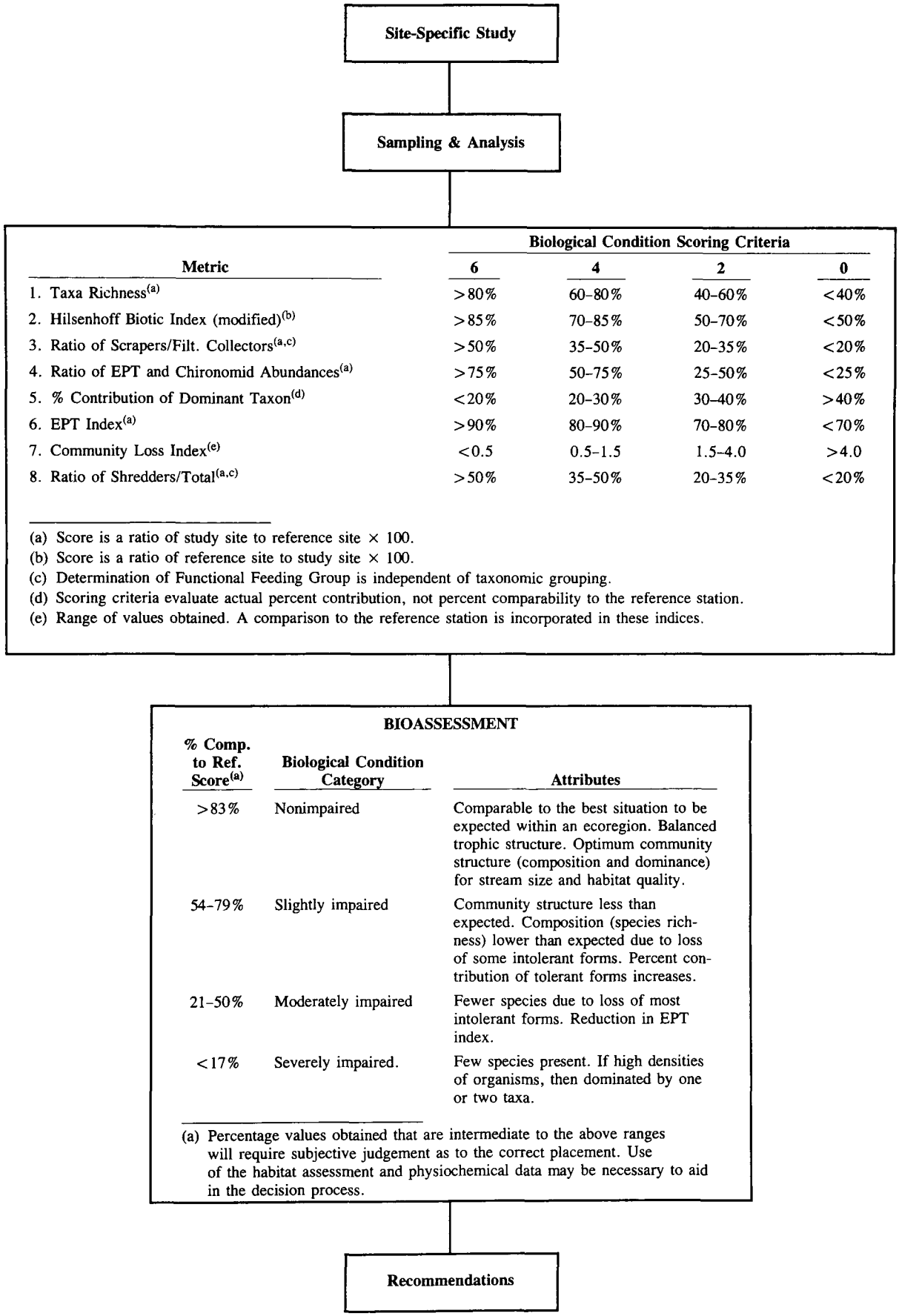
6.4.1 Introduction

A joint survey was conducted by EA Engineering, Science, and Technology and North Carolina Division of Environmental Management (DEM) on 23–24 September 1986. The objective of this study was to investigate several methodological questions raised at the Benthic Rapid Bioassessment Workshop held in July 1986.

The principal questions were

- Is it necessary to integrate sampling across all appropriate habitats at a given site or will sampling a single productive habitat (such as a riffle) suffice for a general characterization of biological integrity?
- Should abundances be characterized as categorical estimates for a total sample or as relative abundances based on a given size subsample? If counts on subsamples are preferred, what is the minimum count needed to detect basic differences among stations?
- Can family-level identifications be useful for a site prioritization or is it necessary to identify all organisms to the lowest taxonomic level?

The purpose of this research project was to assess the use of protocols II and III relative to the above



Metric	Biological Condition Scoring Criteria			
	6	4	2	0
1. Taxa Richness[a]	>80%	60-80%	40-60%	<40%
2. Hilsenhoff Biotic Index (modified)[b]	>85%	70-85%	50-70%	<50%
3. Ratio of Scrapers/Filt. Collectors[a,c]	>50%	35-50%	20-35%	<20%
4. Ratio of EPT and Chironomid Abundances[a]	>75%	50-75%	25-50%	<25%
5. % Contribution of Dominant Taxon[d]	<20%	20-30%	30-40%	>40%
6. EPT Index[a]	>90%	80-90%	70-80%	<70%
7. Community Loss Index[e]	<0.5	0.5-1.5	1.5-4.0	>4.0
8. Ratio of Shredders/Total[a,c]	>50%	35-50%	20-35%	<20%

(a) Score is a ratio of study site to reference site × 100.
(b) Score is a ratio of reference site to study site × 100.
(c) Determination of Functional Feeding Group is independent of taxonomic grouping.
(d) Scoring criteria evaluate actual percent contribution, not percent comparability to the reference station.
(e) Range of values obtained. A comparison to the reference station is incorporated in these indices.

BIOASSESSMENT

% Comp. to Ref. Score[a]	Biological Condition Category	Attributes
>83%	Nonimpaired	Comparable to the best situation to be expected within an ecoregion. Balanced trophic structure. Optimum community structure (composition and dominance) for stream size and habitat quality.
54-79%	Slightly impaired	Community structure less than expected. Composition (species richness) lower than expected due to loss of some intolerant forms. Percent contribution of tolerant forms increases.
21-50%	Moderately impaired	Fewer species due to loss of most intolerant forms. Reduction in EPT index.
<17%	Severely impaired.	Few species present. If high densities of organisms, then dominated by one or two taxa.

(a) Percentage values obtained that are intermediate to the above ranges will require subjective judgement as to the correct placement. Use of the habitat assessment and physiochemical data may be necessary to aid in the decision process.

Figure 6.3-4. Flowchart of bioassessment approach advocated for Rapid Bioassessment Protocol III.

questions. The purpose was not to disprove or discredit any sampling techniques or assessment methods presently in use. As stated earlier, the benthic rapid bioassessment protocols are essentially a synthesis of existing methods that have been employed for some time by various States, (e.g., North Carolina, New York, and Virginia). This guidance, therefore, is meant to provide basic, cost-effective data gathering methods for States that (1) have no established bioassessment procedures, (2) are looking for alternative methodologies, or (3) may need to supplement their existing programs, (not supersede other bioassessment approaches that have already been successfully implemented). Furthermore, the results of the Ararat and Mitchell River Pilot Study should not be viewed as full validation of Rapid Bioassessment Protocols II and III. Subsequent studies and additional refinement in the course of implementation are needed to fully validate the procedures presented in this document.

The Pilot Study was performed in conjunction with North Carolina DEM because their methods were well developed and supported by a large database. Therefore, results from the North Carolina DEM biosurvey provide the basis of evaluation for resolution of the issues listed above.

6.4.2 Methods

The study site was the Ararat River near Mt. Airy, North Carolina, located in the Central Appalachian Ridge and Valley ecoregion. Sampling was conducted at four stations on the Ararat River and one station on the Mitchell River. The Mitchell River served as a reference site representative of excellent biological condition within the region. Two sites above the town of Mt. Airy were selected to be used as site-specific controls. The station selected as a regional reference (Station R) was on the Mitchell River, located near the Ararat River in the same county. A description of the biological sampling stations as illustrated in Figure 6.4-1 are as follows:

Station 1, Ararat River at NC 104. Station 1 served as a control station and was located near the North Carolina-Virginia border. Station 1 was established by North Carolina DEM to monitor the water quality of the Ararat River above the town of Mt. Airy. Land use in this area was a mixture of forestry and agriculture.

Station 2, Ararat River at NC 52 (Bus.). Station 2 provided an alternate control station and was located approximately 1 mi above the WWTP discharge. Station 2 was established by North Carolina DEM to assess the impacts of urban runoff and any unpermitted discharges. Land use was predominantly urban.

Station 3, Ararat River near SR 2116. Station 3 was located just above the confluence of Lovills Creek and the Ararat River and was several hundred meters below the WWTP discharge. Land use was predominantly urban.

Station 4, Ararat River at SR 2019. Station 4 was a site sampled as part of the North Carolina DEM ambient network on 4 August 1986. Station 4, located about 11 miles below the Mt. Airy WWTP discharge, was intended as a primary station for the evaluation of recovery. Land use was primarily forestry with some agriculture.

Station R, Mitchell River at SR 1419. This "reference" station was established in an area known to have good to excellent water quality. Land use was predominantly forestry. Regional reference stations are especially valuable in differentiating between the effects of pollution and natural seasonal and temporal variation, and in estimating the biological potential of waterbodies within a region.

6.4.2.1 Field Collections

Samples were collected concurrently by personnel from North Carolina DEM and EA Engineering, Science, and Technology. At each station, one collecting team from both North Carolina DEM and EA collected two kick-net samples from riffle areas (from both a fast and a slow current velocity area). In addition, North Carolina DEM personnel sampled other habitats according to their standardized collection technique utilizing a variety of sampling methods to collect from all microhabitats present at a station (Lenat 1988). Methods included kick net and sweep net sampling of riffle areas, root masses, "snags," bank areas, and macrophyte beds; use of fine mesh samplers into which invertebrates inhabiting rocks and logs were washed; sieving of leaf pack and sand samples; and visual inspection of large rocks and logs to collect attached organisms (N.C. DNR and Community Development 1983).

EA samples were sorted in the field, with all organisms and sample remains being preserved for additional analysis. North Carolina samples were also sorted in the field (according to their standard procedure), but the organisms collected from the riffle sample were kept separate from those found in all other habitats. These samples were preserved in the event that additional analysis was needed. Additional information collected included a habitat assessment and general physical characterization of the site (e.g.,

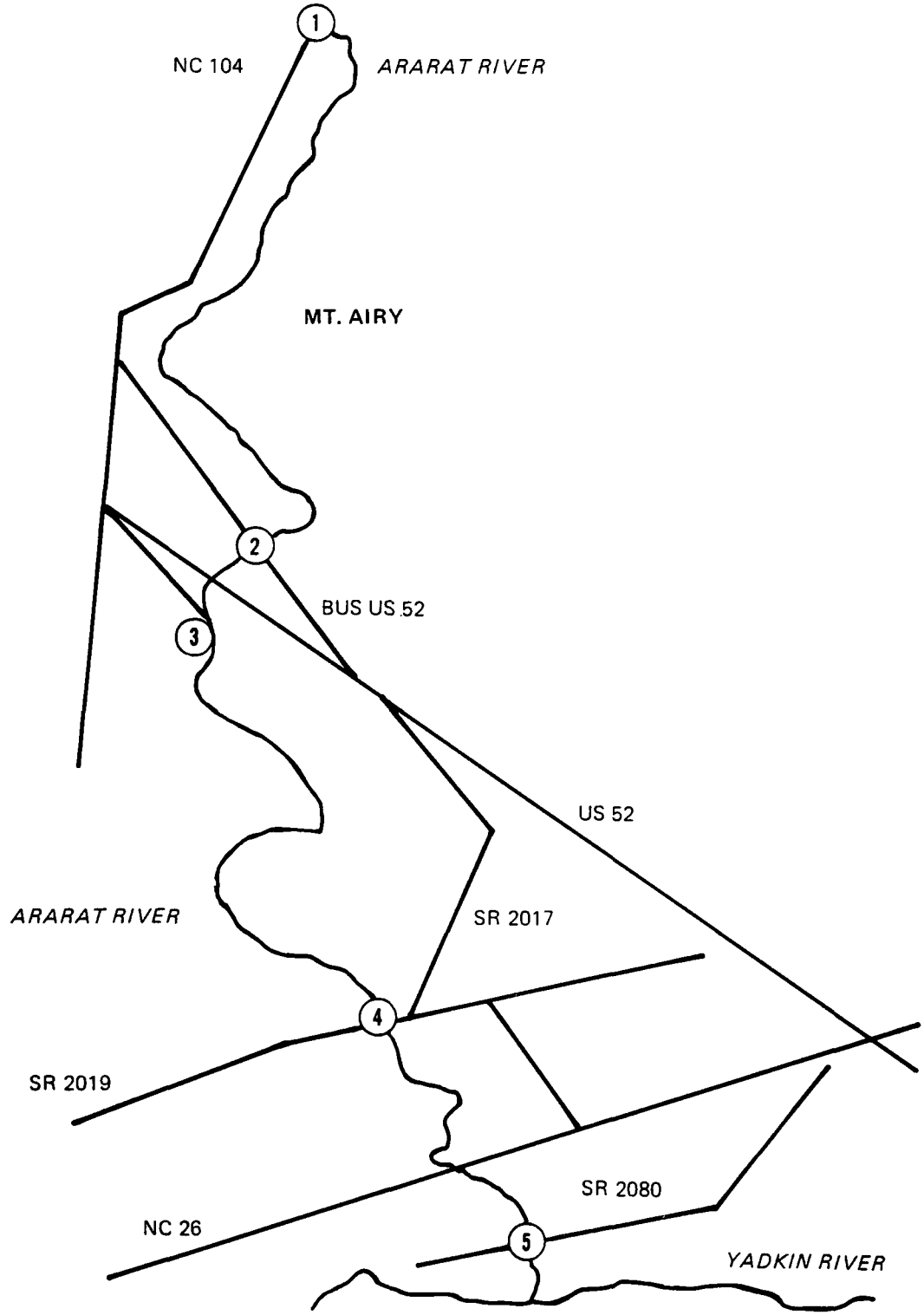


Figure 6.4-1. Pilot study station locations, Ararat River, North Carolina, September 1986. (Taken from 1 October 1986 NC DEM memorandum.)

stream depth, width). The EA sampling effort did not include collection of a CPOM sample. This pilot study pre-dated inclusion of the Shredder metric in RBPs II and III.

6.4.2.2 Laboratory Processing

Samples (vials of organisms) collected by North Carolina DEM were taken to their laboratory and processed according to standard agency procedures (N.C. DNR and Community Development 1983). Organisms were identified to genus or species and tabulated as a total multihabitat sample with the taxa composition for the riffle sample presented separately. Abundances were characterized as categorical estimates of total abundance.

Samples collected by EA were taken to the EA laboratory and processed by a variety of methods to allow comparisons among data sets. First, organisms picked in the field were identified to the lowest positive taxon (species in most cases). Afterwards, these organisms were added to the remainder of the sample brought in from the field. Subsampling to obtain 100 organisms was then performed on the sample using the methods in Appendix B. After picking and separating 100 organisms, an additional 100 were picked and kept separate a second and third time. All three 100-organism subsamples (a total of approximately 300 organisms) were enumerated and identified as separate entities.

6.4.2.3 Quality Assurance

Quality assurance measures were adhered to throughout the pilot study to ensure the reliability of results. Field collection of samples was conducted in conjunction with North Carolina DEM personnel; all samples being collected simultaneously at a given station. Habitat was assessed consistently by the same individual at all stations. All field efforts were thoroughly documented.

All sample processing in the lab was performed by the same individual to ensure consistency in sorting and identification. Subsampling was randomized by using a random numbers table. Number of organisms picked from each block was recorded to verify random distribution and to validate the subsampling procedure. Taxonomic identification was separately documented for each subsample.

6.4.3 Bioclassification of Stations Based on the North Carolina DEM Protocol

Results of the North Carolina DEM study are presented here as described in a memorandum from Dave Lenat to Steve Tedder dated 1 October 1986 (Lenat 1986). These results form the basis for this evaluation of the rapid bioassessment protocols. The biological condition of the Ararat River is discussed in reference to the condition of the Mitchell River (reference station "R").

Although the Ararat River more than doubles in size within the study area, we would expect only minor changes in the composition of the benthic community. Depth and substrate characteristics are similar at all sites, although less sand was observed at the Mitchell River station. This may reflect fewer nonpoint-source problems in the Mitchell River drainage area. Some substrate differences may also be due to differences in soil type.

Taxa richness values (Table 6.4-1) indicated good-fair water quality at Stations 1 and 2. Poor water quality was indicated at Station 3, and fair water quality at Station 4. Only the Mitchell River (Station R) was found to have excellent water quality. There was little indication that urban runoff or unpermitted discharges had any effect on the biota of the Ararat River upstream of Station 2. The Mt. Airy WWTP discharge eliminated all but the most tolerant species, and full recovery appears to take over 25 river miles under low flow conditions.

A Biotic Index[a] has been computed for all sites, using a numeric abundance of 1 for rare species, 3 for common species, and 10 for abundant species. This method will probably give a slightly different value than more quantitative methods, but this type of computation still appears to allow valid between-station comparisons.

Ranking of stations by the Biotic Index values gives results very similar to the taxa richness criteria, i.e.:

$$R > 1 = 2 > 4 > 3.$$

Plecoptera (stoneflies) were completely eliminated at Stations 3 and 4 (Table 6.4-2). Ephemeroptera (mayflies) were largely absent at Station 3 and sharply reduced at Station 4. Trichoptera (caddisflies) were also eliminated at Station 3, but recovered more quickly than the mayflies.

Patterns of numeric abundance for the major groups also have been very roughly indicated (Table 6.4-2) from both field notes and lab counts. These data again indicate a strong similarity between Stations 1 and 2, but note the increased abundance of some Coleoptera (riffle beetles) at

[a]Tolerance characterization developed by North Carolina DEM.

TABLE 6.4-1 BIOCLASSIFICATION RESULTS FOR NORTH CAROLINA DEM MULTIHABITAT BENTHIC SAMPLES COLLECTED FROM THE ARARAT (STATIONS 1-4) AND MITCHELL (STATION R) RIVERS, 23-24 SEPTEMBER 1986

	Stations				
	1	2	3	4	R
Total Taxa Richness	64	63	32	50	94
EPT[a] Taxa Richness	18	20	1	11	31
EPT Abundance[b]	92	75	1	76	133
Biotic Index					
Numeric Value	2.6	2.7	3.4	3.2	2.3
Hilsenhoff Rating	Good	Good	Poor	Fair	V.Good
# Intolerant Taxa[c]	11	7	0	1	15
All	14	11	1	3	24
# Unique Taxa[d]	2	6	2	3	24
Bioclassification[e]	Good-Fair	Good-Fair	V.Poor	Fair	Excel.

(a) Intolerant groups--Ephemeroptera, Plecoptera, and Trichoptera.
(b) Rare=1, common=3, abundant=10, summed for all EPT groups.
(c) Only those intolerant taxa which are common or abundant are counted.
(d) Number of taxa occuring at only one of the five stations, very tolerant species excluded.
(e) Based on DEM Taxa Richness Criteria for Piedmont Rivers.

Station 2. This site also had a greater number of "unique" species (defined here as occurring at only one of the study sites). These between-station differences are probably due to the presence of *Podostemum* (riverweed) at Station 2. Unexplained factors appear to have reduced or eliminated *Podostemum* growths at Station 1.

Organic indicator species were generally not abundant at Ararat River sites. Only *Limnodrilus hoffmeisteri* was found to be abundant, and only at Station 3 (immediately below the WWTP discharge). Note that another oligochaete taxon, Lumbriculidae, was abundant at both Stations 3 and 4. This group is more strongly associated with toxics than with organic pollution.

Toxic indicator species were abundant throughout the Ararat River. The presence of *Cricotopus bicinctus* and *C. infuscatus* gr. at both Stations 1 and 2 suggested some upstream toxicity problems. Although these species were abundant at the upstream stations, they were not dominant taxa. At Stations 3 and 4, however, toxic indicator species clearly dominated the benthic macroinvertebrate community. These data indicate that toxic problems are of greater importance in the Ararat River than organic loading.

Other species-level data are also helpful in making between-station comparisons. These data again indicate comparable water quality at Stations 1 and 2. The loss of some intolerant species at Station 2 (*Heptagenia aphrodite, Helichus, Hydropsyche bronta, Atherix lantha*) seems to be offset by the appearance of other intolerant species (*Baetisca carolina, Promoresia elegans*). It is also evident that Station 3, just below the Mt. Airy discharge, is occasionally influenced by drift from the

TABLE 6.4-2 TAXA RICHNESS, BY GROUP, FOR SAMPLES COLLECTED BY NORTH CAROLINA DEM FROM THE ARARAT (STATIONS 1-4) AND MITCHELL (STATION R) RIVERS

Group	Stations 1	2	3	4	R
Ephemeroptera	12(a)	13(a)	1	6(a)	16(a)
Plecoptera	2	1	0	0	2(a)
Trichoptera	4	6	0	5(a)	13(a)
Coleoptera	6	5(a)	2	2	6
Odonata	3	5	5	4	6
Megaloptera	2	2	1	1(a)	2
Diptera: Misc.	4	3	2	2	4
Diptera: Chiron.	23(a)	22(a)	18(a)	23(a)	32
Oligochaeta	5	5	3(a)	3(a)	4
Crustacea	1	0	0	0	1
Mollusca	1	1	0	2	5(a)
Other	1	0	0	2	3
Subtotal (EPT)	18	20	1	11	31
Total	64	63	32	50	94

(a) Dominant groups (from lab counts and field notes).

upstream sites. This explains the presence of species such as *Baetis pluto* and *Promoresia elegans*. These species did not persist 11 miles downstream.

Several tolerant species were most abundant at Station 4, about 11 miles below the discharge. These taxa included *Rheotanytarsus, Hydropsyche betteni, Argia,* and *Stenacron interpunctatum.* Note that *S. interpunctatum* has replaced the more intolerant *S. pallidum* at this station.

6.4.4 Selection of Metrics

The analysis techniques in this document focus on the use of metrics for assessment of various components of benthic macroinvertebrate community structure and function. Various metrics were evaluated for use in making a biological assessment of the benthic community in the Ararat and Mitchell Rivers. The metrics evaluated were:

- Taxa or species richness—The number of taxa at the lowest identifiable level.
- Percent contribution of the dominant taxon—Intended as a simple measure of evenness.
- Modified Hilsenhoff Biotic Index (HBI)—Integrates tolerance classification with abundance.
- EPT Index—The number of distinct taxa within the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies).
- Ratio of abundance of EPT organisms and chironomid larvae—Intended to measure a shift in organism dominance as a response to toxicants or other pollutants.
- Community Similarity Indices—The Jaccard Coefficient, Pinkham and Pearson Index, weighted Pinkham and Pearson Index, and Community Loss Index.
- Functional Group ratios—Shredders/Collectors, Scrapers/Filterers, and Filterers/Gatherers.

Cluster analyses were used as a means of data evaluation, providing a level of differentiation among varied data sets independent of the rapid bioassessment technique. The few data acquired by this one pilot study do not constitute a rigorous analysis, nor are the results obtained by the cluster analysis intended to be a definitive validation of the rapid bioassessment technique. Hopefully, a larger database will be available in the future to more adequately refine the rapid bioassessment metrics and associated criteria.

Thirteen metrics were calculated using the 100-organism subsample data collected from the riffle habitat of the Ararat and Mitchell River stations. The resulting information was compared using a cluster analysis (Figure 6.4-2). The relative proximity of metrics on the dendrogram, based on distance between cluster centroids, was used to determine the unique information contributed by each metric to an integrated bioassessment.

Seven metrics were considered to add some level of information to the biological assessment, as denoted by the distance between cluster centroids. These are Taxa Richness, Percent Contribution of the Dominant Taxon, Ratio of Scraper to Filtering Collector Functional Feeding Groups, Community Loss Index, Ratio of EPT and Chironomid Abundances, Modified HBI, and EPT Index. The other six metrics appeared to be somewhat redundant to one or more of these seven selected metrics in terms of contributed information. These seven metrics, along with an eighth metric added subsequently to the pilot study, the Ratio of Shredder Functional Group to Total Number of Organisms (derived from a CPOM sample), form the basis of the integrated analysis advocated in this rapid bioassessment approach. These metrics are described in Sections 6.2 and 6.3. The computed data for the original seven metrics are presented in Tables 6.4-3 and 6.4-4.

In addition to cluster analysis, the bioassessment technique described in Sections 6.2 and 6.3 was used to evaluate relationships of biological condition among stations. These results are presented in Section 6.4.8.

6.4.5 Comparison of Multihabitat vs. Single Habitat Collections

From the analysis conducted by North Carolina DEM, the stations were ranked from excellent to poor biological condition as follows:

$$R >> 1 = 2 > 4 >> 3$$

The double brackets, which indicate a greater degree of difference between stations than a single bracket, were added to the North Carolina DEM

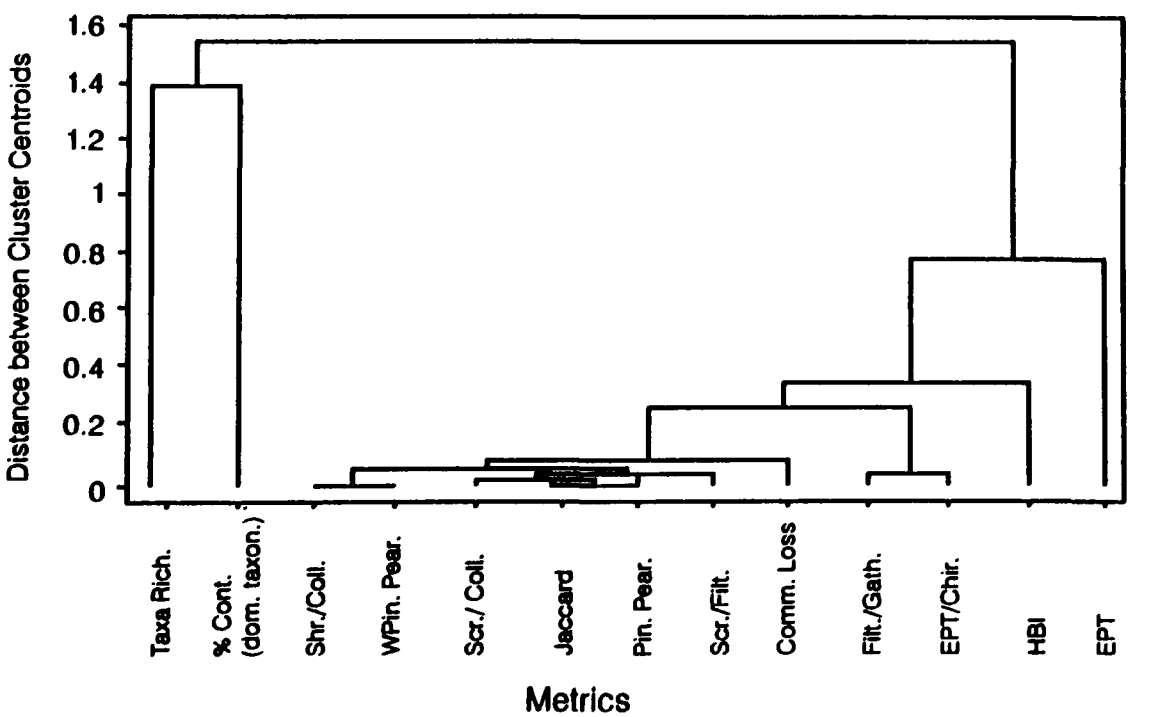


Figure 6.4-2. Cluster analysis results for benthic community metrics, based on 100 organism subsamples from riffle samples collected on the Ararat and Mitchell Rivers.

TABLE 6.4-3 METRIC VALUES, PERCENT COMPARISON, AND BIOASSESSMENT SCORES FOR BENTHIC PILOT STUDY RESULTS: 100-, 200-, AND 300-ORGANISM SUBSAMPLE DATA

Metrics	Metric Value Station					% Comparison Station					Bioassessment Score[a] Station				
	1	2	3	4	R	1	2	3	4	R	1	2	3	4	R
100-ORGANISM SUBSAMPLE															
Taxa Richness	26	26	11	34	34	76	76	32	100	100	4	4	0	6	6
HBI[b]	4.46	4.63	9.34	6.24	3.93	88	85	42	63	100	6	4	0	2	6
Scrapers/Filt. Collect.	0.833	0.604	0.000	0.108	1.500	56	40	0	7	100	6	4	0	0	6
EPT/Chiron. Abundance	2.45	1.47	0.00	0.55	9.28	26	16	0	6	100	2	0	0	0	6
% Contrib. Dom. Taxon[c]	11.2	19.8	53.5	16.5	14.2	11	20	54	16	14	6	4	0	6	6
EPT Index	12	13	0	12	14	86	93	0	86	100	4	6	0	4	6
Community Loss Index[d]	0.64	0.64	2.31	0.62	0	--	--	--	--	--	4	4	2	4	6
Total Score											32	26	2	22	42
Biological Condition											Slightly	Slightly	Sev.	Mod.	Non
200-ORGANISM SUBSAMPLE															
Taxa Richness	32	32	15	38	41	78	78	36	93	100	4	4	0	6	6
HBI[b]	4.55	4.57	9.33	6.06	3.63	80	79	39	60	100	4	4	0	2	6
Scrapers/Filt. Collect.	0.92	0.54	0.00	0.26	3.02	30	18	0	8	100	2	0	0	0	6
EPT/Chiron. Abundance	3.07	1.28	0.00	0.59	13.40	23	10	0	4	100	0	0	0	0	6
% Contrib. Dom. Taxon[c]	11.1	21.1	56.5	16.2	20.1	11	21	56	16	20	6	4	0	6	4
EPT Index	15	15	0	13	17	88	88	0	76	100	4	4	0	2	6
Community Loss Index[d]	0.68	0.73	2.12	0.68	0	--	--	--	--	--	4	4	2	4	6
Total Score											24	20	2	20	40
Biological Condition											Slightly	Mod.	Sev.	Mod.	Non
300-ORGANISM SUBSAMPLE															
Taxa Richness	36	42	19	43	51	70	82	37	84	100	4	6	0	6	6
HBI[b]	4.56	4.60	9.31	6.07	3.55	78	77	38	58	100	4	4	0	2	6
Scrapers/Filt. Collect.	0.84	0.58	0.00	0.20	2.96	28	19	0	7	100	2	0	0	0	6
EPT/Chiron. Abundance	2.91	1.39	0.00	0.49	15.14	19	9	0	3	100	0	0	0	0	6
% Contrib. Dom. Taxon[c]	10.6	19.5	56.3	16.7	24.2	11	20	56	17	24	6	4	0	6	4
EPT Index	16	18	0	13	21	76	86	0	62	100	2	4	0	0	6
Community Loss Index[d]	0.68	0.75	2.00	0.64	0	--	--	--	--	--	4	4	2	4	6
Total Score											22	22	2	18	40
Biological Condition											Slightly	Slightly	Sev.	Mod.	Non

(a) Adjusted for use with seven metrics.
(b) Ratio of reference to station of comparison.
(c) Actual percent contribution evaluated, not percent comparability.
(d) Range of values evaluated, not percent comparability.

TABLE 6.4-4 METRIC VALUES, PERCENT COMPARISON, AND BIOASSESSMENT SCORES FOR BENTHIC PILOT STUDY RESULTS: EA FIELD-SORTED AND FAMILY-LEVEL IDENTIFICATION DATA

Metrics	Metric Value Station					% Comparison Station					Bioassessment Score[a] Station				
	1	2	3	4	R	1	2	3	4	R	1	2	3	4	R
EA FIELD SORTED															
Taxa richness	18	30	7	17	29	62	103	24	59	100	4	6	0	2	6
HBI[b]	4.26	3.98	8.33	5.41	4.19	98	105	50	77	100	6	6	2	4	6
Scrapers/Filt. Collect.	1.47	1.60	0.00	0.32	2.15	68	74	0	15	100	6	6	0	0	6
EPT/Chiron. Abundance	12.6	5.08	0.00	1.96	32.00	39	16	0	6	100	2	0	0	0	6
% Contrib. Dom. Taxon[c]	20.0	12.0	53.8	23.2	10.9	20	12	54	23	11	4	6	0	4	6
EPT Index	11	15	0	10	17	65	88	0	59	100	0	4	0	0	6
Community Loss Index[d]	1.00	0.53	3.50	1.06	0	--	--	--	--	--	4	4	2	4	6
Total Score											26	32	4	14	42
Biological Condition											Slightly	Slightly	Sev.	Mod.	Non
FAMILY-LEVEL IDENTIFICATION															
Taxa richness	12	13	5	12	21	57	62	24	57	100	3	3	0	3	6
FBI[b]	5.15	5.37	9.30	6.14	4.59	89	85	49	75	100	6	3	0	3	6
Scrapers/Filt. Collect.	0.83	0.60	0.00	0.11	1.50	56	40	0	7	100	6	3	0	0	6
EPT/Chiron. Abundance	2.45	1.474	0.000	0.550	9.286	26	16	0	6	100	3	0	0	0	6
% Contrib. dom. family[c]	19.0	34.2	70.9	54.5	34.0	19	34	71	54	34	6	3	0	0	3
EPT Index	5	6	0	4	8	62	75	0	50	100	0	3	0	0	6
Community Loss Index[d]	0.83	0.85	3.60	1.08	0	--	--	--	--	--	3	3	3	3	6
Total Score											27	18	3	9	39
Biological Condition											Mod.	Mod.	Sev.	Sev.	Non

(a) Adjusted for use with seven metrics.
(b) Ratio of reference to station of comparison.
(c) Actual percent contribution evaluated, not percent comparability.
(d) Range of values evaluated, not percent comparability.

results to provide another level of differentiation of biological condition.

The North Carolina DEM analysis was conducted by sampling several habitats and making an integrated assessment focusing primarily on taxa richness and EPT Index. These results were used as a basis for comparison to results obtained only from the riffle habitat. Comparison of taxa richness for the multihabitat and riffle samples that were analyzed using the same method indicates that more taxa were collected using the multihabitat approach (Figure 6.4-3). The general trend of taxa richness among stations was similar. Although the multihabitat approach provides a distinct separation of benthic diversity among stations, data obtained from the riffle habitat alone is sufficient for a discrimination among the stations with regard to taxa richness. A comparison of the EPT Index between the multihabitat and riffle samples was highly similar at all stations except at Station R (Figure 6.4-3).

The use of combined information from the seven metrics was evaluated by performing independent cluster analyses and comparing station relationship results with those obtained from the North Carolina DEM

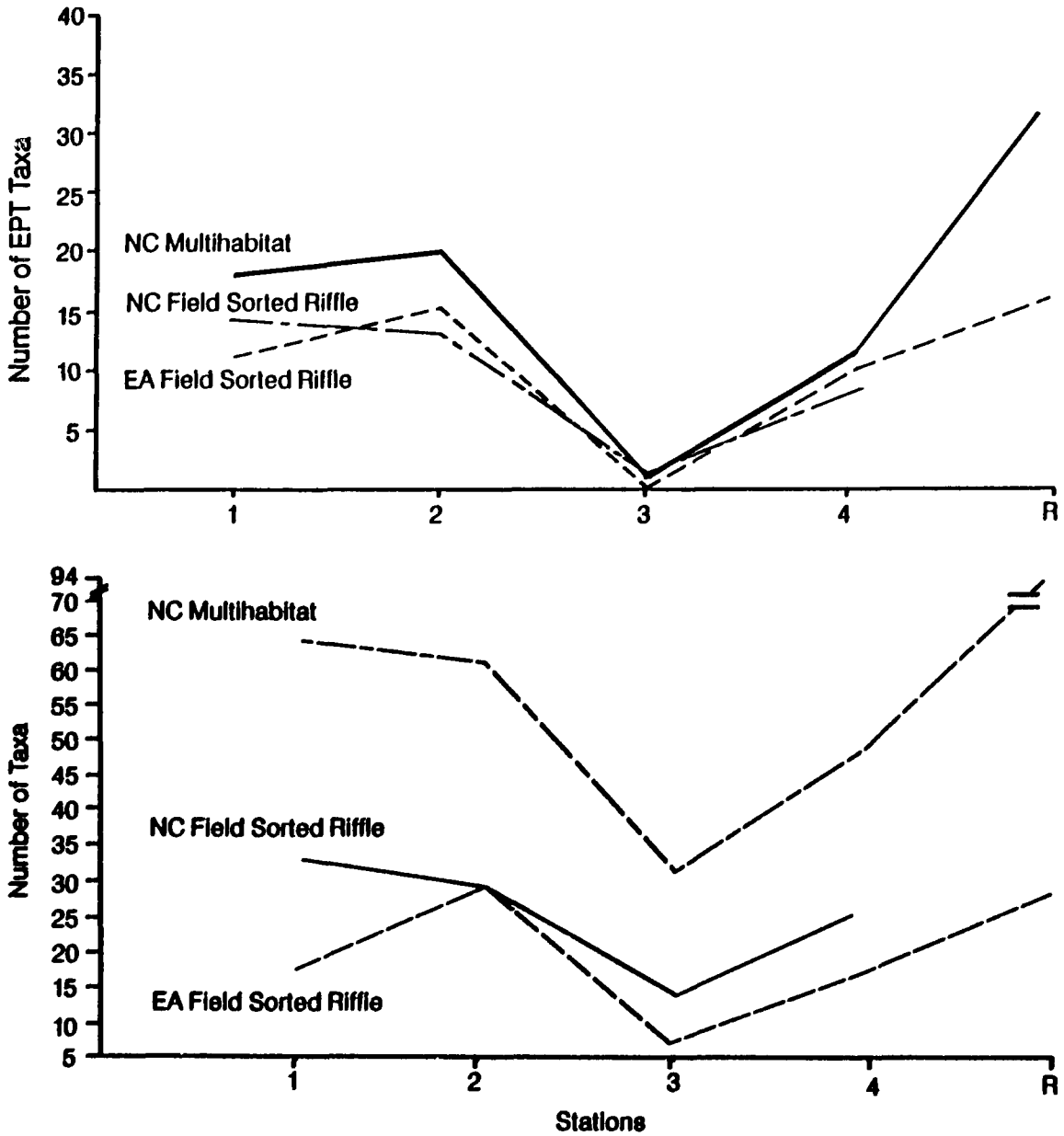


Figure 6.4-3. Comparison of taxa richness for all field sorted samples collected on the Ararat (Stations 1 – 4) and Mitchell (Station R) Rivers.

approach. Results of the cluster analysis performed on the EA field-sorted riffle sample (Figure 6.4-4) indicate a station relationship based on similarity of attributes from the seven metrics. However, the relationships of the stations in terms of biological condition cannot be determined using only the clusters. Therefore, a knowledge of the biological results obtained using the North Carolina DEM analysis technique is used to put the station relationships in perspective.

$$R >> 1 = 2 > 4 >> 3$$

A further analysis was conducted on the riffle sample by subsampling to 100 organisms in the laboratory, identifying and enumerating, and performing a cluster analysis on the computed metrics. The results of the clusters indicate that Stations 1 and 2 are most similar, with Station 4 being next most similar to the centroid of 1 and 2. The reference station was unlike any of the others, as was Station 3. Rearranging these results in the context of the biological data to provide an interpretation of impairment, these results (Figure 6.4-5) also indicate a strong similarity with the classification presented by the North Carolina DEM. The ranking of stations according to biological condition from the laboratory-processed samples using

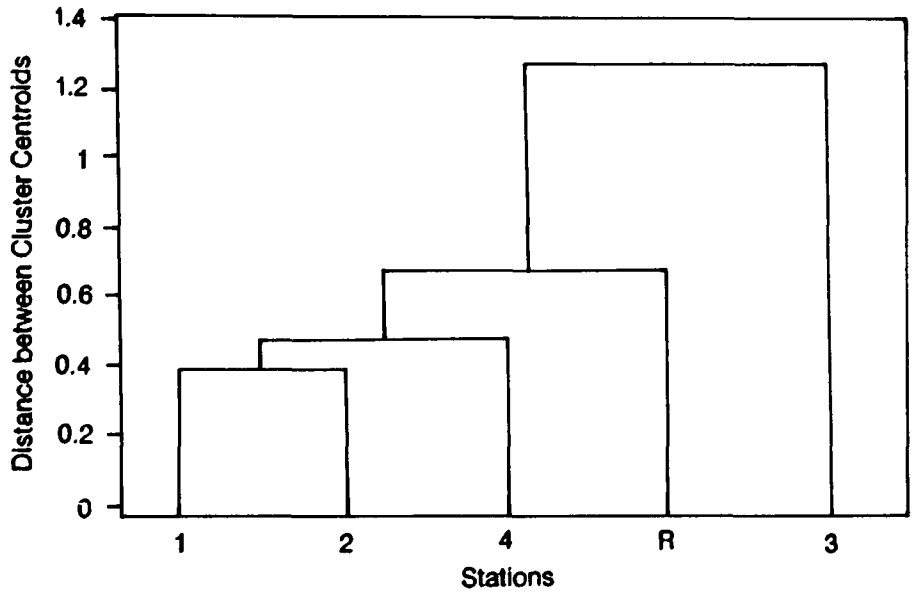


Figure 6.4-4. Station cluster analysis results for field sorted riffle samples collected on the Ararat (Station 1 – 4) and Mitchell (Station R) Rivers.

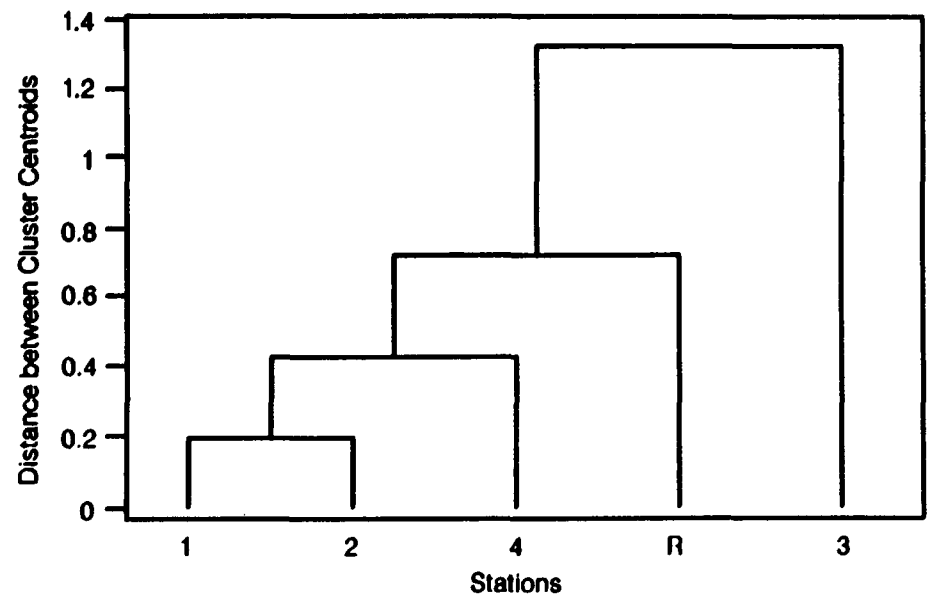


Figure 6.4-5. Station cluster analysis results for 100 organism subsamples from riffle samples collected on the Ararat (Stations 1 – 4) and Mitchell (Station R) Rivers.

the clusters is:

$$R >> 1 = 2 > 4 >> 3$$

This preliminary analysis of multihabitat versus single habitat conducted at one site suggests that the single habitat approach can provide a representative sample for an evaluation of biological condition.

6.4.6 Evaluation of the 100-Organism Subsample

To determine if a 100-organism subsample provides an adequate estimate of community structure, comparisons were made between 100-, 200-, and 300-organism subsamples. Although comparison of the cumulative taxa richness and EPT values for the 100-, 200-, and 300-organism subsamples (Table 6.4-3) indicates that additional information is gained with each incremental increase in organism count, results of a cluster analysis of the seven metrics performed on the 300-organism count data (Figure 6.4-6) showed the same station relationship as that obtained with the 100-organism data (Figure 6.4-5). The greater sensitivity demonstrated with the 300-organism data was subtle and may not warrant the additional time expenditure required.

Laboratory sorting of each 100-organism subsample was estimated to require between 1 and 1.5 hours. If a 300-organism subsample was used, approximately 3 to 4.5 hours would be necessary to pick the organisms from the sample. The time-savings estimated for the 100-organism subsample, combined with the minimal additional information provided with additional subsamples, supports the contention that a 100-organism subsample is adequate for assessment of the benthic community. Other researchers have also found that a 100-organism subsample will provide sufficient data to detect impact (Nuzzo 1986, Bode 1988, Shackleford 1988).

6.4.7 Family-Level vs. Species-Level Identification

Additional tabulation was done to determine if family-level identifications resulted in similar site classifications. A cluster analysis was performed on all of the 13 metrics described in Section 6.4.4 for the family-level data of the 100-organism riffle sample. Results obtained with the cluster analysis (Figure 6.4-7) showed the same relationship in terms of contribution of the metrics to the assessment as did the species-level (lowest taxon) analysis. Therefore, the seven metrics used for the lowest taxonomic level assessment were used for the family-level assessment.

Data for the seven computed metrics using family-level identification are presented in Table 6.4-4. Results of station clusters illustrate a spatial trend (Figure 6.4-8) that is the same as that obtained using the RBP III approach on the 100-organism (lowest taxon) data (Figure 6.4-5). Stations 1 and 2 were most similar; Station 4 clustered next, then R, and finally 3. However, the bioassessment scheme of the family-level protocol is more general than that provided by

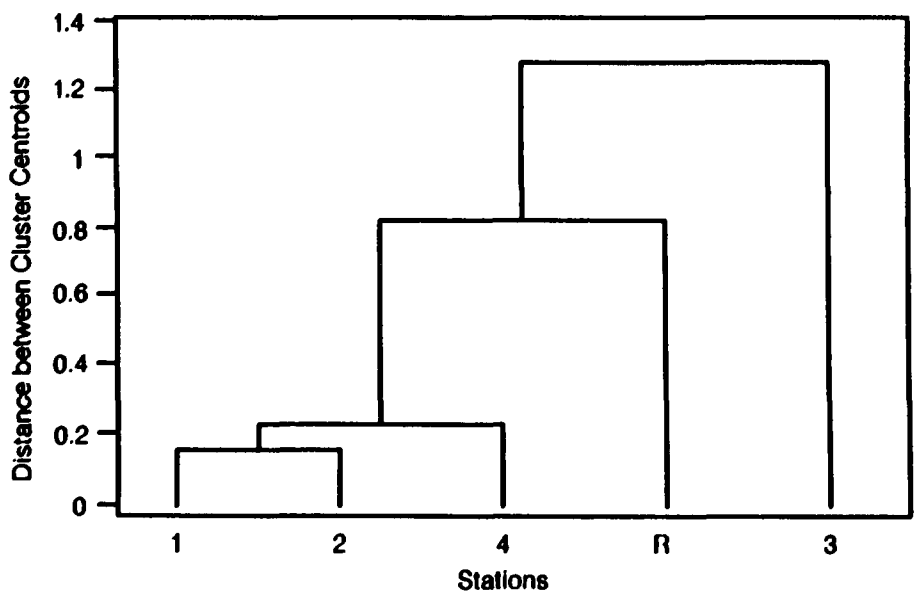


Figure 6.4-6. Station cluster analysis results for 300 organism subsamples from riffle samples collected on the Ararat (Station 1 – 4) and Mitchell (Station R) Rivers.

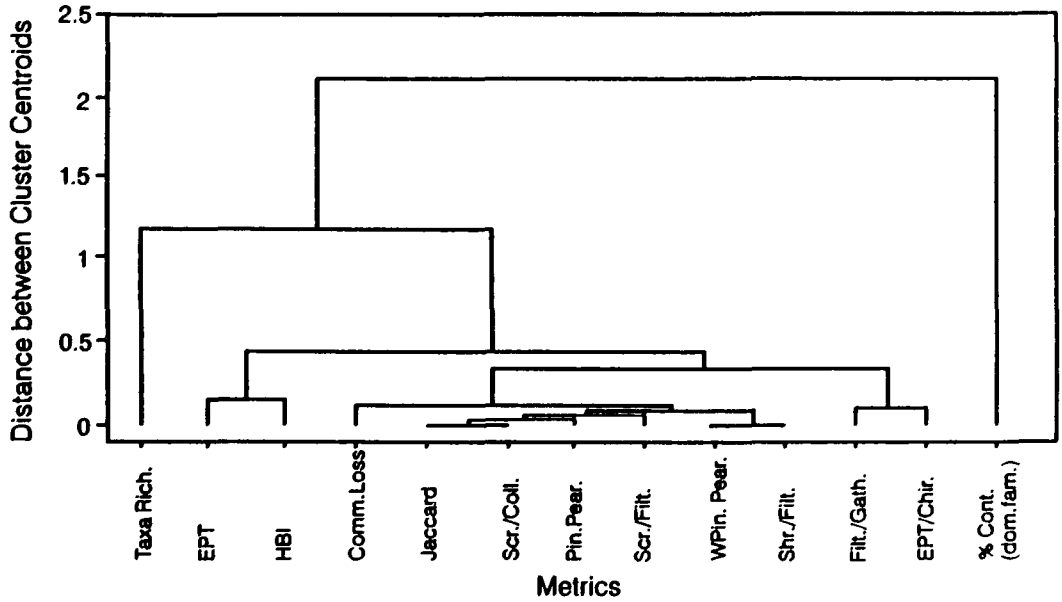


Figure 6.4-7. Cluster analysis results for benthic community metrics, based on family level identifications of 100 organism subsamples from riffle samples collected on the Ararat (Stations 1 – 4) and Mitchell Rivers (Station R).

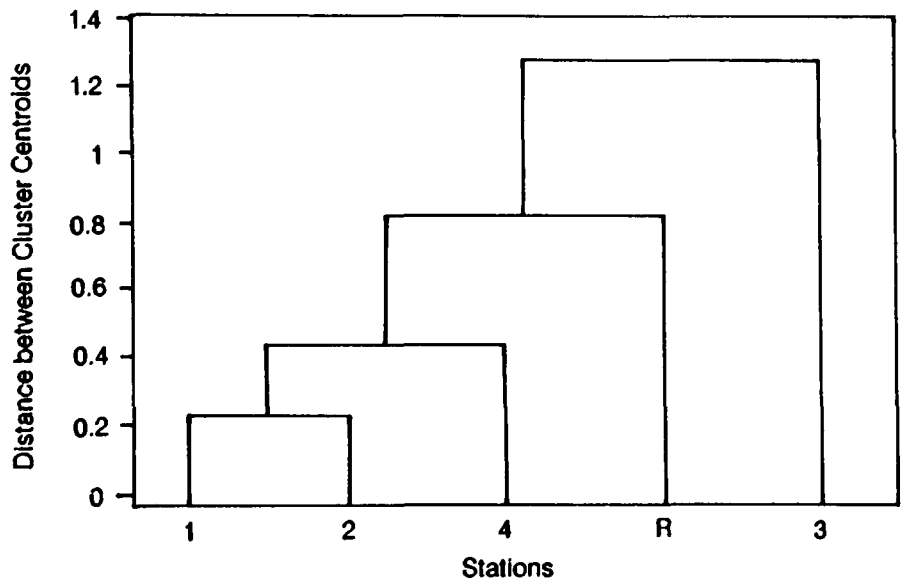


Figure 6.4-8. Station cluster analysis results for benthic community metrics, based on family-level identifications of 100 organism subsamples from riffle samples collected on the Ararat (Stations 1 – 4) and Mitchell (Station R) Rivers.

the species level and subtle differences in biological impairment will not be readily discerned. In this particular pilot study, the family-level bioclassification provided station relationships similar to those of the species level. The bioclassification is:

$$R >> 1 = 2 > 4 >> 3$$

The family-level data differed slightly in level of station similarity compared to that for the species-level (100-organism riffle), which is to be expected with different taxonomic levels of identification. However, results indicate that a reasonably good evaluation can be obtained with family-level identifications. The relative sensitivity of a family-level identification effort is sufficient for a prioritization or site ranking protocol that would differentiate between non-impaired, moderately impaired, and severely impaired conditions.

6.4.8 Integrated Bioassessment

A summary of the bioclassification scheme for the stations that was derived from the cluster analysis performed on all of the data sets and the North Carolina DEM analysis technique is presented in Table 6.4-5.

TABLE 6.4-5 SUMMARY OF THE BIOCLASSIFICATION DERIVED FROM AN ANALYSIS OF SAMPLES COLLECTED FROM THE ARARAT AND MITCHELL RIVERS

Data Set	Bioclassification
Cluster Analysis (Based on NC DEM Classifications)	
NC multihabitat; NC analysis	R >> 1 = 2 > 4 >> 3
EA field-sorted riffle; RBP III analysis	R >> 1 = 2 > 4 >> 3
100-organism riffle; RBP III analysis	R >> 1 = 2 > 4 >> 3
300-organism riffle; RBP III analysis	R >> 1 = 2 $\geq$ 4 >> 3
100-organism riffle; RBP II (family) analysis	R >> 1 = 2 > 4 >> 3
Bioassessment Technique	
NC multihabitat; NC analysis	R >> 1 = 2 > 4 >> 3
EA field sorted riffle; RBP III analysis	R > 1 = 2 > 4 > 3
100-organism riffle; RBP III analysis	R > 1 = 2 $\geq$ 4 >> 3
300-organism riffle; RBP III analysis	R >> 1 = 2 $\geq$ 4 > 3
100-organism riffle; RBP II (family) analysis	R > 1 = 2 > 4 = 3

Very little variation existed in the relationship of Stations R, 1, and 2, whereby R was always of greater quality than Station 1, and essentially the same quality existed between Stations 1 and 2. In addition, the orientation of the stations in terms of biological condition was the same for all data sets. The subjectivity in these analyses exists in the fact that some judgment has to be used in interpreting the biological relationships from the station similarity information illustrated by the dendrograms of the cluster analysis. It is possible that the close proximity of Station 4 to the centroid of Stations 1 and 2 could indicate equality rather than a slightly lower quality. This situation occurred particularly in the data sets of the 300-organism riffle subsample and the multihabitat RBP III analysis.

Using the scoring criteria described for RBP II (Section 6.2) and RBP III (Section 6.3), the bioassessment metrics were calculated. The bioclassification for the 100-organism subsample species-level identification resulted in Station R being classified as non-impaired, Stations 1 and 2 as slightly impaired, Station 4 as moderately impaired, and Station 3 as severely impaired (Table 6.4-3). Therefore, $R > 1 = 2 \geq 4 >> 3$. Bioclassification for the 300-organism subsample resulted in a classification similar to that of the 100-organism count samples. The station relationship results based on biological condition using the bioassessment approach are not unlike those obtained using the cluster analysis (assuming the biological condition as identified by NC DEM) on the same data sets. However, the amount of data was limited for an adequate cluster analysis. Station ranking based on results of the field-sorted riffle sample is similar to that resulting from the North Carolina DEM multihabitat bioclassification. Although differences among stations were more conservative using the RBP approach, these are the same station trends observed in the cluster analysis for this data set (Table 6.4-5).

The family-level bioclassification results suggest an orientation slightly different from that obtained with the 100-organism (species-level) sample (Table 6.4-4).

Both the species-level bioassessment and the station cluster for the family-level data indicated that Station 3 is different from Station 4, which reflects the lesser sensitivity associated with the family-level identification used in RBP II (Table 6.4-5). The difference between family-level bioclassification (moderate impairment) and species-level bioclassification (slight impairment) at Stations 1 and 2 is attributable to the fact that the RBP II classification scheme is based on only three levels of impairment as opposed to the four levels used in RBP III.

The bioassessment technique appears to be more conservative than the clustering technique, which may be beneficial from a water quality management point of view. Subtle differences in structure and function will be regarded as rationale for further confirmative study, to ascertain the significance of complex impairment problems. If an evaluation of biological condition was based on a straight percent-of-reference, a slightly different scenario might be obtained. A greater differentiation between Stations R and 1 and between Stations 3 and 4 would be one outcome. However, based on this single pilot study, the ranges of biological condition are necessarily conservative. With a good reference database, these ranges can be modified, making them either more or less protective.

7. FISH BIOSURVEY AND DATA ANALYSIS

Two levels of fish biosurvey analyses are presented: Rapid Bioassessment Protocol IV constitutes a questionnaire approach where local and State fisheries experts are canvassed for existing data and information; Rapid Bioassessment Protocol V consists of collecting fish at selected sites for biosurvey analyses. The data analysis used in RBP V is based on the IBI (Karr et al. 1986) and the IWB (Gammon 1980). This document only provides an overview of the IBI and IWB and their conceptual foundations. Effective use of RBP V requires information presented in Karr et al. (1986) and Gammon (1980). Sample field and data sheets are presented as guidance.

Pilot studies based on use of the fish biosurvey (RBP V) have been published. An overview of two of these studies is presented in Section 7.3.

7.1 RAPID BIOASSESSMENT PROTOCOL IV—FISH

The intent of RBP IV is to serve as a screening tool and to maximize the use of existing knowledge of fish communities. The questionnaire polls State fish biologists and university ichthyologists believed knowledgeable about the fish assemblages in stream reaches of concern. The proposed questionnaire (Figure 7.1-1) is modeled after one used in a successful national survey of 1,300 river reaches or segments (Judy et al. 1984). Unlike field surveys, questionnaires can provide information about tainting or fish tissue contamination and historical trends and conditions. Disadvantages of questionnaires include inaccuracy caused by hasty responses, a desire to report conditions as better or worse than they are, and insufficient knowledge. The questionnaire provides a qualitative assessment of a large number of waterbodies quickly and inexpensively. Its quality depends on the survey design (the number and location of waterbodies), the questions presented, and the knowledge and cooperation of the respondents.

This document provides guidance on the design and content of the questionnaire survey. Judy et al. (1984) found that State fish and game agencies have a vested interest in assuring the quality of the data, and they generally provide reliable information.

7.1.1 Design of Fish Assemblage Questionnaire Survey

Selection of stream reaches requires considerable forethought. If the survey program is statewide or regional in scope, a regional framework is advisable. Regional reference reaches can be selected to serve as benchmarks for comparisons (Hughes et al. 1986). These sites should be characteristic of the waterbody types and sizes in the region and should be minimally impacted. The definition of minimal impact varies from region to region, but includes those waters that are generally free of point sources, channel modifications, and diversions, and have diverse habitats, complex bottom substrate, considerable instream cover, and a wide buffer of natural riparian vegetation.

Remaining sites should also be selected carefully. If the questionnaire focuses on larger streams, a 1:1,000,000 scale topographic map should be used for reach selection. Reaches of small streams should be selected from the largest scale map possible; reaches selected from 1:250,000 versus 1:24,000 scale topographic maps may omit as much as 10 percent of the permanent streams in humid, densely forested areas. Small, medium, and large streams should be selected based on their importance in the region.

The potential respondent (or the agency chief if a number of agency staff are to be questioned) should be contacted initially by telephone to identify appropriate respondents. To ensure maximum response, the questionnaire should be sent at times other than the field season and the beginning and end of the fiscal year. The questionnaire should be accompanied by a personalized cover letter written on official stationary, and closed by an official title below the signature. A stamped, self-addressed return envelope increases the response rate. Materials mailed first or priority class are effective; special delivery and certified letters are justified in follow-up mailings. Telephone contact is advisable after three follow-up notes.

7.1.2 Response Analysis

Questionnaire response should provide the following information:

1. The integrity of the fish community

FISH ASSEMBLAGE QUESTIONNAIRE

INTRODUCTION

This questionnaire is part of an effort to assess the biological health or integrity of the flowing waters of this state. Our principle focus is on the biotic health of the designated waterbody as indicated by its fish community. You were selected to participate in the study because of your expertise in fish biology and your knowledge of the waterbody identified in this questionnaire.

Using the scale below, please circle the rank (at left) corresponding to the explanation (at right) that best describes your impression of the condition of the waterbody. Please complete all statements. If you feel that you cannot complete the questionnaire, check here [ ] and return it. If you are unable to complete the questionnaire but are aware of someone who is familiar with the waterbody, please give this person's name, address, and telephone number in the space provided below.

_____

_____

_____

Waterbody code _____

Waterbody name _____

Waterbody location (also see map)

State _____ County _____ Long/Lat_____

Ecoregion _____

Waterbody size

Stream (<1 cfs, 1-10 cfs, >10 cfs)

(Answer questions 1-4 using the scale below.)

5 Species composition, age classes, and trophic structure comparable to non (or minimally) impacted sites of similar waterbody size in that ecoregion.

4 Species richness somewhat reduced by loss of some intolerant species; young of the year of top carnivores rare; less than optimal abundances, age distributions, and trophic structure for waterbody size and ecoregion.

3 Intolerant species absent, considerably fewer species and individuals than expected for that waterbody size and ecoregion, older age classes of top carnivores rare, trophic structure skewed toward omnivory.

Figure 7.1-1. Fish assemblage questionaire for use with Rapid Bioassessment Protocol IV.

2 Dominated by highly tolerant species, omnivores, and habitat generalists; top carnivores rare or absent; older age classes of all but tolerant species rare; diseased fish and anomalies relatively common for that waterbody size and ecoregion.

1 Few individuals and species present, mostly tolerant species and small individuals, diseased fish and anomalies abundant compared to other similar-sized waterbodies in the ecoregion.

0 No fish

(Circle one number using the scale above.)

1. Rank the current conditions of the reach

5 4 3 2 1 0

2. Rank the conditions of the reach 10 years ago

5 4 3 2 1 0

3. Given present trends, how will the reach rank 10 years from now?

5 4 3 2 1 0

4. If the major human-caused limiting factors were eliminated, how would the reach rank 10 years from now?

5 4 3 2 1 0

(Complete each subsection by circling the single most appropriate limiting factor and probable cause.)

Subsection 1--Water Quality

Limiting factor		Probable cause	
5	Temperature too high	18	Primarily upstream
6	Temperature too low	19	Within reach
7	Turbidity	20	Point source discharge
8	Salinity	21	Industrial
9	Dissolved oxygen	22	Municipal
10	Gas supersaturation	23	Combined sewer
11	pH too acidic	24	Mining
12	pH too basic	25	Dam release
13	Nutrient deficiency	26	Nonpoint source discharge
14	Nutrient surplus	27	Individual sewage
15	Toxic substances	28	Urban runoff
16	Other (specify below)	29	Landfill leachate
	_____	30	Construction
17	Not limiting	31	Agriculture
		32	Feedlot
		33	Grazing
		34	Silviculture
		35	Mining
		36	Natural
		37	Unknown
		38	Other (specify below)
			_____

Figure 7.1-1. (Cont.).

Subsection 2--Water Quantity

Limiting factor	Probable source
39 Below optimum flows	45 Dam
40 Above optimum flows	46 Diversion
41 Loss of flushing flows	47 Watershed conversion
42 Excessive flow fluctuation	48 Agriculture
43 Other (specify below)	49 Silviculture
_____	50 Grazing
	51 Urbanization
44 Not limiting	52 Mining
	53 Natural
	54 Unknown
	55 Other (specify below)
	_____

Subsection 3--Habitat Structure

Limiting factor	Probable cause
56 Excessive siltation	64 Agriculture
57 Insufficient pools	65 Silviculture
58 Insufficient riffles	66 Mining
59 Insufficient shallows	67 Grazing
60 Insufficient concealment	68 Dam
61 Insufficient reproductive habitat	69 Diversion
	70 Channelization
62 Other (specify below)	71 Snagging
_____	72 Other channel modifications
	73 Natural
63 Not limiting	74 Unknown
	75 Other (specify below)
	_____

Subsection 4--Fish Community

Limiting factor	Probable source
76 Overharvest	84 Fishermen
77 Underharvest	85 Aquarists
78 Fish stocking	86 State agency
79 Non-native species	87 Federal agency
80 Migration barrier	88 Point source
81 Tainting	89 Nonpoint source
82 Other (specify below)	90 Natural
_____	91 Unknown
	92 Other (specify below)
83 Not limiting	_____

Subsection 5--Major Limiting Factor

93 Water quality
94 Water quantity
95 Habitat structure
96 Fish community
97 Other (specify)
_____

Your name (please print)_____

Figure 7.1-1. (Cont.).

2. The frequency of occurrence of particular limiting factors and causes
3. The frequency of occurrence of particular fish community condition characterizations for the past, present, and future.
4. The geographic patterns in these variables
5. The temporal trends in the variables
6. Effect of waterbody type and size on the spatial and temporal trends and the associated limiting factors
7. The likelihood of improvement and degradation
8. The major limiting factor

The questionnaire data are most effectively entered and analyzed by using a microcomputer and interactive database management software (e.g., dBase III or Revelation). This software reduces data entry errors and facilitates the qualitative analysis of numerous variables. Results can be reported as histograms, pie graphs, or box plots. If such a system is unavailable data can be analyzed and the results plotted by hand. RBP IV allows considerable flexibility.

7.2 RAPID BIOASSESSMENT PROTOCOL V—FISH

Rapid Bioasssessment Protocol V (RBP V) is a rigorous approach similar to macroinvertebrate RBP III in accuracy and effort, but focuses on the fish community. RBP V involves careful, standardized field collection, species identification and enumeration, and community analyses using biological indices or quantification of the biomass and numbers of key species. The RBP V survey yields an objective, discrete measure of the health of the fish community that usually can be completed onsite by qualified fish biologists (difficult species identifications may require laboratory confirmation). Data provided by RBP V can serve to assess use attainment, develop biological criteria, prioritize sites for further evaluation, provide a reproducible impact assessment, and assess fish community status and trends. RBP V is based primarily on the Index of Biotic Integrity (IBI), a fish community assessment approach developed by Karr (1981). A more detailed description of this approach is presented in Karr et al. (1986) and Ohio EPA (1987b). Regional modifications and applications are described in Hughes and Gammon (1987), Leonard and Orth (1986), Steedman (1988), Wade and Stalcup (1987), and Miller et al. (1988a).

7.2.1 Field Survey Methods

RBP V involves field evaluation of the same physicochemical and habitat characteristics as RBPs I, II, and III (Figures 5.1-1 and 5.2-1), a similar impairment assessment (Figure 7.2-1), and a fish community biosurvey. Because it provides critical information for evaluating the cause and source of impairment, the habitat and physical characterization (described in Chapter 5 of this document) are essential to RBP V. The approach for conducting a RBP V site-specific fish community analysis is based on the use of the IBI (Figure 7.2-2).

7.2.1.1 Sample Collection

Electrofishing, the most common technique used by agencies that monitor fish communities, and the most widely applicable approach for stream habitats, is the sampling technique recommended for use with RBP V. However, pilot studies may indicate the need for different or multiple gear.

The fish community biosurvey data are designed to be representative of the fish community at all station habitats, similar to the "representative qualitative sample" proposed by Hocutt (1981). The sampling station should be representative of the reach, incorporating at least one (preferably two) riffle(s), run(s), and pool(s) if these habitats are typical of the stream in question. Sampling of most species is most effective near shore and cover (macrophytes, boulders, snags, brush). The biosurvey is not an exhaustive inventory, but it provides a realistic sample of fishes likely to be encountered in the waterbody. Sampling procedures effective for large rivers are described in Gammon (1980), Hughes and Gammon (1987), and Ohio EPA (1987b).

Typical sampling station lengths range from 100–200 meters for small streams to 500–1000 meters in rivers, but are best determined by pilot studies. The size of the reference station should be sufficient to produce 100–1000 individuals and 80–90 percent of the species expected from a 50 percent increase in sampling distance. Sample collection is usually done during the day, but night sampling can be more effective if the water is especially clear and there is little cover (Reynolds 1983). Use of block nets set (with as little wading as possible) at both ends of the reach increases sampling efficiency for large, mobile species sampled in small streams.

The RBP V fish community assessment requires that all fish species (not just gamefish) be collected. This reduces the effects of stocking and fishing and acknowledges the growing public interest in nongame

Field crew electrofishing with a pram-towed unit.

IMPAIRMENT ASSESSMENT SHEET

1. Detection of impairment: Impairment detected (Complete Items 2-6) No impairment detected (Stop here)

2. Biological impairment indicator:

Fish	Other aquatic communities
___ sensitive species reduced/absent	___ Macroinvertebrates
___ dominance of tolerant species	___ Periphyton
___ skewed trophic structure	___ Macrophytes
___ abundance reduced/unusually high	
___ biomass reduced/unusually high	
___ hybrid or exotic abundance unusually high	
___ poor size class representation	
___ high incidence of anomalies	

3. Brief description of problem: _____

Year and date of previous surveys: _____

Survey data available in: _____

4. Cause (indicate major cause): organic enrichment toxicants flow

sediment temperature poor habitat

other _____

5. Estimated areal extent of problem (m^2) and length of stream reach affected (m) where applicable: _____

6. Suspected source(s) of problem

___ point source	___ mine
___ urban runoff	___ dam or diversion
___ agricultural runoff	___ channelization or snagging
___ silvicultural runoff	___ natural
___ livestock	___ other
___ landfill	___ unknown

Comments: _____

_____

Figure 7.2-1. Impairment Assessment Sheet for use with fish Rapid Bioassessment Protocol V.

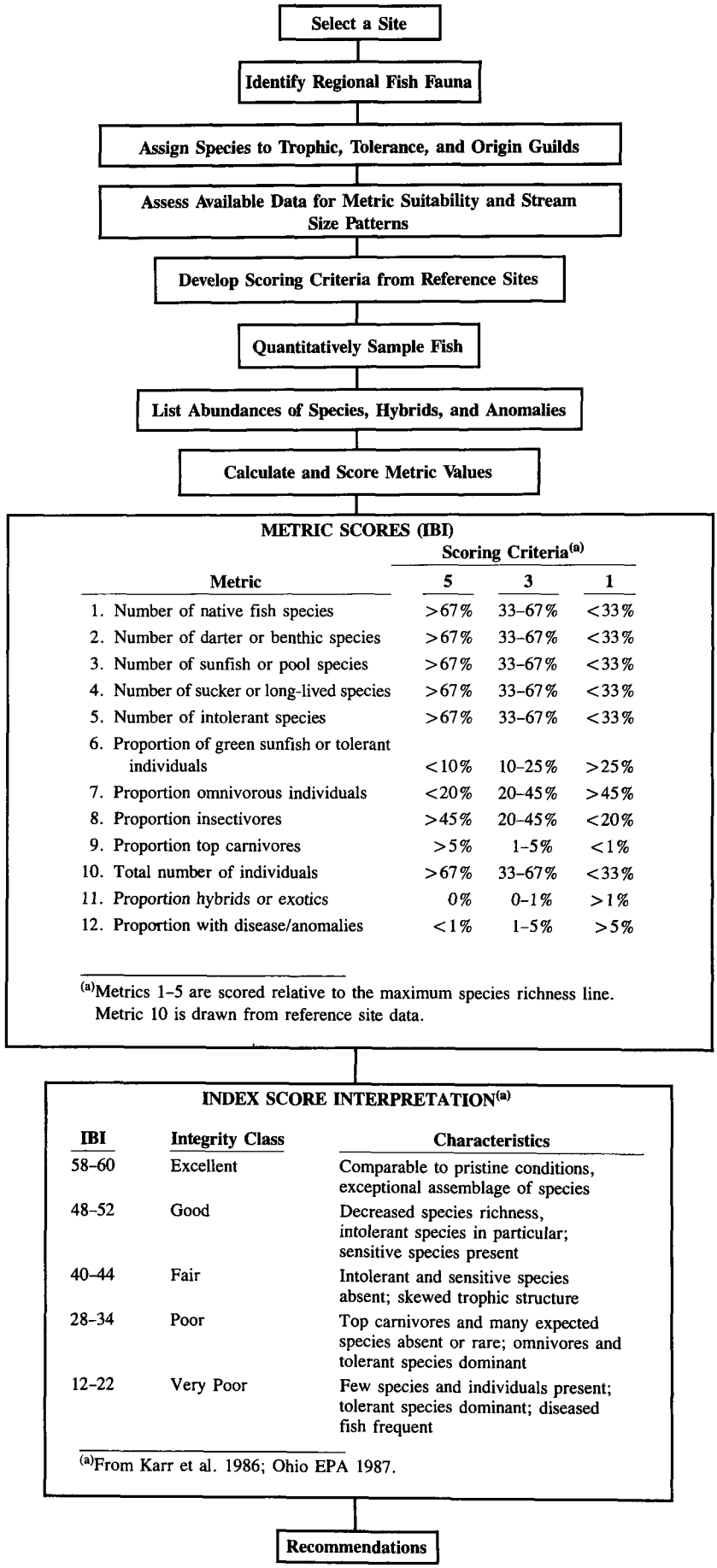


Figure 7.2-2. Flowchart of bioassessment approach advocated for Rapid Bioassessment Protocol V.

species. Small fish that require special gear for their effective collection may be excluded. Exclusion of young-of-the-year fish during collection can have a minor effect on IBI scores (Angermeier and Karr 1986), but lowers sampling costs and reduces the need for laboratory identification. Karr et al. (1986) recommended exclusion of fish less than 20 mm in length. This recommendation should be considered on a regional basis and is also applicable to large fish requiring special gear for collection (e.g., sturgeon). The intent of the sample (as with the entire protocol) is to obtain a representative estimate of the species present, and their abundances, for a reasonable amount of effort.

Sampling effort among stations is standardized as much as possible. Regardless of the gear used, the collection method, site length (or area), and work hours expended must be comparable to allow comparison of fish community status among sites. Major habitat types (riffle, run, and pool) sampled at each site and the proportion of each habitat type sampled should also be comparable. Generally 1 to 2 hours of actual sampling time are required, but this varies considerably with the gear used and the size and complexity of the site.

Atypical conditions, such as high flow, excessive turbidity or turbulence, heavy rain, drifting leaves, or other unusual conditions that affect sampling efficiency, are best avoided. Glare, a frequent problem, is reduced by wearing polarized glasses during sample collection.

7.2.1.2 Sample Processing

A field collection data sheet (Figure 7.2-3) is completed for each sample. Sampling duration and area or distance sampled are recorded in order to determine level of effort. Species may be separated into adults and juveniles by size and coloration; then total numbers and weights and the incidence of external anomalies are recorded for each group. Reference specimens of each species from each site are preserved in 10 percent formaldehyde, the jar labeled, and the collection placed with the State ichthyological museum to confirm identifications and to constitute a biological record. This is especially important for uncommon species, for species requiring laboratory identification, and for documenting new distribution records. If retained in a live well, most fish can be identified, counted, and weighed in the field by trained personnel and returned to the stream alive. In warmwater sites, where handling mortality is highly probable, each fish is identified and counted, but for abundant species, subsampling may be considered. When subsampling is employed, the subsample is extrapolated to obtain a final value. Subsampling for weight is a simple, straightforward procedure, but failure to examine all fish to determine frequency of anomalies (which may occur in about 1 percent of all specimens) can bias results. The trade off between handling mortality and data bias must be considered on a case-by-case basis. If a site is to be sampled repeatedly over several months (i.e., monitoring), the effect of sampling mortality may outweigh data bias. Holding fish in liveboxes in shaded, circulating water will substantially reduce handling mortality. More information on field methods is presented in Karr et al. (1986) and Ohio EPA (1987b).

7.2.2 Data Analysis Techniques

Based on observations made in the assessment of habitat, water quality, physical characteristics, and the fish biosurvey, the investigator concludes whether impairment is detected. If impairment is detected, the probable cause and source is estimated and recorded on an Impairment Assessment Sheet (Figure 7.2-1). A preliminary judgment on the presence of biological impairment is particularly important if RBP IV is not used prior to RBP V.

Data can be analyzed using the IBI (or individual IBI metrics), the IWB (Gammon 1980), and multivariate statistical techniques to determine community similarities. Detrended correspondence analysis (DCA) is a useful multivariate analysis technique for revealing regional community patterns and patterns among multiple sites. It also demonstrates assemblages with compositions differing from others in the region or reach. See Gauch (1982) and Hill (1979) for descriptions of, and software for, DCA. Data analyses and reporting, including parts of the IBI, can be computer generated. Computerization reduces the time needed to produce a report and increases staff capability to examine data patterns and implications. Illinois EPA has developed software to assist professional aquatic biologists in calculating IBI values in Illinois streams (Bickers et al. 1988). (Use of this software outside Illinois without modification is not recommended.) However, hand calculation in the initial use of the IBI promotes understanding of the approach and provides insight into local inconsistencies.

The IBI is a broadly-based index firmly grounded in fisheries community ecology (Karr 1981; Karr et al. 1986). The IBI incorporates zoogeographic, ecosystem, community, population, and individual perspectives. It can accommodate natural differences in the distribution and abundance of species that result from differences in waterbody size, type, and region of occurrence (Miller et al. 1988a). Use of the IBI allows national comparisons of biological integrity

page___ of___

FISH FIELD COLLECTION DATA SHEET

Drainage _____ Date _____
Sampling Duration (min) _____
Sampling Distance (m) _____ Sampling Area (m^2) _____ Crew _____
Habitat Complexity/Quality (excellent good fair poor very poor)
Weather _____ Flow (flood bankfull moderate low)
Gear Used _____ Gear/Crew Performance _____
Comments _____
Fish (preserved) Number of Individuals ____ Number of Anomalies _____

Genus/Species	Adults		Juveniles		Anomalies(*)
	No.	Wt.	No.	Wt.	No.

(*) Discoloration, deformities, eroded fins, excessive mucus, excessive external parasites, fungus, poor condition, reddening, tumors, and ulcers

Figure 7.2-3. Fish Field Collection Data Sheet for use with Rapid Bioassessment Protocol V.

without the traditional bias for small coldwater streams (e.g., a salmon river in Alaska and a minnow stream in Georgia both could be rated excellent if they were comparable to the best streams expected in their respective regions).

Karr et al. (1986) provided a consistent theoretical framework for analyzing fish community data. The IBI uses 12 biological metrics to assess integrity based on the fish community's taxonomic and trophic composition and the abundance and condition of fish. Such multiple-parameter indices are necessary for making objective evaluations of complex systems. The IBI was designed to evaluate the quality of small midwestern streams but has been modified for use in many regions of the country and in large rivers (Section 7.2.2.1).

The metrics attempt to quantify an ichthyologist's best professional judgment of the quality of the fish community. The IBI utilizes professional judgment, but in a prescribed manner, and it includes quantitative standards for discriminating fish community condition. Judgment is involved in choosing the most appropriate population or community element that is representative of each metric and in setting the scoring criteria. This process can be easily and clearly modified, as opposed to judgments that occur after results are calculated. Each metric is scored against criteria based on expectations developed from appropriate regional reference sites. Metric values approximating, deviating slightly from, or deviating greatly from values occurring at the reference sites are scored as 5, 3, or 1, respectively. The scores of the 12 metrics are added for each station to give an IBI of 60 (excellent) to 12 (very poor). Trophic and tolerance classifications of midwestern and northwestern fish species are listed in Appendix D. Additional classifications can be derived from information in State and regional fish texts or by objectively assessing a large statewide database. Use of the IBI in the southeastern and southwestern United States and its widespread use by water resource agencies may result in further modifications. Past modifications have occurred (Section 7.2.2.1; Miller et al. 1988a) without changing the IBI's basic theoretical foundations. Sample calculations of the IBI are given in Section 7.3.

The steps in calculating the IBI (Figure 7.2-2) are explained below:

1. Assign species to trophic guilds; identify and assign species tolerances. Where published data are lacking, assignments are made based on knowledge of closely related species and morphology.
2. Develop scoring criteria for each IBI metric. Maximum species richness (or density) lines are developed from a reference database.
3. Conduct field study and identify fish; note anomalies, eroded fins, poor condition, excessive mucous, fungus, external parasites, reddening, lesions, and tumors. Complete field data sheets.
4. Enumerate and tabulate number of fish species and relative abundances.
5. Summarize site information for each IBI metric.
6. Rate each IBI metric and calculate total IBI score.
7. Translate total IBI score to one of the five integrity classes.
8. Interpret data in the context of the habitat assessment (see Chapter 8). Individual metric analysis may be necessary to ascertain specific trends.

The IBI is based on an integrated analysis of the metrics. However, individual IBI metrics may serve as separate variables to aid in data interpretation. Comparison of commonly-occurring and dominant species are revealing, especially when related to their ecological requirements and tolerances. Larsen et al. (1986) and Rohm et al. (1987) provide examples of such regional characterizations of common and abundant species. The IWB (Gammon 1980; Hughes and Gammon 1987) incorporates two abundance and two diversity estimates in approximately equal fashion, thereby representing fish assemblage quality more realistically than a single diversity or abundance measure. The IWB is calculated as

$$IWB = 0.5 \ln N + 0.5 \ln B + H'_N + H'_B$$

where N equals the number of individuals caught per kilometer, B equals the biomass of individuals caught per kilometer, and H′ is the Shannon diversity index. Ohio EPA and J.R. Gammon (Gammon 1989) found that subtracting highly tolerant species from the number and biomass variables increases sensitivity of the index in degraded environments (Ohio EPA 1987b).

If the size of a particular fish population (e.g., trout or bass species) is of concern, it can be estimated with known confidence limits by several methods. One of the most popular approaches is the removal method (Seber and LeCren 1967; Seber and Whale 1970, Seber 1982). This approach assumes a closed population, equal probability of capture for all fish, and a constant probability of capture from sample to sample (equal sampling effort and conditions). The removal method is applicable to situations in which the total catch is large relative to the total population. If subsequent samples produce equal or greater numbers than previous samples, the population must be resampled. Population size in the two sample cases is estimated by

$$N = C_1^2/(C_1 - C_2)$$

where C_1 and C_2 are the number of fish captured in

the first and second samples, respectively.
In the three sample cases, population size is estimated by

$$N = \frac{6X^2 - 3XY - Y^2 + Y(Y^2 + 6XY - 3X^2)^{1/2}}{18(X - Y)}$$

where $X = 2C_1 + C_2$, and $Y = C_1 + C_2 + C_3$.

Many methods are available to calculate population statistics from removal data including regression, maximum likelihood, and maximum weighted-likelihood. Public-domain software is available to assist in calculating these and other fisheries population statistics (Van Deventer and Platts 1989).

7.2.2.1 Description of IBI Metrics

The IBI serves as an integrated analysis because individual metrics may differ in their relative sensitivity to various levels of biological condition. A description and brief rationale for each of the 12 IBI metrics is outlined below. The original metrics described by Karr (1981) for Illinois streams (underlined) are followed by substitutes used in or proposed for different geographic regions and stream sizes. Because of zoogeographic differences, different families or species are evaluated in different regions, with regional substitutes occupying the same general habitat or niche. The source for each substitute is footnoted below. Table 7.2-1 presents an overview of the IBI metric variations for six areas of the United States and Canada and their sources. Scoring criteria for the 12 original IBI metrics (Karr 1986) are included in Figure 7.2-1 as an example of the assessment approach for evaluating fish community condition.

Species Richness and Composition Metrics

These metrics assess the species richness component of diversity and the health of the major taxonomic groups and habitat guilds of fishes. Two of the metrics assess community composition in terms of tolerant or intolerant species. Scoring for the first five of these metrics and their substitutes, requires development of species-waterbody size relationships for different zoogeographic regions. Development of this relationship requires data sufficient to plot the number of species collected from regional reference sites of various stream sizes against a measure of stream size (watershed area, stream order) of those sites. A line is then drawn with slope fit by eye to include 95 percent of the points. Finally the area under the line is trisected into areas that are scored as 5, 3, or 1 (Figure 7.2-4). A detailed description of these methods can be found in Fausch et al. (1984), Ohio EPA (1987b), and Karr et al (1986).

Metric 1. **Total number of fish species(1,4,5).** Substitutes: Total number of native fish species(2,8), and salmonid age classes(6)

This number decreases with increased degradation; hybrids and introduced species are not included. In coldwater streams supporting few fish species, the age classes of the species found represent the suitability of the system for spawning and rearing. The number of species is strongly affected by stream size at small stream sites, but not at large river sites (Karr et al. 1986; Ohio EPA 1987b). Thus, scoring depends on developing species/waterbody size relationships.

Metric 2. **Number and identity of darter species(1).** Substitutes: Number and identity of sculpin species(2,4), benthic insectivore species(3,4), salmonid yearlings (individuals)(6); number of sculpins (individuals)(4); percent round-bodied suckers(5), sculpin and darter species(8)

These species are sensitive to degradation resulting from siltation and benthic oxygen depletion because they feed and reproduce in benthic habitats (Kuehne and Barbour 1983; Ohio EPA 1987b). Many smaller species live within the rubble interstices, are weak swimmers, and spend their entire lives in an area of 100–400 m^2 (Hill and Grossman 1987; Matthews 1986). Darters are appropriate in most Mississippi Basin streams; sculpins and yearling trout occupy the same niche in western streams. Benthic insectivores and sculpins or darters are used in small Atlantic slope streams that have few sculpins or darters, and round-bodied suckers are suitable in large midwestern rivers. Scoring requires development of species/waterbody size relationships.

Metric 3. **Number and identity of sunfish species(1).** Substitutes: Number and identity of cyprinid species(2,4), water column species(3,4), salmonid species(4), headwater species(5), and sunfish and trout species(8)

These pool species decrease with increased degradation of pools and instream cover (Gammon et al. 1981; Angermeier 1983; Platts et al. 1983). Most of these fishes feed on drifting and surface invertebrates and are active swimmers. The sunfishes and salmonids are important sport species. The sunfish metric works

TABLE 7.2-1 REGIONAL VARIATIONS OF IBI METRICS[a]

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento-San Joaquin
1. Total Number of Species	X	X		X	X		X
# native fish species			X			X	
# salmonid age classes[b]						X	X
2. Number of Darter Species	X			X	X		
# sculpin species						X	
# benthic insectivore species		X					
# darter and sculpin species			X				
# salmonid yearlings (individuals)[b]						X	X
% round-bodied suckers	X						
# sculpins (individuals)							X
3. Number of Sunfish Species	X				X		
# cyprinid species						X	
# water column species		X					
# sunfish and trout species			X				
# salmonid species							X
# headwater species	X						
4. Number of Sucker Species	X	X				X	
# adult trout species[b]						X	X
# minnow species	X				X		
# sucker and catfish species			X				

(a) Taken from Karr et al. (1986), Hughes and Gammon (1987), Miller et al. (1988a), Miller et al. (1988b In Review), Ohio EPA (1987b), Steedman (1988).
(b) Metric suggested by Moyle or Hughes as a provisional replacement metric in small western salmonid streams.
(c) An optional metric found to be valuable by Hughes and Gammon (1987).

Note: X = metric used in region. Many of these variations are applicable elsewhere.

TABLE 7.2-1 (Cont.)

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento-San Joaquin
5. Number of Intolerant Species	X	X			X	X	
# sensitive species	X						
# amphibian species							X
presence of brook trout			X				
6. % Green Sunfish	X						
% common carp						X	
% white sucker		X			X		
% tolerant species	X						
% creek chub				X			
% dace species			X				
7. % Omnivores	X	X	X	X	X	X	
% yearling salmonids[(b)]					X	X	
8. % Insectivorous Cyprinids	X						
% insectivores		X				X	
% specialized insectivores				X	X		
# juvenile trout							X
% insectivorous species	X						
9. % Top Carnivores	X	X	X				
% catchable salmonids						X	
% catchable wild trout							X
% pioneering species	X						
Density catchable wild trout							X

TABLE 7.2-1 (Cont.)

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento-San Joaquin
10. Number of Individuals	X		X	X	X	X	X
Density of individuals		X					
11. % Hybrids	X	X					
% introduced species					X	X	
% simple lithophils	X						
# simple lithophilic species	X						
% native species							X
% native wild individuals							X
12. % Diseased Individuals	X	X	X	X	X	X	
13. Total Fish Biomass[c]						X	

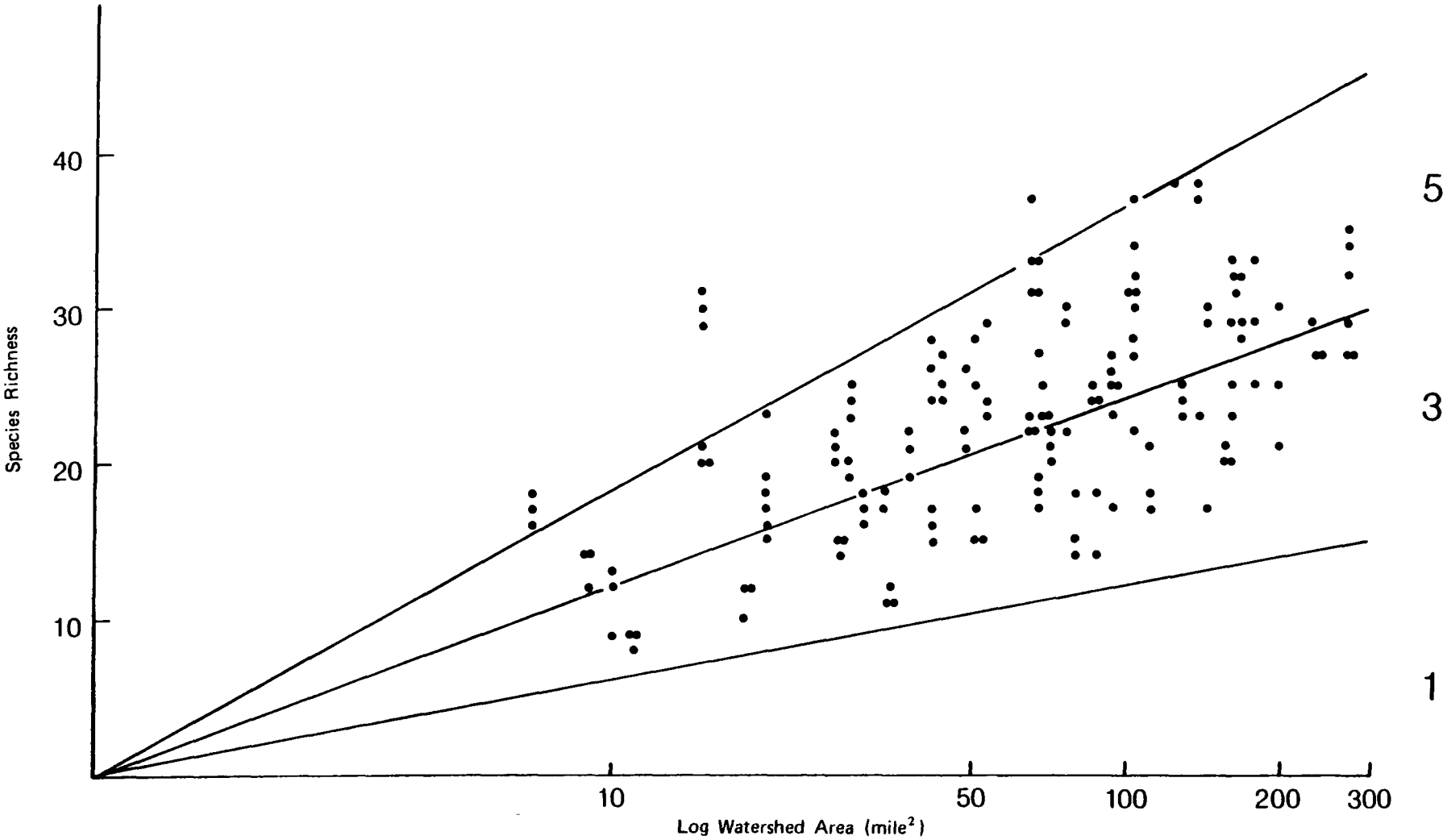


Figure 7.2-4. Total number of fish species versus watershed area for Ohio regional reference sites.

for most Mississippi Basin streams, but where sunfish are absent or rare, other groups are used. Cyprinid species are used in coolwater western streams; water column species occupy the same niche in northeastern streams; salmonids are suitable in coldwater streams; headwater species serve for midwestern headwater streams and trout and sunfish species are used in southern Ontario streams. Karr et al. (1986) and Ohio EPA (1987b) found the number of sunfish species to be dependent on stream size in small streams, but Ohio EPA (1987b) found no relationship between stream size and sunfish species in medium to large streams, nor between stream size and headwater species in small streams. Scoring of this metric requires development of species/waterbody size relationships.

Metric 4. **Number and identity of sucker species(1).** Substitutes: Number of adult trout species(6), number of minnow species(5); and number of suckers and catfish(8)

These species are sensitive to physical and chemical habitat degradation and commonly comprise most of the fish biomass in streams. All but the minnows are long-lived species and provide a multiyear integration of physicochemical conditions. Suckers are common in medium and large streams; minnows dominate small streams in the Mississippi Basin; and trout occupy the same niche in coldwater streams. The richness of these species is a function of stream size in small and medium sized streams, but not in large rivers. Scoring of this metric requires development of species/waterbody size relationships.

Metric 5. **Number and identity of intolerant species(1).** Substitutes: Number and identity of sensitive species(5), amphibian species(4); and presence of brook trout(8)

This metric distinguishes high and moderate quality sites using species that are intolerant of various chemical and physical perturbations. Intolerant species are typically the first species to disappear following a disturbance. Species classified as intolerant or sensitive should only represent the 5–10 percent most susceptible species, otherwise this becomes a less discriminating metric. Candidate species are determined by examining regional ichthyological books for species that were once widespread but have become restricted to only the highest quality streams. Ohio EPA (1987b) uses number of sensitive species (which includes highly intolerant and moderately intolerant species) for headwater sites because highly intolerant species are generally not expected in such habitats. Moyle (1976) suggested using amphibians in northern California streams because of their sensitivity to silvicultural impacts. This also may be a promising metric in Appalachian streams which may naturally support few fish species. Steedman (1988) found that the presence of brook trout had the greatest correlation with IBI score in Ontario streams. The number of sensitive and intolerant species increases with stream size in small and medium sized streams but is unaffected by size of large rivers. Scoring of this metric requires development of species/waterbody size relationships.

Metric 6. **Proportion of individuals as green sunfish(1).** Substitutes: Proportion of individuals as common carp(2,4), white sucker(3,4), tolerant species(5), creek chub(7), and dace(8)

This metric is the reverse of Metric 5. It distinguishes low from moderate quality waters. These species show increased distribution or abundance despite the historical degradation of surface waters, and they shift from incidental to dominant in disturbed sites. Green sunfish are appropriate in small Midwestern streams; creek chubs were suggested for central Appalachian streams; common carp were suitable for a coolwater Oregon river; white suckers were selected in the northeast and Colorado where green sunfish are rare to absent; and dace (Rhinichthys species) were used in southern Ontario. To avoid weighting the metric on a single species, Karr et al. (1986) and Ohio EPA (1987b) suggest using a small number of highly tolerant species. Scoring of this metric may require development of expectations based on waterbody size.

Trophic Composition Metrics

These three metrics assess the quality of the energy base and trophic dynamics of the community. Traditional process studies, such as community production and respiration, are time consuming to conduct and the results are equivocal; distinctly different situations can yield similar results. The trophic

composition metrics offer a means to evaluate the shift toward more generalized foraging that typically occurs with increased degradation of the physicochemical habitat.

Metric 7. **Proportion of individuals as omnivores(1,2,3,4,5,8).** Substitutes: Proportion of individuals as yearlings(4)

The percent of omnivores in the community increases as the physical and chemical habitat deteriorates. Omnivores are defined as species that consistently feed on substantial proportions of plant and animal material. Ohio EPA (1987b) excludes sensitive filter feeding species such as paddlefish and lamprey ammocoetes and opportunistic feeders like channel catfish. Where omnivorous species are nonexistent, such as in trout streams, the proportion of the community composed of yearlings, which initially feed omnivorously, may be substituted.

Metric 8. **Proportion of individuals as insectivorous cyprinids(1).** Substitutes: Proportion of individuals as insectivores (2,3,5), specialized insectivores(4), and insectivorous species(5); and number of juvenile trout(4)

Insectivores or invertivores are the dominant trophic guild of most North American surface waters. As the invertebrate food source decreases in abundance and diversity due to physicochemical habitat deterioration, there is a shift from insectivorous to omnivorous fish species. Generalized insectivores and opportunistic species, such as blacknose dace and creek chub were excluded from this metric by Ohio EPA (1987b). This metric evaluates the midrange of biotic integrity.

Metric 9. **Proportion of individuals as top carnivores(1,3,8).** Substitutes: Proportion of individuals as catchable salmonids(2), catchable wild trout(4), and pioneering species(5)

The top carnivore metric discriminates between systems with high and moderate integrity. Top carnivores are species that feed as adults predominantly on fish, other vertebrates, or crayfish. Occasional piscivores, such as creek chub and channel catfish, are not included. In trout streams, where true piscivores are uncommon, the percent of large salmonids is substituted for percent piscivores. These species often represent popular sport fish such as bass, pike, walleye, and trout. Pioneering species are used by Ohio EPA (1987b) in headwater streams typically lacking piscivores.

Fish Abundance and Condition Metrics

The last three metrics indirectly evaluate population recruitment, mortality, condition, and abundance. Typically, these parameters vary continuously and are time consuming to estimate accurately. Instead of such direct estimates, the final results of the population parameters are evaluated. Indirect estimation is less variable and much more rapidly determined.

Metric 10. **Number of individuals in sample(1,2,4,5,8).** Substitutes: Density of individuals(3,4)

This metric evaluates population abundance and varies with region and stream size for small streams. It is expressed as catch per unit effort, either by area, distance, or time sampled. Generally sites with lower integrity support fewer individuals, but in some nutrient poor regions, enrichment increases the number of individuals. Steedman (1988) addressed this situation by scoring catch per minute of sampling greater than 25 as a three, and less than 4 as a one. Unusually low numbers generally indicate toxicity, making this metric most useful at the low end of the biological integrity scale. Hughes and Gammon (1987) suggest that in larger streams, where sizes of fish may vary in orders of magnitude, total fish biomass may be an appropriate substitute or additional metric.

Metric 11. **Proportion of individuals as hybrids(1).** Substitutes: Proportion of individuals as introduced species(2,4), simple lithophils(5); and number of simple lithophilic species(5)

This metric is an estimate of reproductive isolation or the suitability of the habitat for reproduction. Generally as environmental degradation increases, the percent of hybrids and introduced species also increases, but the proportion of simple lithophils decreases. However, minnow hybrids are found in some high quality streams, hybrids are often absent from

highly impacted sites, and hybridization is rare and difficult for many to detect. Thus, Ohio EPA (1987b) substitutes simple lithophils for hybrids. Simple lithophils spawn where their eggs can develop in the interstices of sand, gravel, and cobble substrates without parental care. Hughes and Gammon (1987) and Miller et al. (1988a) propose using percent introduced individuals. This metric is a direct measure of the loss of species segregation between midwestern and western fishes that existed before the introduction of midwestern species to western rivers.

Metric 12. **Proportion of individuals with disease, tumors, fin damage, and skeletal anomalies(1).**

This metric depicts the health and condition of individual fish. These conditions occur infrequently or are absent from minimally impacted reference sites but occur frequently below point sources and in areas where toxic chemicals are concentrated. They are excellent measures of the subacute effects of chemical pollution and the aesthetic value of game and nongame fish.

Metric 13. **Total fish biomass (optional).**

Hughes and Gammon (1987) suggest that in larger areas where sizes of fish may vary in orders of magnitude this additional metric may be appropriate.

Because the IBI is an adaptable index, the choice of metrics and scoring criteria is best developed on a regional basis through use of available publications (Karr et al. 1986; Ohio EPA 1987b; Miller et al. 1988a). Several steps are common to all regions. The fish species must be listed and assigned to trophic and tolerance guilds. Scoring criteria are developed through use of high quality historical data and data from minimally-impacted regional reference sites. This has been done for much of the country, but continued refinements are expected as more fish community ecology data become available. Once scoring criteria have been established, a fish sample is evaluated by listing the species and their abundances (Figure 7.2-3), calculating values for each metric, and comparing these values with the scoring criteria. Individual metric scores are added to calculate the total IBI score (Figure 7.2-5). Hughes and Gammon (1987) and Miller et al. (1988a) suggest that scores lying at the extremes of scoring criteria can be modified by a plus or minus; a combination of three pluses or three minuses results in a two point increase or

Station No. _____

Site _____

Metrics[a]	Scoring Criteria[b] 5 (%)	3 (%)	1 (%)	Metric Value	Metric Score
1. Number of Native Fish Species	>67	33-67	<33		
2. Number of Darter or Benthic Species	>67	33-67	<33		
3. Number of Sunfish or Pool Species	>67	33-67	<33		
4. Number of Sucker or Long-Lived Species	>67	33-67	<33		
5. Number of Intolerant Species	>67	33-67	<33		
6. % Green Sunfish or Tolerant Individuals	<10	10-25	>25		
7. % Omnivores	<20	20-45	>45		
8. % Insectivores or Invertivores	>45	20-45	<20		
9. % Top Carnivores	>5	1-5	<1		
10. Total Number of Individuals	>67	33-67	<33		
11. % Hybrids or Exotics	0	0-1	>1		
12. % Anomalies	<1	1-5	>5		

Scorer _____ IBI Score _____

Comments: _____

(a) Karr's original metrics or commonly used substitutes. See text and Table 7.2-1 for other possibilities.
(b) Karr's original scoring criteria or commonly used substitutes. These may require refinement in other ecoregions.

Figure 7.2-5. Data Summary Sheet for Rapid Bioassessment Protocol V.

decrease in IBI. Ohio EPA (1987b) scores proportional metrics as 1 when the number of species and individuals in samples are fewer than 6 and 75, respectively, when their expectations are of higher numbers.

7.3 RESULTS OF PILOT STUDIES IN OHIO AND OREGON

Surveys of 109 regional reference sites in five Ohio ecoregions and of 26 sites on the mainstem Willamette River, Oregon, were conducted during the summers of 1983 and 1984. The Ohio survey was a cooperative project between Ohio EPA, EPA Region V, and EPA's Environmental Research Laboratory at Corvallis (ERL-C); the Willamette survey was a cooperative project between DePauw University, EPA-Region X, Oregon Department of Environmental Quality, U.S. Fish and Wildlife Service, and ERL-C. The objectives of the Ohio survey were to evaluate the correspondence between ecoregions and stream ecosystem attributes and to assess regional patterns in attainable water resource quality. The Willamette study was intended to measure the effects of water resource quality on the fish community and to examine the usefulness of the IBI and IWB in a large western river. The results of these two surveys have been published elsewhere (Larsen et al. 1986; Hughes and Gammon 1987; Ohio EPA 1987b; Whittier et al. 1987; Larsen et al. 1988) and only the fisheries aspects will be summarized here.

7.3.1 Methods

Ohio

Minimally-impacted regional reference sites were selected based on: census and point-source data; maps of population density, land use, and mining; and aerial and ground inspection of the watershed and site (Figure 7.3-1).

Ohio EPA collected fish at half the sites two to three times at 1 month intervals each summer (1983 and 1984). Rivers were sampled via boat-mounted electrofishers for 500 meters, headwaters were sampled with backpack electrofishers for 200 meters, most streams were sampled with a towed electrofisher for 300 meters. All captured fish were identified to species, counted, and examined for disease; a subsample was weighed at the site. The data were analyzed through use of the IBI, IWB, and identification of regionally characteristic species.

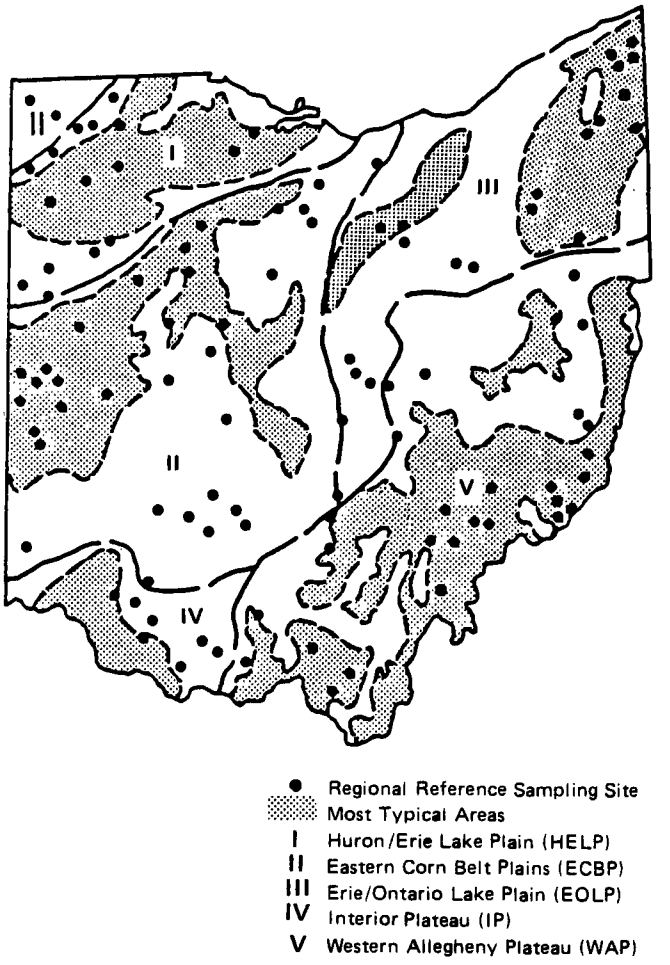


Figure 7.3-1. Locations of regional reference sites in Ohio (from Whittier et al. 1987).

Willamette River

Sites were selected along one side of the river about 2 yd offshore to bracket large point sources and to space sites approximately 16 km apart (Figure 7.3-2). Each site was 500 meters long and included slow, deep water; shallow, fast, water; and cover.

Each site was sampled twice in the summer of 1983 with a boat-mounted electrofisher. All fish were identified to species, counted, and examined for anomalies, and a subsample was weighed. Data were analyzed through use of IBI, IWB, and DCA.

7.3.2 Results and Interpretation

The tolerance and trophic guilds, and origin of selected species are given in Appendix D. For the sake of brevity, IBI and IWB calculations are presented for four selected sites only.

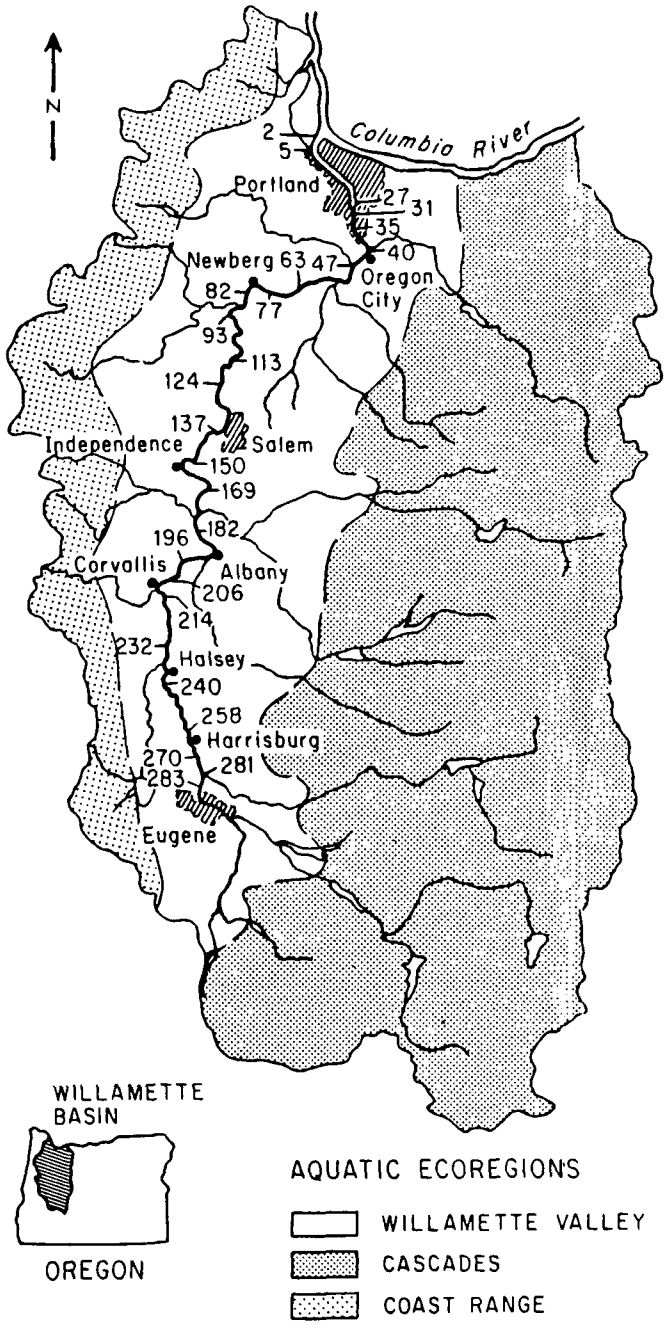


Figure 7.3-2. Locations of sampling sites on the mainstem Willamette River, Oregon (taken from Hughes and Gammon 1987).

Table 7.3-1 COLLECTION DATA FOR TWO OHIO ECOREGION REFERENCE SITES

Species	Site WAP	Site HELP
Grass pickerel	--	19
White sucker	21	24
Black redhorse	2	--
Golden redhorse	24	--
Northern hogsucker	26	--
Common carp	--	26
Blacknose dace	3	--
Creek chub	94	2
Golden shiner	--	42
Redfin shiner	--	22
Silver shiner	6	--
Rosyface shiner	41	--
Striped shiner	443	--
Sand shiner	264	--
Mimic shiner	1	--
Silverjaw shiner	93	--
Bluntnose minnow	101	8
Fathead minnow	--	3
Central stoneroller	559	--
Yellow bullhead	--	23
Black bullhead	--	13
Tadpole madtom	--	2
Brindled madtom	4	--
Blackstripe topminnow	--	66
White crappie	--	6
Green sunfish	--	156
Bluegill	--	1
Rock bass	16	--
Longear sunfish	3	--
Smallmouth bass	11	--
Largemouth bass	--	2
Orangespotted sunfish	--	2
Blackside darter	3	--
Logperch	3	--
Johnny darter	40	--
Greenside darter	56	--
Banded darter	25	--
Rainbow darter	60	--
Fantail darter	113	--

Ohio

The unmodified IBI metrics and criteria (Karr et al. 1986) were used to analyze the Ohio data. Species richness metrics were determined as suggested by Karr et al. 1986 (Figure 7.2-4). Sample data, scoring criteria, and scores are given in Tables 7.3-1 and 7.3-2 for a site in the Huron Erie Lake Plain (HELP) and Western Allegheny Plateau (WAP) ecoregions. The fish communities of the Ohio regional reference sites showed distinct ecoregional differences between the Western Allegheny Plateau and the Huron Erie Lake Plain ecoregions. Differences among the three transitional ecoregions were less obvious. This was true whether examining patterns in IBI scores (Figure 7.3-3) or dominant species (Figure 7.3-4).

TABLE 7.3-2 SCORING CRITERIA AND IBI AND IWB SCORES FOR TWO OHIO ECOREGION REFERENCE SITES

Metric (Criteria)	Value(Score) WAP	HELP
Total Number of Species (<9=1, 9-18=3, >18=5)	25(5)	17(3)
Number of Darter Species (<2=1, 2-5=3, >5=5)	7(5)	0(1)
Number of Sunfish Species (<2=1, 2-4=3, >4=5)	2(3)	4(3)
Number of Sucker Species (<2=1, 2-4=3, >4=5)	4(3)	1(1)
Number of Intolerant Species (<4=1, 4-8=3, >8-5)	8(3)	0(1)
% Green Sunfish (>20=1, 5-20=3, <5=5)	0(5)	37(1)
% Omnivores (>45=1, 20-45=3, <20=5)	5(5)	9(5)
% Insectivorous Minnows (<20=1, 20-45=3, >45=5)	42(3)	15(1)
% Top Carnivores (<1=1, 1-5=3, >5=5)	1(3)	5(3)
Number of Individuals (<200=1, 200-800=3, >800=5)	2,012(5)	417(3)
% Hybrids (>1=1, 0-1=3, 0=5)	0(5)	0(5)
% Diseased (>5=1, 2-5=3, <2=5)	1(5)	4(3)
Total IBI Score	50	30
IWB Score	10	9
Integrity	Good	Poor

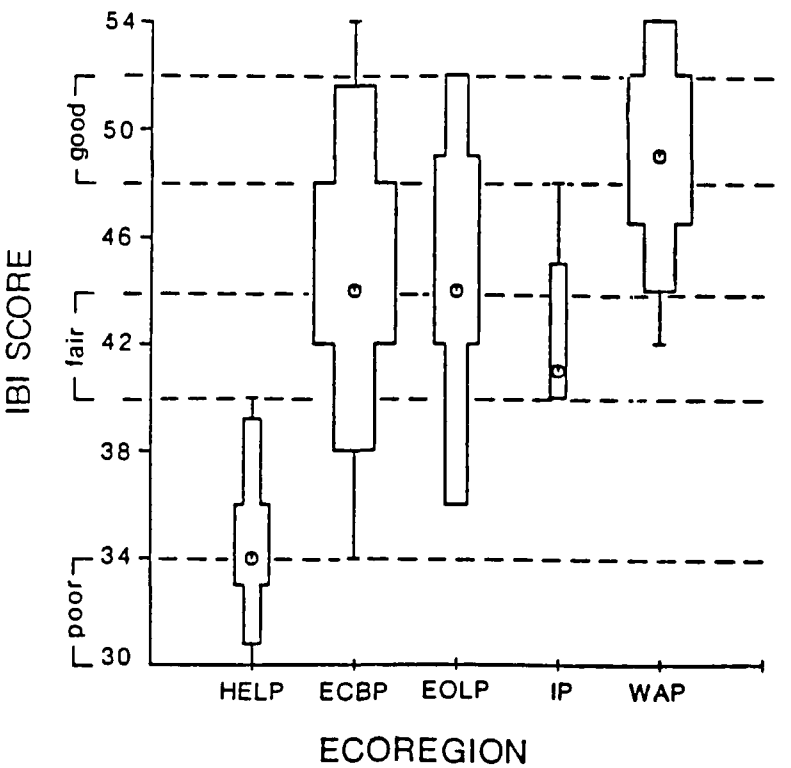


Figure 7.3-3. Index of biotic integrity scores by Ohio ecoregion (from Whittier et al. 1987). Vertical lines represent ranges; horizontal lines represent 10th, 25th, 75th, and 90th percentiles; open circles are medians.

1 bluntnose minnow
2 creek chub
3 green sunfish
4 blackstripe topminnow
5 fathead minnow
6 yellow bullhead
7 johnny darter
8 white sucker
9 rockbass
10 rosefin shiner
11 greenside darter
12 bluegill
13 mottled sculpin
14 common shiner
15 rainbow darter
16 fantail darter
17 spotfin shiner
18 longear sunfish
19 sand shiner
20 golden redhorse
21 emerald shiner
22 central stoneroller
23 striped shiner

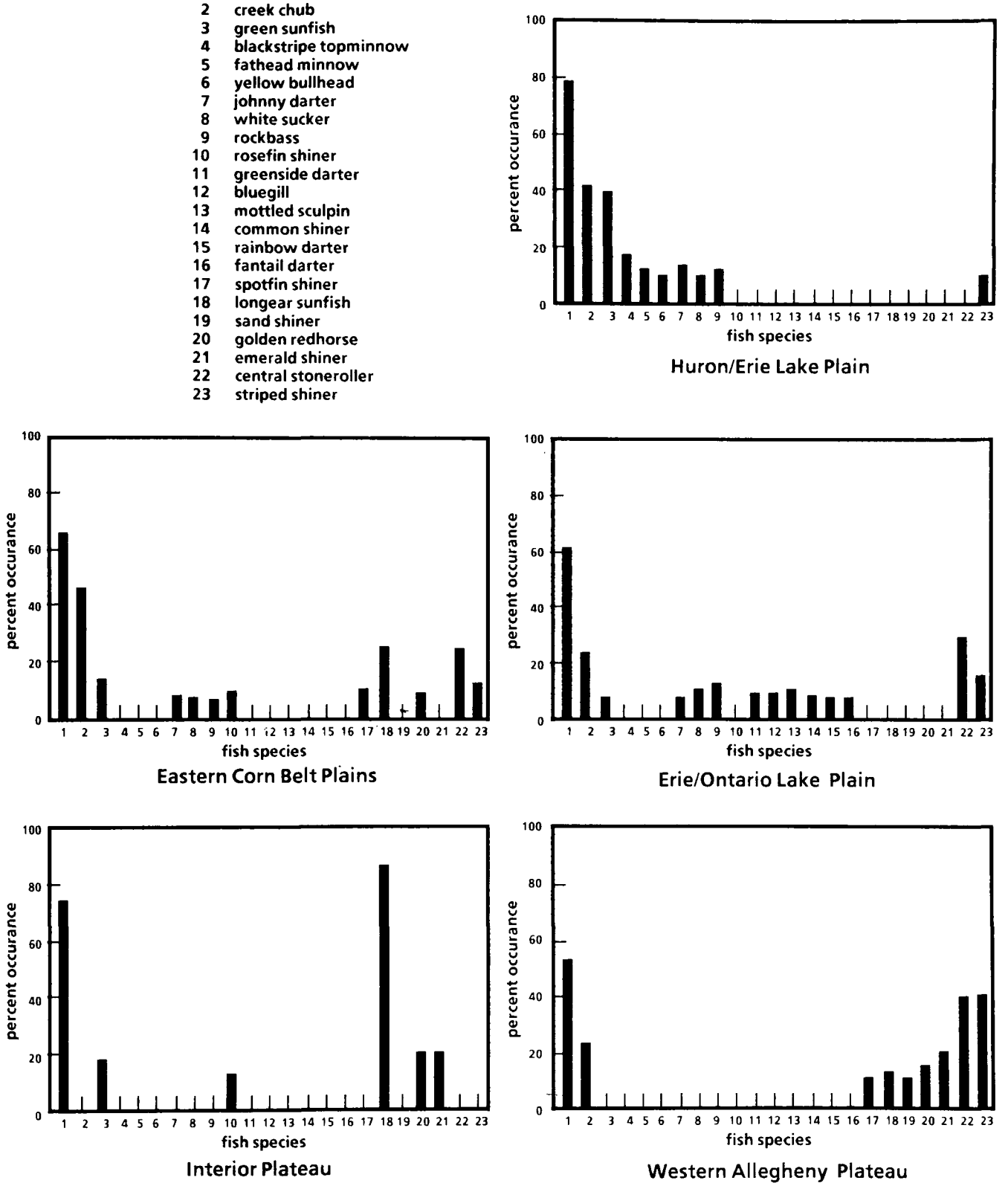


Figure 7.3-4. Dominant Ohio fish species by ecoregion (from Whittier et al. 1987).

Willamette River

Five of the original 12 metrics that Karr et al. (1986) found appropriate for midwestern streams were inappropriate for a large western river. These five metrics were modified by making the following substitutions: (1) The number of sculpin species replaced the number of darter species as suggested by Karr et al. (1986); (2) The number of native minnow species replaced the number of sunfish species. In cool and warmwater western streams, the introduced sunfish increase and native minnows decline when habitat structure deteriorates (Minckley 1973; Moyle 1976); (3) Percent common carp replaced percent green sunfish. No green sunfish were collected in the Willamette, the other relatively tolerant species were either rarely captured or dominant, and common carp increased as the physicochemical habitat deteriorated; (4) Percent catchable salmonids (longer than 20 cm) replaced percent piscivores. The dominant piscivore in the Willamette is a relatively tolerant species and is not indicative of integrity; most Willamette salmonids (juvenile whitefish and anadromous salmon) are not piscivorous in freshwater rivers; and (5) Percent introduced individuals replaced percent hybrids. Hybrids are too rare in the Willamette to make this a useful metric for discriminating degradation. Percent introduced individuals do increase with degradation (Moyle and Nichols 1973; Holden and Stalnaker 1975; Leidy and Fiedler 1985) and represent a loss of the segregation existing before midwestern species were brought to western waters.

Scoring criteria were based on the Willamette data due to the lack of sufficient quantitative historical data; no adjustments were required for stream size because it changed little. Criteria were based on characteristics of the fish assemblages at the minimally impacted sites of the upper mainstem (Table 7.3-3). Like the metrics, criteria were adjusted in the following ways to reflect western conditions (Table 7.3-4): (1) Criteria for common carp were reduced because carp are much less common in Oregon than the green sunfish is in the Midwest; (2) Omnivore criteria were increased because a dominant species (largescale sucker) is an omnivore; (3) The catchable salmonids criteria were increased because salmonids are more common than midwestern piscivores; (4) Percent introduced individuals had

TABLE 7.3-3 COLLECTION DATA (NUMBER OF INDIVIDUALS) FOR TWO SITES ON THE WILLAMETTE RIVER, OREGON

Species	River Kilometer 270	31
Mountain whitefish	25	--
Chinook salmon	4	2
Longnose dace	1	--
Speckled dace	3	--
Northern squawfish	2	2
Common carp	--	7
Largescale sucker	44	10
Mountain sucker	6	--
Largemouth bass	--	2
Yellow perch	--	1
Prickly sculpin	--	1
Reticulate sculpin	2	--
Torrent sculpin	3	--
Paiute sculpin	5	--

TABLE 7.3-4 SCORING CRITERIA AND IBI AND IWB SCORES FOR TWO SITES ON THE WILLAMETTE RIVER, OREGON

Metric (Criteria)	Value(Score) River Kilometer 270	31
Number of Native Species (<5=1, 5-9=3, >9=5)	10(5)	4(1)
Number of Sculpin Species (<2=1, 2=3, >2=5)	3(5)	1(1)
Number of Native Minnow Species (<3=1, 3-5=3, >5=5)	3(3)	1(1)
Number of Sucker Species (0=1, 1=3, >1=5)	2(5)	1(3)
Number of Intolerant Species (0=1, 1-2=3, >2=5)	2(3)	1(3)
% Common Carp (>9%=1, 1-9%=3, <1%=5)	0(5)	28(1)
% Omnivores (>50%=1, 25-50%=3, <25%=5)	46(3)	68(1)
% Insectivores (<20%=1, 20-40%=3, >40%=5)	22(3)	16(1)
% Catchable Salmonids (0%=1, 1-9%=3, >9%=5)	23(5)	0(1)
Number of Individuals/km (<50=1, 50-99=3, >99=5)	95(3)	25(1)
% Introduced (>9%=1, 1-9%=3, <1%=5)	0(5)	40(1)
% Anomalies (>5%=1, 2-5%=3, <2%=5)	0(5)	8(1)
Total IBI Score	50	16
IWB Score	6.1	4.7
Integrity	Good	Very Poor

higher criteria than expected for percent hybrids; and (5) Percent anomalies criteria were higher because, where they occurred, they were above the 1 percent maximum as referenced in Karr et al. (1986).

A detrended correspondence analysis (DCA) showed four distinct clusters among the 26 sites corresponding to upper river, middle river, Newberg pool, and Portland metropolitan fish assemblages (Figure 7.3-5). These differences and the longitudinal trends shown by the IBI (Figure 7.3-6) corresponded to declining physical and chemical habitat quality and increased nonpoint-source pollution.

(1)Karr et al. (1986); (2)Hughes and Gammon (1987); (3)Miller et al., (1988b In Review); (4)Miller et al. (1988a); (5)Ohio EPA (1987b); (6)Metric suggested by Moyle or Hughes as a provisional replacement metric in small western salmonid streams; (7)Leonard and Orth (1986); (8) Steedman (1988).

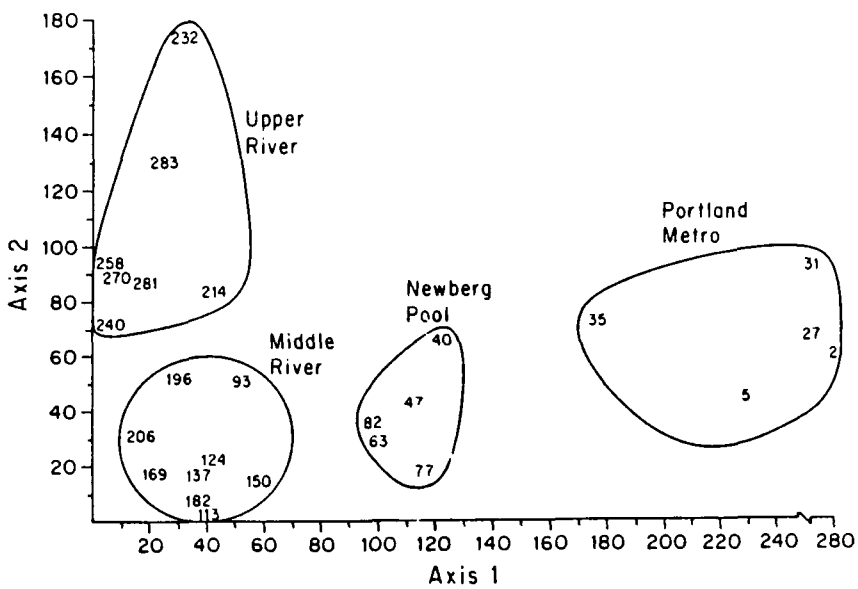


Figure 7.3-5. Patterns in mainstem Willamette River fish assemblages as revealed by detrended correspondence analysis (taken from Hughes and Gammon 1987).

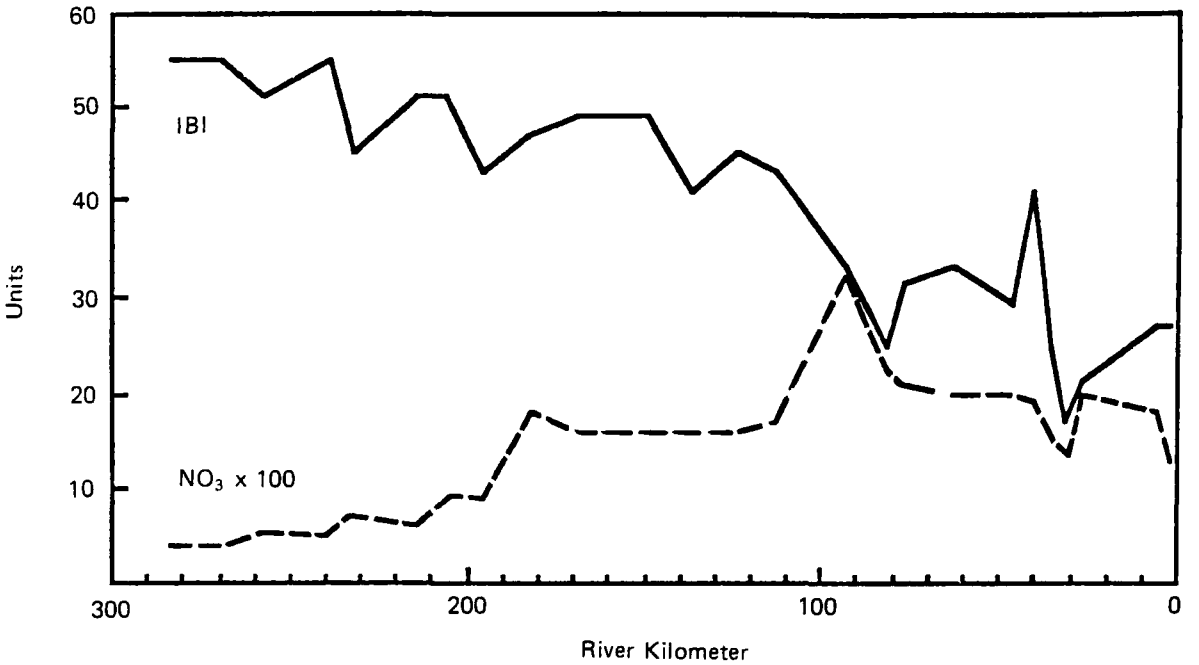


Figure 7.3-6. Longitudinal trends in Index of Biotic Integrity and nitrate in the Willamette River (modified from Hughes and Gammon) 1987.

8. INTEGRATION OF HABITAT, WATER QUALITY, AND BIOSURVEY DATA

The overall assessment of ecological condition first focuses on the evaluation of habitat quality, then analyzes the biological components of the system in light of these data. Habitat, as the principal determinant of biological potential, sets the context for interpreting biosurvey results and can be used as a general predictor of biological condition. Routine water chemistry can also help to characterize certain impacts.

In Rapid Bioassessment Protocols (RBPs) I and IV, the habitat evaluation carries considerable weight in the final assessment because minimal effort is expended on the collection and analysis of biological data. In RBPs II, III, and V, however, the biological evaluations are more rigorous and appropriately take precedence. The habitat assessment plays a supporting role within these protocols. It is used to identify obvious constraints on the attainable potential of the site, help in the selection of appropriate sampling stations, and provide basic information for interpreting biosurvey results.

8.1 THE RELATIONSHIP BETWEEN HABITAT QUALITY AND BIOLOGICAL CONDITION

The attainable biological potential of a site is primarily determined by the quality of the habitat at that site. The relationship between habitat quality and biological condition can be envisioned as a sigmoid curve (Figure 8.1-1) with community response varying with habitat quality. In the upper segment of the curve, good quality habitat (supporting or excellent) will support high quality communities, and responses to minor alterations in habitat will be only subtle and of little consequence. As habitat quality continues to decline, however, discernible biological impairment results, and, in the absence of confounding water quality effects, the relationship is roughly linear.

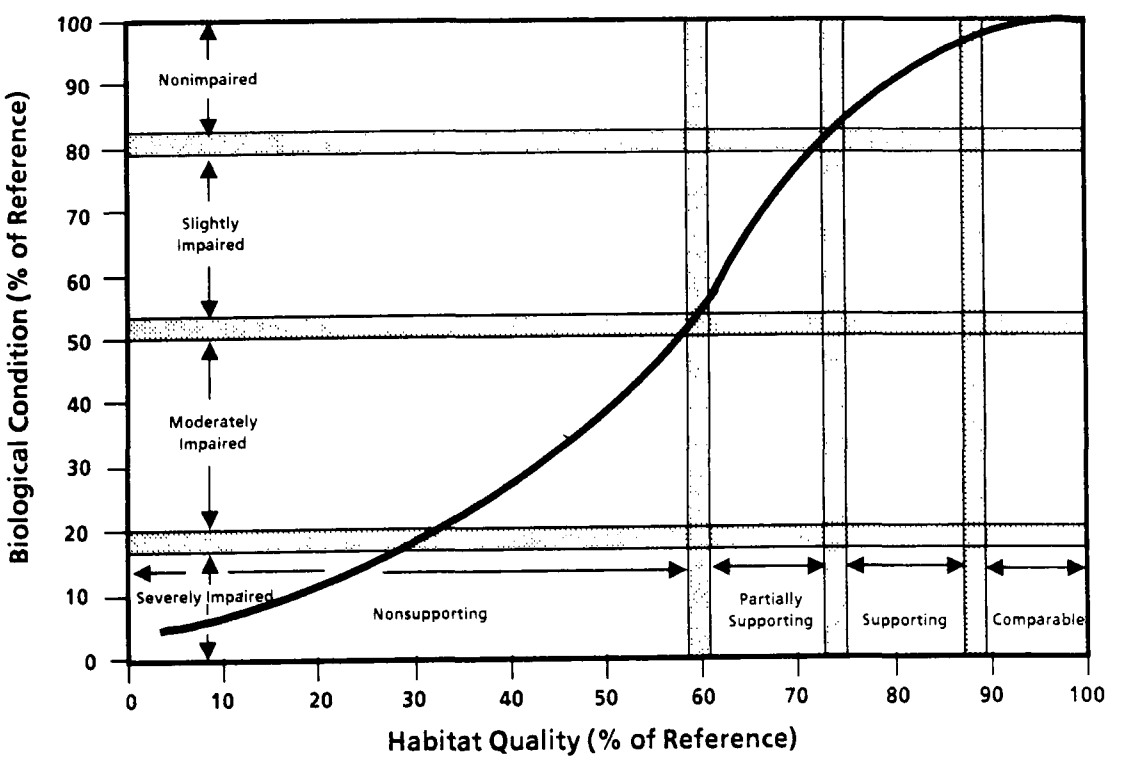


Figure 8.1-1. The relationship between habitat and biological condition.

In areas of severe habitat degradation, predicting the degree of biological impairment becomes more difficult. Community structure is less dependent on habitat diversity, which is effectively simplified by degradation, and more dependent on the opportunistic colonization strategies of a relatively few tolerant species. These opportunists are adapted to environmental conditions that are unfavorable to most other species and, in the absence of competition, thrive (or at least survive) in these marginal conditions. Therefore, biological measures, particularly those used in the RBPs, are relatively insensitive to habitat variations in this range, and a nonsupporting characterization may correspond to either a moderately or severely impaired biological condition, depending on the specific site.

When habitat and biological data are systematically collected together, empirical relationships can be quantified and subsequently used for screening impact sites, scoping field activities, and discriminating water quality impacts from habitat degradation. With the acquisition of a multiple-site database, confidence bounds can be established for the habitat/indigenous community relationship.

A theoretical relationship of habitat quality and biological condition as affected by water quality problems (organic or toxicant loadings) also can be hypothesized (Figure 8.1-2). Curve II in Figure 8.1-2 indicates the general relationship of biological condition to habitat quality in the absence of water quality effects. Curve II may, in fact, resemble a sigmoid curve as illustrated by Figure 8.1-1. Curve III represents a situation where organic pollution or toxicants will adversely affect biological condition regardless of the quality of the habitat.

In areas of good or excellent habitat, biological communities will reflect degraded conditions when water quality effects are present. However, as habitat degrades to a poor condition in the presence of water quality problems, response of the communities may be less dramatic because the community is composed of tolerant and generally opportunistic species. Curve I is representative of a situation indicative of nutrient enrichment, which will artificially sustain a more diverse fauna than dictated by the habitat quality. However, at some point along the curve as habitat degradation proceeds, nutrient enrichment will no longer support a diverse community, and a drastic decrease in biological condition will result.

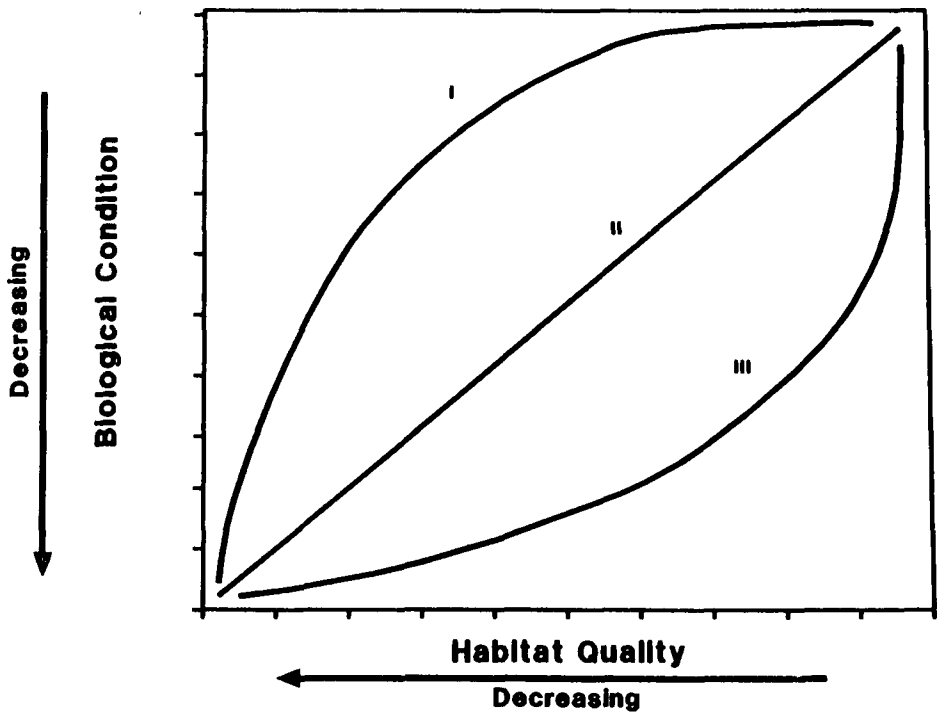


Figure 8.1-2. Relationship of habitat quality and biological condition in the context of water quality.

8.2 BIOASSESSMENT TECHNIQUE

As described in Chapters 6 and 7, the biological assessment involves an integrated analysis of both functional and structural components of the aquatic communities. These functional and structural components are evaluated through the use of eight metrics for benthic RBPs II and III and 12 metrics for fish RBP V. The range of pollution sensitivity exhibited by each metric differs among metrics (Figures 8.2-1 and 8.2-2); some are sensitive across a broad range of biological conditions, others only to part of the range. Sensitivity of metrics may also vary depending on whether organic or toxicant impacts are being evaluated (Figure 8.2-1). The considerable overlap in the ranges of sensitivity helps reinforce final conclusions regarding biological condition, while metrics that are better able to differentiate responses at the extremes of the range of impairment enable a more complete bioassessment. The integrated analysis approach thus allows a broader assessment of condition than an analysis using any single metric. However, information from individual metrics will be useful in enhancing overall data interpretation.

Certain metrics are designed to be better estimators of either organic or toxicant effects. For instance in the benthic protocols, the Hilsenhoff Biotic Index (HBI) utilizes a tolerance classification scheme that is based on organic pollution effects, while Functional Group representation can be altered by either organics or toxicants (Figure 8.2-1). Although Scrapers and Filterers are affected by toxicants to a certain extent, their ratio can best be used to assess organic enrichment (Cummins 1987, personal communication). As discussed in Chapter 6, a reduction in the value

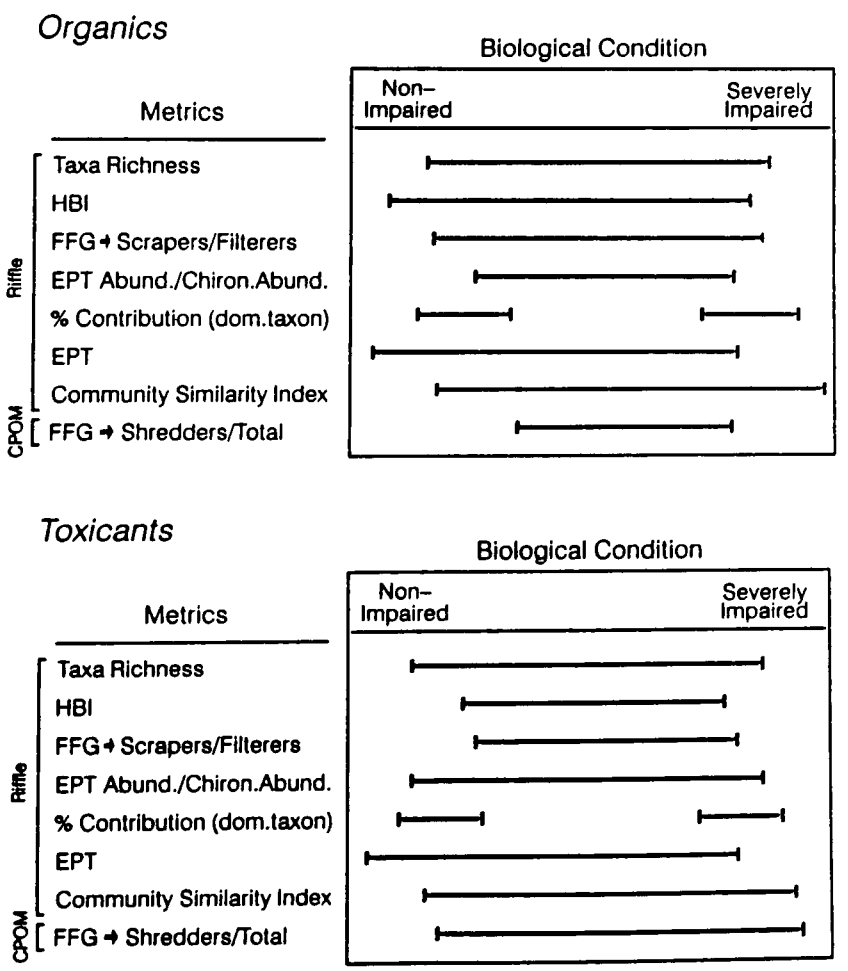


Figure 8.2-1. Range of sensitivities of Rapid Bioassessment Protocol II and III benthic metrics in assessing biological condition in response to organics and toxicants.

obtained for Scrapers/Filterers can be indicative of either a reduction in the quality of the periphyton as a food source and/or an increase in the suspended FPOM. Filterers are also affected by FPOM contaminated by toxicants.

The relative abundance of Shredders in the benthic community is a good indicator of toxicant problems because of the sensitivity of the Shredder community to toxic conditions (Cummins 1987, personal communication). Vegetation sprayed with pesticides eventually becomes a CPOM food source for Shredders. In sufficient concentrations, toxicants bound to CPOM may affect Shredders directly through ingestion, as well as indirectly by killing attached microbes that serve as a nutrition base for Shredders. The ratio of the abundances of EPT taxa and chironomids may also function as a toxicant indicator, since some midge species such as *Cricotopus* sp. become abundant in areas affected by metals (Winner et al. 1980; Mount et al. 1986).

The 12 IBI metrics used in fish Protocol V also represent differing sensitivities (Figure 8.2-2). For example, municipal effluents typically affect total abundance and trophic structure (Karr et al. 1986), while unusually low total abundance generally indicates a toxicant effect. However, some nutrient-deficient environments support a limited number of individuals, and an increase in abundance may indicate organic enrichment. Bottom dwelling species (e.g., darters, sculpins) that depend upon benthic habitats for feeding and reproduction are particularly sensitive to the effects of siltation and benthic oxygen depletion (Kuehne and Barbour 1983; Ohio EPA 1987b) and are good indicators of habitat degradation.

For the benthic and fish biosurveys and habitat assessment, scores are assigned to each metric or parameter based on a decision matrix. In the case of habitat assessment, evaluation of the quality of the parameter is based on visual observation. The score assigned to each habitat parameter is a function of a range of scores and is weighted in terms of its contribution to the total habitat quality. The scores assigned to the benthic and fish metrics are based on computed values of the metrics and a station comparison, where the regional or stream reference station serves as the highest attainment criterion. Comparison of the total score computed for the metrics or parameters with that of the reference station provides a judgment as to impairment of biological condition.

Effects indicated by the aquatic community need to be evaluated in the context of habitat quality. A poor habitat in terms of riparian vegetation, bank stability, stream substrate, etc., would not be conducive to supporting a well-balanced community structure. The attainment of a higher quality biological condition may be prohibited by the constraints of habitat quality.

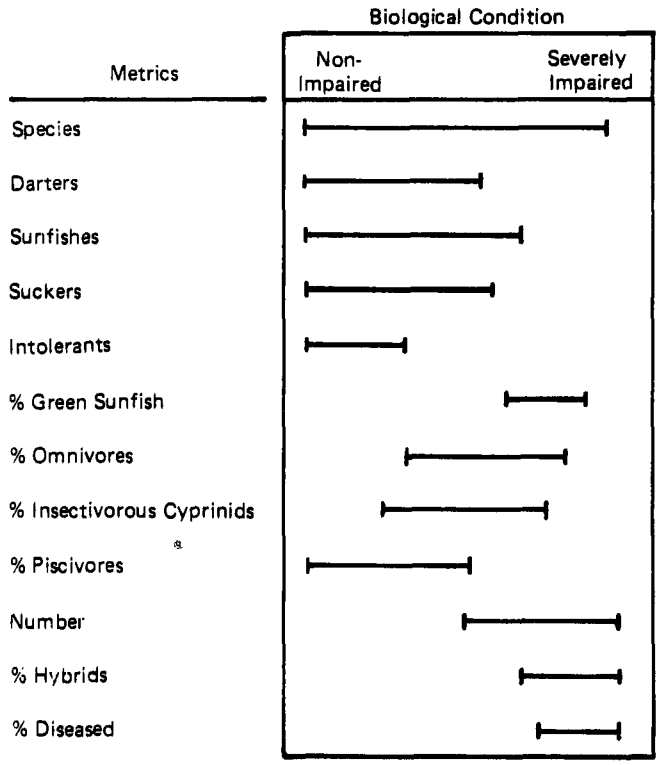


Figure 8.2-2. Range of sensitivities of Rapid Bioassessment Protocol V fish metrics in assessing biological condition (from Karr et al. 1986).

8.3 AN INTEGRATED ASSESSMENT APPROACH

The initial focus of a bioassessment should be on habitat quality. Based on a regional reference, the habitat at an impacted site may be equal to or less than the desired quality for that particular system. As discussed in Section 1.4, if the habitat at the impact site and reference are equal, then a direct comparison of biological condition can be made. If the habitat at the impact site is lower in quality than the reference, the habitat potential should be evaluated as a first step. A site-specific control may be more appropriate than a regional reference for an assessment of an impact site. Once a determination of the appropriate reference site type is made, possible outcomes of the bioassessment are: (1) no biological effects; (2) effects due to habitat degradation; (3) effects due to water quality; or (4) effects due to a combination of water quality and habitat degradation. Once habitat problems are identified, in most cases, separating the cause of impairment from water quality problems is difficult. The following decision matrix illustrates the approach to assessing biological effects.

Evaluation of Habitat at a Site-Specific Control Relative to that at a Regional Reference (Figure 8.3-1)

Selection of an appropriate station of comparison for evaluation of biological impact begins with an evaluation of habitat at the potential control station.

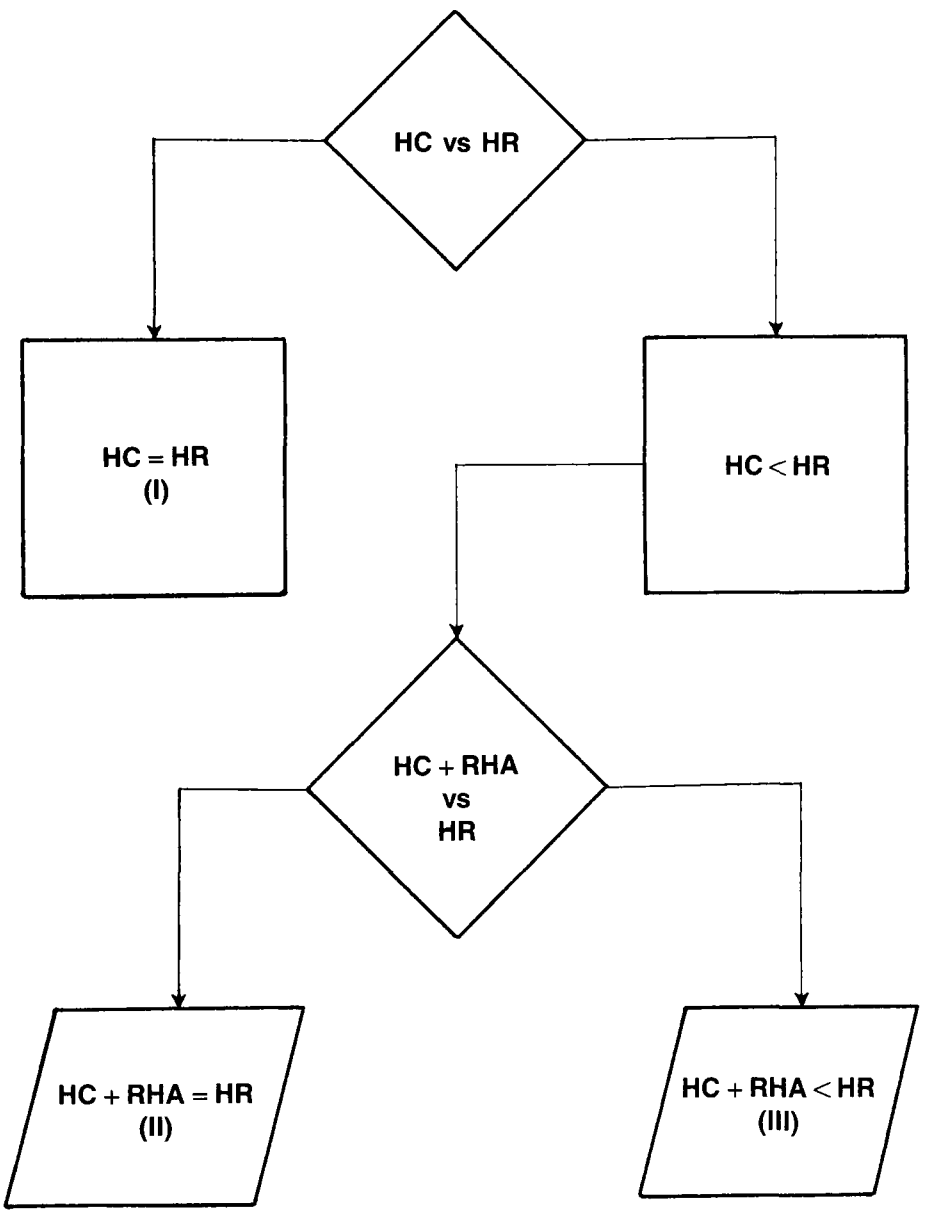


Figure 8.3-1. Evaluation of habitat at a site-specific control relative to that at a regional reference.

This comparison assumes that a regional reference database is available for the particular site being studied. Reference data used for comparison may be obtained from a single reference site. However, a reference database derived from numerous sites is much preferred and strongly recommended.

Scenario I depicts the situation where the habitat quality at the control station (HC) is equivalent to that at the regional reference (HR). If the control station habitat is degraded relative to that at the regional reference, it becomes necessary to consider the effect that reversible habitat alterations (RHA) may have on habitat quality (Scenarios II and III). Reversible habitat alterations are those habitat parameters that can potentially be altered by remedial action (i.e., bank stability, bank vegetation, streamside cover, and sometimes embeddedness).

Evaluation of Water Quality Effects (Figure 8.3-2)

A determination may be made that the habitat quality at the site-specific control station (C) is equivalent to that at the reference (R) (Scenario I). In this case, a biological assessment can be used to evaluate potential water quality effects at C (Figure 8.3-2).

(1) If impairment is not detected in a comparison of biological condition at the site-specific control station (BC) to biological condition at the reference (BR), then C should be included in the R database and either C or R may be used as a reference for biological assessment at the impact site (I). The site-specific control would be the best indicator of a site-specific situation. In addition, C would be more appropriate for use in determining water quality effects of point-source pollutants since it would be located on the same waterbody (or a nearby waterbody) and would integrate all other background sources of impairment other than the point source being evaluated. This allows segregation of effects of a particular point source. The reference would be more appropriate in an assessment of nonpoint-source effects since it is virtually impossible to find a nearby site-specific control which would not be impacted by the impact sources being studied. If R is based on an extensive database, then use of R as a reference would provide a better estimate of acceptable variation in a data set. Confidence intervals could be derived and used to put bounds on the data from C and/or the impact site (I).

(2) If biological impairment is detected at C relative to R, the impairment may be attributed to water quality effects. The use designation at C is probably appropriate, but R should be used as the bioassessment reference because of impairment at C.

Evaluation of Biological Impairment Due to Reversible Habitat Alterations (Figure 8.3-3)

If the habitat quality at C is degraded relative to that at R, but habitat quality could potentially be improved by reversing those degraded habitat parameters which are reversible (Scenario II), biological assessment at C will indicate whether C, R, or an alternative control site (C*) should be used as a bioassessment reference for the impact site (I) (Figure 8.3-3).

(3) When BC is equal to BR, the use designation at C may be considered appropriate, and C should be used as the bioassessment reference. This is a potential situation since reversible habitat parameters are mainly tertiary characteristics and should have the least effect on the biological community. However, in this situation benthic RBP III or fish RBP V should be utilized as a minimum. A more rigorous biological analysis (e.g., quantitative sampling) may be warranted to ensure that the approach is sufficiently sensitive to detect impairment.

(4) In situations where BC is less than BR, impairment may be due to either reversible habitat alterations, water quality effects, or a combination of the two. Selection of a bioassessment reference is dependent on the purpose of the assessment and the suspected source of impairment.

(5) If point source effects are being assessed, a habitat independent approach (e.g., toxicological testing, sampling with artificial substrates) may be warranted, using R as a reference. C could be considered an impact site.

(6) It may be appropriate to continue the rapid bioassessment (and habitat evaluation) approach using R as the reference because of impairment at C. Assuming that impairment at C indicates impairment also at I, the degree of impairment at I can be assessed relative to C. An *a priori* knowledge of potential water quality problems from an existing database would enhance interpretation of findings in this case.

(7) Another alternative would be to eliminate the confounding effects of the reversible habitat alterations by selecting another site-specific control station (C*), which, if possible, would then be evaluated relative to R (Figure 8.3-1).

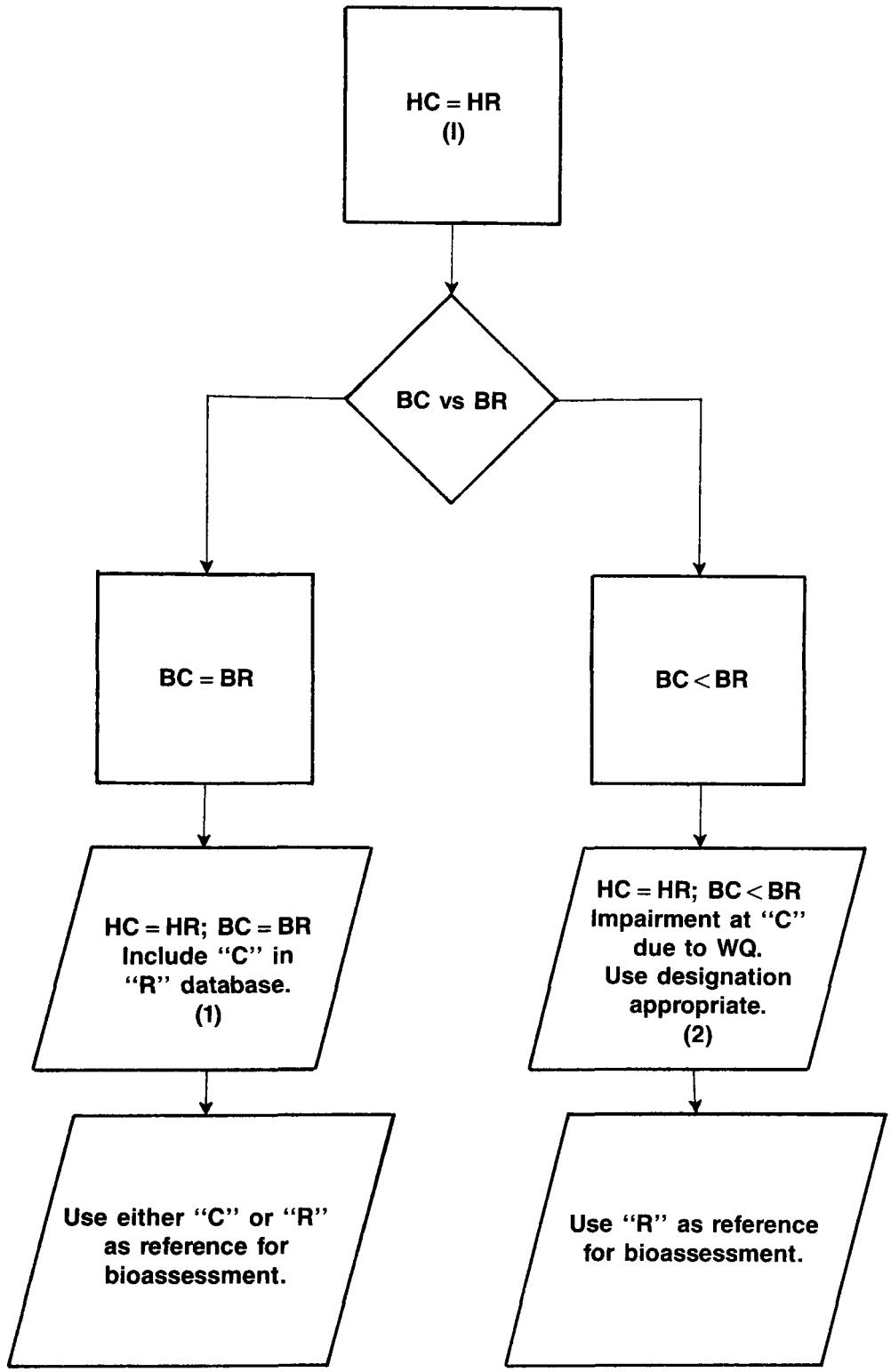


Figure 8.3-2. Evaluation of water quality effects. (Numbers in parentheses refer to points of discussion in text.)

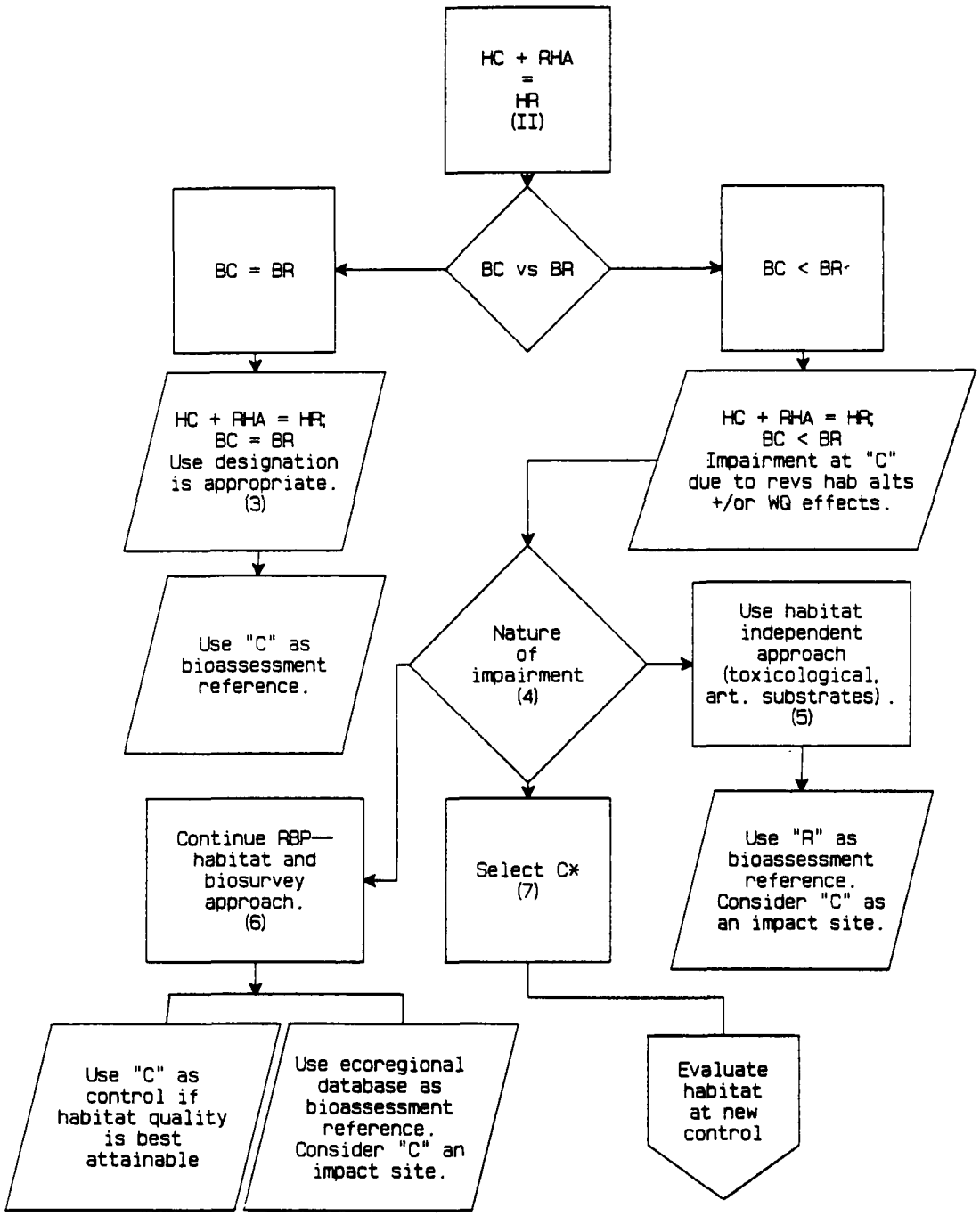


Figure 8.3-3. Evaluation of biological impairment due to reversible habitat alterations (RHAs). (Numbers in parentheses refer to points of discussion in text.)

Evaluation of an Alternative Site-Specific Control Station (Figure 8.3-4)

If the habitat quality at C is degraded relative to that at R, and reversible habitat alterations do not account for all of the habitat differences, then it is necessary to select an alternative site-specific control station (C*), if one is available (Figure 8.3-4).

(8) If a more appropriate control is located (where HC* = HR) then use C* as the reference and proceed with the bioassessment.

(9) If HC* is also degraded relative to HR, and it appears that a better quality site-specific control is not available, then biological condition should be evaluated at C* relative to R.

(10) Where reversible habitat alterations account for all differences in habitat quality between C* and R, then use C* as the reference and proceed with the bioassessment.

(11) In the unlikely situation where the degraded habitat (reversible and/or irreversible parameters) at C* is not limiting to biological condition, and BC* = BR, then either C* or R would be an appropriate reference.

(12) If biological impairment is detected at C*, the effects may be attributable to either degraded habitat (reversible and/or irreversible parameters) and/or water quality effects. Three possible alternatives should be considered: (a) A Use Attainability Analysis (UAA) is needed to determine the appropriate use classification of the system. In this case, the system as represented by C or C* is aberrant to R. A UAA will be needed to redefine R*, or a subset of R, for interpretation of an appropriate bioassessment. (b) C* would be used as a reference for bioassessment because it represents the best attainable condition for that system. However, interpretation of effects would be in the context of a control that does not meet the criteria of the region. (c) A prediction of the expected biological condition can be made from an extrapolation of the regression line formed from the reference database and the best potential habitat quality at C*.

Bioassessment Using a Site-Specific Control Station (Figure 8.3-5)

Once the decision is made to use a site-specific control (C or C*) then evaluation of the impact site relative to C (or C*) proceeds as in Figure 8.3-5. As indicated in the previous flowcharts, C is used when it is biologically representative of the region or is considered to represent the best attainable condition. A matrix of conclusions from the potential scenarios is presented in Table 8.3-1. The general bioassessment approaches are as follows:

(13) If HC = HI, then bioassessment for the purpose of detecting water quality effects at I (impact site) would proceed similarly to the evaluation of BC relative to BR (Figure 8.3-2).

(14) Where reversible habitat alterations account for all habitat differences between C and I, then bioassessment would proceed as in Figure 8.3-3.

(15) If habitat degradation is due to reversible and/or irreversible alterations, then bioassessment would proceed as in Figure 8.3-4.

Bioassessment Using a Regional Reference (Figure 8.3-6)

In situations where R is to be used as a reference, reference data could be obtained either from a single reference site or a regional database made up of numerous sites, and evaluation of the impact site would proceed as in Figure 8.3-6. As data are accumulated and processed, regional databases will provide refinement to the criteria and enhance bioassessments. A matrix of conclusions that would result from the possible scenarios is presented in Table 8.3-1.

(16) If HR = HI, then the approach in assessing potential water quality effects at I would be similar to that followed in evaluating BC relative to BR (Figure 8.3-2).

(17) Where reversible habitat alterations account for all habitat differences between I and R, then bioassessment would proceed as in Figure 8.3-3.

(18) Where habitat degradation may be caused by reversible and/or irreversible alterations, then bioassessment would proceed according to Figure 8.3-4.

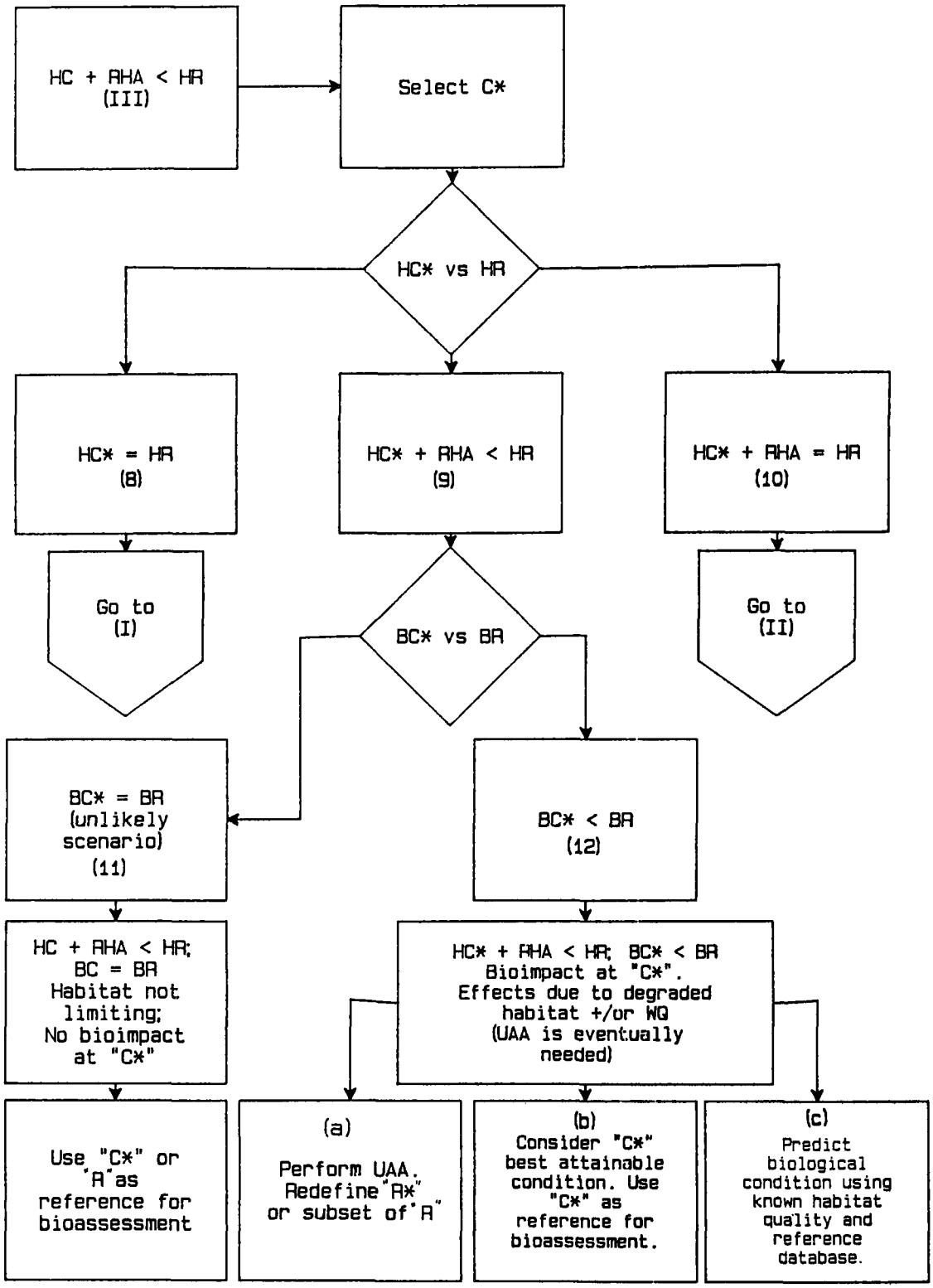


Figure 8.3-4. Evaluation of an alternative site-specific control station (C*). (Numbers in parentheses refer to points of discussion in text.)

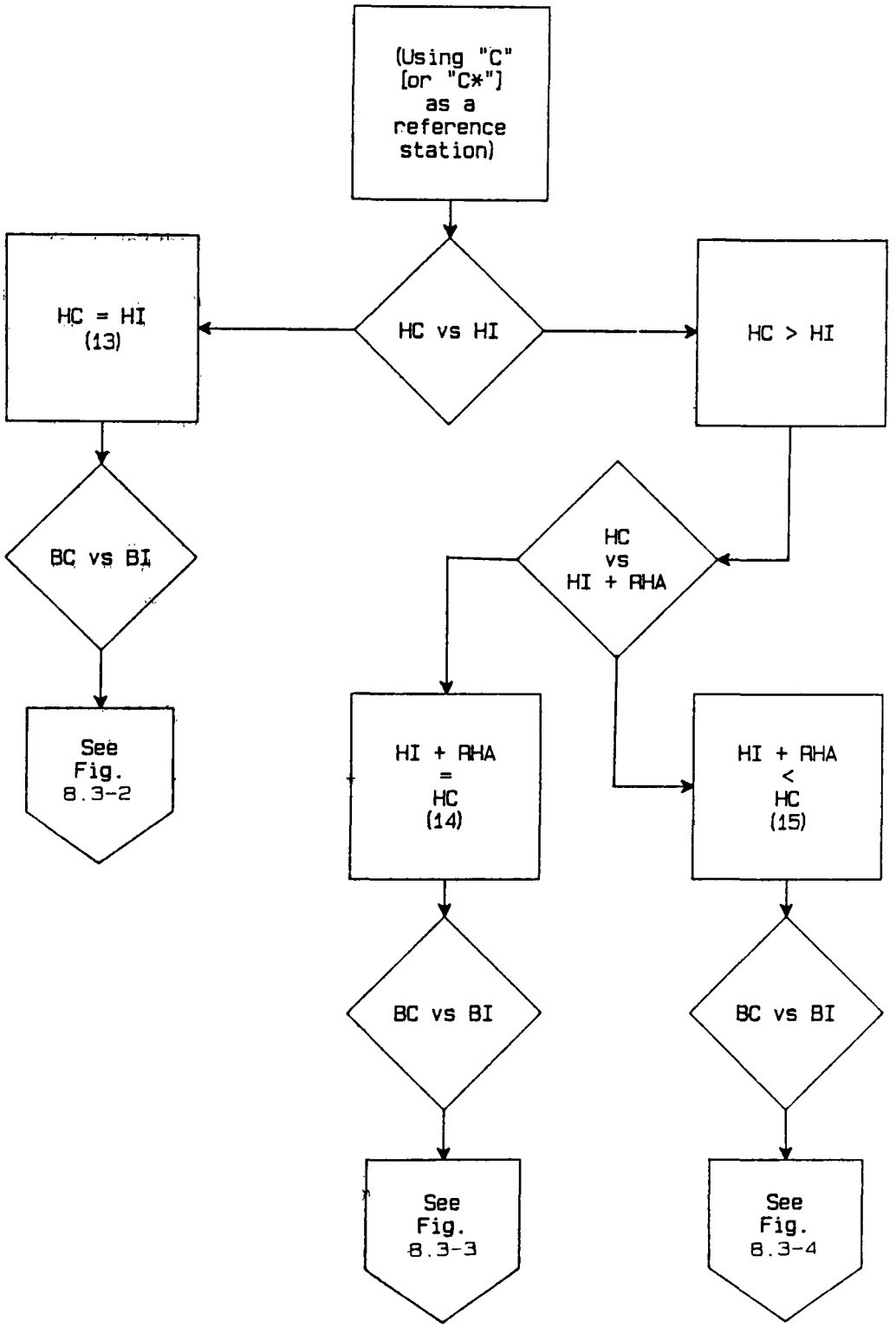


Figure 8.3-5. Bioassessment using a site-specific control station. (Numbers in parentheses refer to points of discussion in text.)

TABLE 8.3-1 BIOASSESSMENT CONCLUSIONS RELATIVE TO USE OF A SITE-SPECIFIC CONTROL OR REGIONAL REFERENCE

		HC = HR		HC + RHA = HR		HC + RHA < HR	
		BC = BR	BC < BR	BC = BR	BC < BR(a)	BC = BR	BC < BR(a)
HC = HI	BC = BI	No bioimpairment at C or I; I is a candidate for inclusion in the R database.		No bioimpairment at C or I; use designation appropriate. I is a candidate for inclusion in R database. Reversible habitat alterations are present but not limiting		Unlikely scenario	Bioimpairment at C and I due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b)
	BC > BI	Bioimpairment at I due to WQ effects.		Bioimpairment at I due to WQ effects(b) and maybe to reversible habitat alterations.(c)		Unlikely scenario	Both C and I impaired relative to R due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b) Additional bioimpairment at I due to WQ.
HI + RHA = HC	BC = BI	No bioimpairment at I; reversible habitat alterations are present but not limiting.		Unlikely scenario		Unlikely scenario	Bioimpairment at C and I due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b)
	BC > BI	Bioimpairment at I due to degraded habitat (reversible parameters) and/or WQ.(b)		Bioimpairment at I due to degraded habitat (reversible parameters) and/or WQ effects.		Bioimpairment at I due to degraded habitat (reversible w/respect to C, and both reversible and irreversible parameters w/respect to R) and/or WQ effects.(b)	Both C and I impaired relative to R due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b) Additional bioimpairment at I due to degraded habitat (reversible parameters).

(a) Use attainability analysis eventually needed.
(b) To differentiate water quality effects from habitat effects, a fairly extensive database on water quality parameters is necessary. This information, if it exists, should be available for prior review. Thus, an agency would be aware of a potential water quality problem prior to biological assessment, which would aid in the determination of course and effects relative to habitat constraints or water quality problems.
(c) Unlikely, but may occur in response to organic enrichment.

TABLE 8.3-1 (Cont.)

		HC = HR		HC + RHA = HR		HC + RHA < HR	
		BC = BR	BC < BR	BC = BR	BC < BR(a)	BC = BR	BC < BR(a)
HI + RHA < HC	BC = BI	No bioimpairment at I, but reversible and/or irreversible habitat alterations present.(c)		Unlikely scenario		Unlikely scenario	Bioimpairment at I, and C due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b)
	BC < BI	Bioimpairment at I, due to degraded habitat (reversible and/or irreversible parameters)(b) and/or WQ effects.		Bioimpairment at I, due to degraded habitat (reversible parameters)(b) and/or WQ effects.		Bioimpairment at I, due to degrded habitat (reversible and/or irreversible parameters)(b) and/or WQ effects.	Both C and I impaired relative to R due to degraded habitat (reversible and/or irreversible parameters)(b) and/or WQ effects. Additional bioimpairment at I due to degraded habitat (reversible and/or irreversible habitat parameters)(b) and/or WQ.
HR = HI	BR = BI	Nc impairment at I. Include I in R database.	Biological impairment at C, but not I.		Unlikely scenario	Unlikely scenario	Unlikely scenario
	BR > BI	Bioimpairment at I due to WQ effects.	Both C and I impaired relative to R due to WQ effects.		C impaired relative to R due to degraded habitat (reversible parameters) and/or WQ effects. Bioimpairment at I due to WQ effects.	Bioimpairment at I due to WQ. Habitat not limiting at C.	C impaired relative to R due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b) Bioimpairment at I due to degraded habitat (reversible parameters) and/or WQ.

TABLE 8.3-1 (Cont.)

		HC = HR		HC + RHA = HR		HC + RHA < HR	
		BC = BR	BC < BR	BC = BR	BC < BR(a)	BC = BR	BC < BR(a)
HI + RHA = HR	BR = BI	No bioimpairment at I; reversible habitat alterations present, but not limiting.	Bioimpairment at I due to reversible habitat parameters and/or WQ effects.(b) Bioimpairment at C due to WQ.		Unlikely scenario	Unlikely scenario	Unlikely scenario
	BR > BI	Bioimpairment at I due to reversible habitat alterations and/or WQ effects.(c)	C impaired relative to R due to WQ effects. Bioimpairment at I due to degraded habitat (reversible parameters) and/or WQ.(b)		Bioimpairment at C and I relative to R due to reversible habitat alterations and/or WQ.	Bioimpairment at I due to reversible habitat alterations and/or WQ. Habitat not limiting at C.	Bioimpairment at C and I relative to R due to degraded habitat (reversible and irreversible for C, reversible for I) and/or WQ.
HI + RHA < HR	BR = BI	No bioimpairment at I, but reversible and/or irreversible habitat alterations are present.(c)	Bioimpairment at I due to degraded habitat (reversible and/or irreversible parameters) and/or WQ.(b) Bioimpairment at C due to WQ.		Unlikely scenario	Unlikely scenario	Unlikely scenario
	BR < BI	Bioimpairment at I due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b)	C impaired relative to R due to WQ effects. Bioimpairment at I due to degraded habitat (reversible and/or irreversible parameters) and/or WQ.(b)		Bioimpairment at C relative to R due to reversible habitat alterations and/or WQ. Bioimpairment at I due to degraded habitat (reversible and/or irreversible parameters) and/or WQ effects.(b)	Bioimpairment at I due to degraded habitat and/or WQ. Habitat at C not limiting.	Bioimpairment at C and I relative to R due to degraded habitat (irreversible and reversible parameters) and/or WQ.

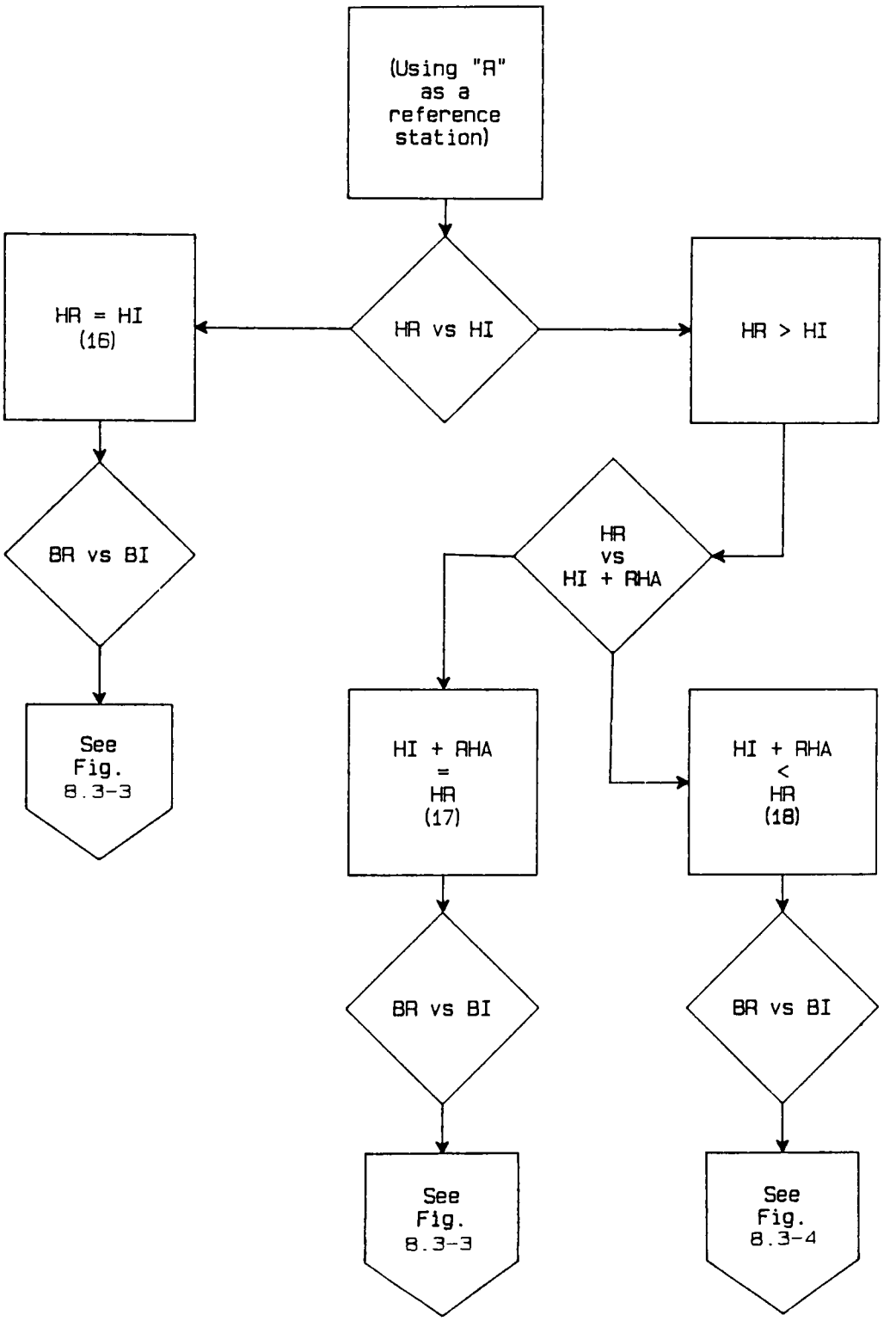


Figure 8.3-6. Bioassessment using a regional reference. (Numbers in parentheses refer to points of discussion in text.)

8.4 CASE STUDY

Using the data from the North Carolina DEM pilot study (discussed in Section 6.4), an integrated assessment can be performed using the decision matrix approach described in Section 8.3. This case study is presented in a step-by-step fashion to illustrate the concepts of the decision matrix. Only data from Stations 3 and 4 are compared to Stations 1 (site-specific control) and R (regional reference).

1. The habitat quality of the site-specific control is first compared to that of the regional reference.

   HC = HR ← HC VS HR → HC < HR

   Results: HC is 81% of HR → (HC = HR); HC = Supporting category (see Table 8.4-1).

2. Compare the biological communities between the site-specific control and the reference.

   BC = BR ← BC VS BR → BC < BR

   Results: BC = 32, BR = 42; BC is slightly impaired (see Table 8.4-2); therefore, BC < BR.

   - Results of the comparison are: HC = HR; BC < BR
   - Bioimpairment is present at C. Effects are due to water quality
   - Station "R" will be used as the reference station for bioassessment because of impairment at C.

3. Now that the reference station has been selected, a comparison of the habitat between the reference and impact station is made.

   HI = HR ← HI VS HR → HI < HR

   Results: For Station 3, HI is 50% of HR → (HI < HR); HI = Nonsupporting category.

   For Station 4, HI is 86% of HR → (HI = HR); HI = Supporting category.

4. For Station 3, Reversible Habitat Alterations (RHA) are considered. The RHA pertinent to Station 3 would be embeddedness (primary parameter) and the tertiary parameters of bank stability, bank vegetation, and streamside cover. The decision as to which parameters might be reversible is subjective and may vary on a case-by-case basis. Generally, the tertiary parameters, if degraded, would potentially be reversible.

   HI + RHA = HR ← HI + RHA VS HR → HI + RHA < HR

   Results: For Station 3, HI + RHA is 65% of HR → (HI + RHA < HR); HI = Partially supporting category.

   Note: Because the habitat at Station 4 is equal to the habitat at the reference, reversible habitat alterations do not need to be considered.

5. Compare the biological condition between the reference and the impact station.

   BI = BR ← BI VS BR → BI < BR

   Results: For Station 3: BI = 2, BR = 42; therefore, BI = Severely impaired → BI < BR.

   For Station 4: BI = 22, BR = 42; therefore, BI = Moderately impaired → BI < BR.

6. Conclusions for Station 3:

   HI + RHA < HR; BI < BR

   Biological impairment is present at Station 3 and is due to degraded habitat (irreversible and reversible parameters) and water quality. Thus, a water quality problem clearly exists, but degraded habitat is also a factor.

7. Conclusions for Station 4:

   HI = HR; BI < HR

   Biological impairment is present at Station 4 in addition to that noted at C. This additional bioimpairment at Station 4 is due to water quality.

Evaluation of habitat quality allows for some variability regarding control conditions. The habitat of the site-specific control was not exactly comparable to the regional reference, but was regarded as supporting. However, the biological condition of the site-specific control was classified as slightly impaired relative to the reference. This probably indicates a nonpoint-source water quality problem. Because of this bioimpairment noted at C, it is best to use the regional reference for bioassessment.

Judgment of bioimpairment in this case study was done in the strictest sense, where specific comparability to the reference conditions needed to be attained at the site of comparison. Conditions at Station 4 indicated that habitat quality was supporting relative to the reference, and biological conditions were moderately impaired. Therefore, bioimpairment at Station 4 is not as severe as that noted at Station 3.

TABLE 8.4-1 SUMMARY OF HABITAT ASSESSMENT SCORING FOR ARARAT AND MITCHELL RIVERS BENTHIC CASE STUDY DATA

Habitat Category/Parameter	Stations C(1)	3	4	R(6)
Primary--Substrate and Instream Cover				
1. Bottom substrate and available cover	14	8	18	18
2. Embeddedness[a]	18	6 (18)	10	18
3. Flow/velocity	16	9	18	19
Secondary--Channel Morphology				
4. Channel alteration	7	2	11	13
5. Bottom scouring and deposition	10	4	13	13
6. Pool/riffle, run/bend ratio	11	10	11	14
Tertiary--Riparian and Bank Structure				
7. Bank stability[a]	6	7 (10)	9	10
8. Bank vegetation[a]	9	9 (10)	10	10
9. Streamside cover[a]	10	8 (10)	8	10
Subtotal for tertiary parameters	25	24 (30)[b]	27	30
Score =	101	63 (81)	108	125
Proportion (%) of ecoregional reference	81	50 (65)	86	100
Classification =	S	P	S	E

Criteria:

≥ 90% excellent (comparable to reference)
75-89 supporting
60-74 partially supporting
≤ 59 nonsupporting

(a) Reversible habitat alteration (RHA) parameters.
(b) Parentheses indicate adjustment for RHAs pertinent to ecoregional reference.

TABLE 8.4-2 SUMMARY OF METRIC VALUES, PERCENT COMPARISON, AND BIOASSESSMENT SCORES FOR ARARAT AND MITCHELL RIVERS BENTHIC CASE STUDY DATA

	100-Organism Subsample											
	Metric Value Station				% Comparison to Reference Station				Bioassessment Score Station			
Metrics	C(1)	3	4	R(6)	C(1)	3	4	R(6)	C(1)	3	4	R(6)
Taxa richness	26	11	34	34	76	32	100	100	4	0	6	6
HBI	4.46	9.34	6.24	3.93	88[a]	42[a]	63[a]	100[a]	6	0	2	6
Scrapers/Filt. Collect.	0.833	0.000	0.108	1.500	56	0	7	100	6	0	0	6
EPT/Chiron. Abundance	2.45	0.00	0.55	9.28	26	0	6	100	2	0	0	6
% Contrib. Dom. Taxon	11.2	53.5	16.5	14.2	--[b]	--[b]	--[b]	--[b]	6	0	6	6
EPT Index	12	0	12	14	86	0	86	100	4	0	4	6
Community Loss Index	0.64	2.31	0.62	0.00	--[c]	--[c]	--[c]	--[c]	4	2	4	6
Total score									32	2	22	42
Biological condition									Slight	Sev.	Mod.	Non

(a) HBI comparison is ratio of reference to station evaluated.
(b) Actual percent contribution is evaluated, not percent comparability.
(c) Range of values is evaluated, not percent comparability.

It is apparent from a general comparison of habitat quality and biological condition at this North Carolina site that there is a close relationship between habitat and biological condition (Figure 8.4-1). As habitat quality declines, so does the value of the benthic index (based on the RBP approach). If these data from the pilot study are plotted against the theoretical curve depicting the relationship between habitat and biological condition, the deviation from the predicted relationship for each station can be discerned (Figure 8.4-2). The development of a substantial reference database would allow for the development of an empirical line with statistical confidence intervals around the line. From this information, predictions of water quality effects beyond the habitat constraints are possible. In this manner, cause of the degradation of biological condition at Stations 3 and 4 could be refined.

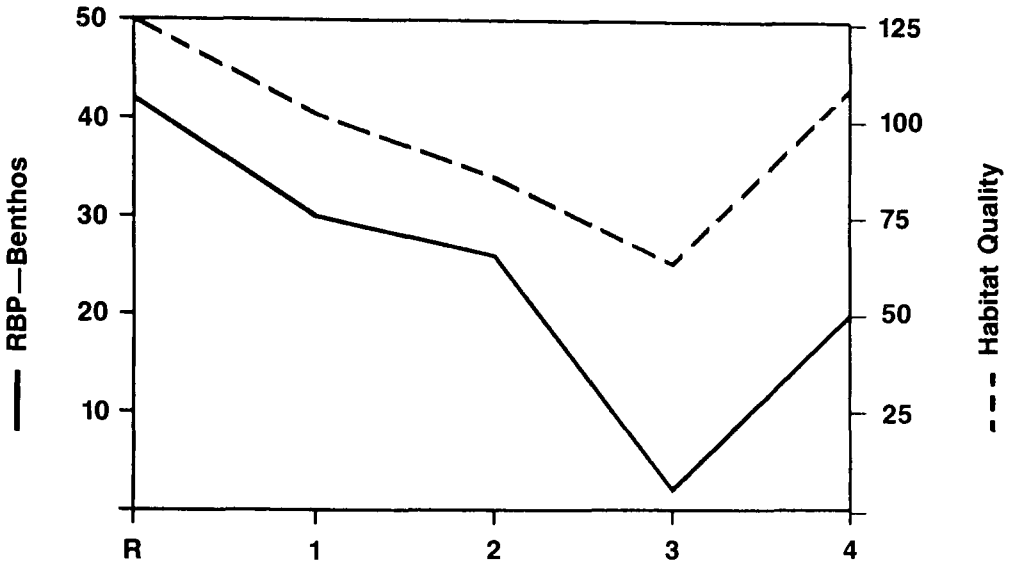


Figure 8.4-1. The relationship between habitat quality and benthic community condition at the North Carolina pilot study site.

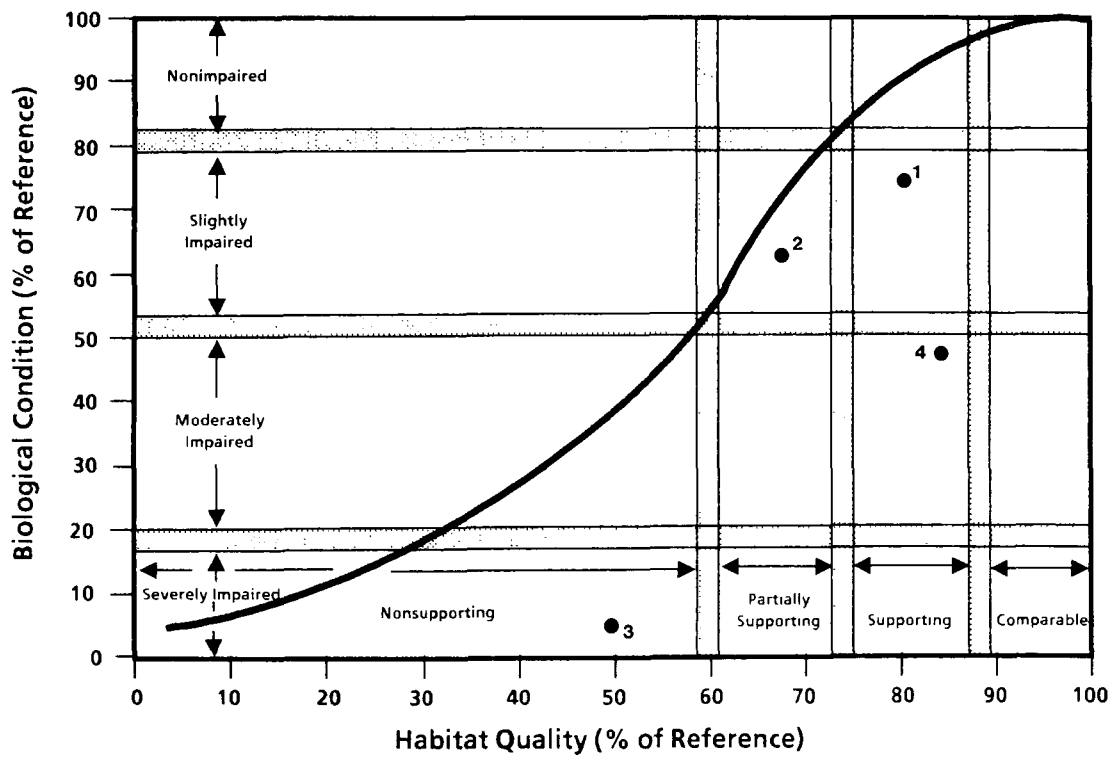


Figure 8.4-2. Pilot study results applied to the theoretical habitat vs. biological condition curve.

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APPENDIX A

GUIDANCE FOR USE OF FIELD AND LABORATORY DATA SHEETS

APPENDIX A
GUIDANCE FOR USE OF FIELD AND LABORATORY DATA SHEETS

This appendix provides guidance for use of the rapid bioassessment field and laboratory data sheets. The guidance sheets give brief descriptions of the information required for each data sheet.

A.1 GUIDANCE FOR HEADER INFORMATION (Figure 2.6-1)

Waterbody Name:
Name of river, stream, or drain.

Location:
Township, range, section, county where problem area is located. For rivers, streams, or drains, road crossings or outfall locations should be referenced where applicable.

Reach/Milepoint:
Indicate station reach/milepoint.

Latitude/Longitude:
Indicate station latitude/longitude.

County/State:
Name of county and state where station is located.

Aquatic Ecoregion:
Name of ecoregion.

Station:
Agency name or number for station.

Investigators:
List field personnel involved.

Date:
Date of survey.

Agency:
Agency name or affiliation (academic, private consulting)

Hydrologic Unit Code:
Indicate the USGS cataloging unit number in which the station is located.

Form Completed By:
List personnel completing form.

Reason for Survey:
Reason survey was conducted.

A.2 GUIDANCE FOR BIOSURVEY FIELD DATA SHEET FOR BENTHIC RBPs I, II, AND III (Figures 6.1-1, 6.2-1, and 6.3-1)

Rapid Bioassessment Protocol I (Figure 6.1-1)

Estimated Abundance Level of Aquatic Biota: Record estimated abundance level of biota found in the sampling area. Circle the number (corresponding to the descriptions just below on the data sheet) that best indicates the estimated size of each population found in the sampling area. Each agency should develop its own abundance level criteria.

Macrobenthos Qualitative Sample: Using the guidelines of rare, common, abundant, or dominant, record the estimated abundance level of each major taxa found in the sampling area. Each agency should develop its own abundance level criteria.

Observations: Information included here would include abundance of fish nesting sites; notes concerning biota present; type of game fish observed; location or presence of noteworthy physical structures such as bridges, rip-rap, culverts; habitat alteration due to construction activities; or any other observations pertinent to an impact assessment.

Rapid Bioassessment Protocol II (Figure 6.2-1)

Estimated Abundance Level of Aquatic Biota: Record estimated abundance level of biota found in the sampling area. Circle the number (corresponding to the descriptions just below on the data sheet) that best indicates the estimated size of each population found in the sampling area. Each agency should develop its own abundance level criteria.

Macrobenthos Qualitative Sample List: List families found in 100-organism subsample and the number of individuals within each family.

Riffle Sample Functional Feeding Groups: Record the number of individuals collected in the 100-organism riffle subsample which represent the Scraper and Filtering Collector Functional Feeding Groups.

CPOM Sample: Record the number of individuals collected in the supplemental CPOM sample which represent the Shredder Functional Feeding Group, and total number of individuals sorted.

Observations: Information included here would include abundance of fish nesting sites; notes concerning biota present; type of game fish observed; location or presence of noteworthy physical structures such as bridges, rip-rap, culverts; habitat alteration due to construction activities; or any other observations pertinent to an impact assessment.

Rapid Bioassessment Protocol III (Figure 6.3-1)

Estimated Abundance Level of Aquatic Biota: Record estimated abundance level of biota found in the sampling area. Circle the number (corresponding to the descriptions just below on the data sheet) that best indicates the estimated size of each population found in the sampling area. Each agency should develop its own abundance level criteria.

Macrobenthos Qualitative Sample: Using the guidelines of rare, common, abundant, or dominant, record the estimated abundance level of each major taxa found in the sampling area. Each agency should develop their own abundance level criteria.

CPOM Sample: Record the number of individuals collected in the supplemental CPOM sample which represents the Shredder Functional Feeding Group, and total number of individuals sorted.

Observations: Information recorded here would include abundance of fish nesting sites; notes concerning biota present; type of game fish observed; location or presence of noteworthy physical structures such as bridges, rip-rap, culverts; habitat alteration due to construction activities; or any other observations pertinent to an impact assessment.

A.3 GUIDANCE FOR IMPAIRMENT ASSESSMENT SHEET FOR RBPs I, II, III, AND V (Figures 6.1-2 and 7.2-1)

Rapid Bioassessment Protocols I, II, III, and V

1. **Detection of Impairment:** Circle the one that applies.
2. **Biological Impairment Indicator:** Circle those that apply, as indicated by the benthos, fish, and other aquatic biota.
3. **Brief Description of Problem:** Briefly explain the biological nature of the problem, based on field investigation and sampling. List the year and date of previous biological data and reports, and where the information can be found (state file, BIOS).
4. **Cause:** Circle those that apply. Indicate which is the major cause of the stream problem.
5. **Estimated Areal Extent of Problem:** Record estimated downstream extent of impact (in m) and multiply by approximate stream width (in m) to estimate areal width.
6. **Suspected Source(s) of Problem:** Check those that are suspected. Briefly explain why you suspect a specific source, and reference other surveys or studies done to document the problem and its source. Give title of applicable report, author(s), and year published or completed. Use back of sheet if necessary.

A.4 GUIDANCE FOR DATA SUMMARY SHEET FOR BENTHIC RBPs II AND III (Figure 6.2-2 and 6.3-3)

Rapid Bioassessment Protocol II (Figure 6.2-2)

Station Number: Indicate station number for each data set recorded.

Station Location: Record brief description of sampling site relative to established landmarks (i.e., roads, bridges).

Taxa Richness: Record total number of families (or higher taxa) collected in the 100-organism riffle subsample.

FBI (modified): Record the Family Biotic Index value (Hilsenhoff 1988) calculated for the 100-organism riffle subsample using the formula presented in RBP II. Tolerance classification values can be entered into the computer database to simplify calculation.

Functional Feeding Group: Functional Feeding Group classifications may be entered into the computer database to simplify calculations.

Riffle Community; Scrapers/Filtering Collectors: Enter the value obtained by dividing the number of individuals in the riffle subsample representing the Scraper Functional Group, by the number representing the Filtering Collector Functional Group.

CPOM Community; Shredders/Total: Enter the value obtained by dividing the number of individuals in the CPOM sample (or subsample) representing the Shredder Functional Group, by the total number of organisms in the sample (or subsample).

EPT/Chironomidae: Enter the value obtained by dividing the number of individuals in the 100-organism riffle subsample in the family Chironomidae, by the total number of individuals in the orders Ephemeroptera, Plecoptera, and Trichoptera.

Percent Contribution (Dominant Family): Record the value obtained by dividing the number of individuals in the family that is most abundant in the 100-organism riffle subsample, by the total number of individuals in the sample.

EPT Index: Record the total number of taxa in the 100-organism riffle subsample representing the orders Ephemeroptera, Plecoptera, and Trichoptera.

Community Similarity Index: Enter the value calculated for the appropriate community similarity index, using data from the 100-organism riffle subsample.

Values obtained for each metric should be assigned a score based on percent comparability to the control or reference station data. Scores are summed for both the impaired and reference station. The percent comparison between the total scores provides the final evaluation of biological condition.

Rapid Bioassessment Protocol III (Figure 6.3-3)

Station Number: Indicate station number for each data set recorded.

Station Location: Record brief description of sampling site relative to established landmarks (i.e., roads, bridges).

Species Richness: Record total number of species (or higher taxa) collected in the 100-organism riffle subsample.

HBI (modified): Record the species level Hilsenhoff Biotic Index value (Hilsenhoff 1987b) calculated for the 100-organism riffle subsample using the formula presented in Rapid Bioassessment Protocol III. Tolerance classification values can be entered into the computer database to simplify calculation.

Functional Feeding Group: Functional Feeding Group classifications may be entered into the computer database to simplify calculations.

Riffle Community; Scrapers/Filtering Collectors: Enter the value obtained by dividing the number of individuals in the riffle subsample representing the Scraper Functional Group, by the number representing the Filtering Collector Functional Group.

CPOM Community; Shredders/Total: Enter the value obtained by dividing the number of individuals in the CPOM sample (or subsample) representing the Shredder Functional Group, by the total number of organisms in the sample (or subsample).

EPT/Chironomidae: Enter the value obtained by dividing the total number of individuals in the 100-organism riffle subsample in the orders Ephemeroptera, Plecoptera, and Trichoptera, by the number of individuals in the family Chironomidae.

Percent Contribution (Dominant Taxon): Record the value obtained by dividing the number of individuals in the taxon that is most abundant in the 100-organism riffle subsample, by the total number of individuals in the sample.

EPT Index: Record the total number of taxa in the 100-organism riffle subsample representing the orders Ephemeroptera, Plecoptera, and Trichoptera.

Community Similarity Index: Enter the value calculated for the appropriate community similarity index, using data from the 100-organism riffle subsample.

Values obtained for each metric should be assigned a score based on percent comparability to the control or reference station data. Scores are summed for both the impacted and reference station. The percent comparison between the total scores provides the final evaluation of biological condition.

Note: To maximize time efficiency, the metric values entered on the Data Summary Sheets are intended to be generated by use of a computerized database.

A.5 GUIDANCE FOR LABORATORY BENCH SHEET FOR BENTHIC RBP III (Figure 6.3-2)

Station Number: Indicate agency assigned number for each sample dataset recorded.

Station Location: Record brief description of sampling site relative to established landmarks (i.e., roads, bridges).

Species: List species identified from the 100-organism riffle subsample.

Number of Organisms: Indicate number of individuals of each species collected in the 100-organism riffle subsample at each station.

Total Organisms: Record total number of individuals collected in the 100-organism riffle subsample at each station.

Number of Taxa: Record the total number of taxa collected in the 100-organism riffle subsample at each station.

A.6 GUIDANCE FOR FIELD COLLECTION DATA SHEET FOR FISH RBP V (Figure 7.2-3)

Drainage: Give name of stream or river and its basin, site descriptor, and unique site code.

Date: Enter day, month, and year of collection.

Sampling Duration: Record length of time in minutes actually collecting fish. If replicates are taken, record them separately.

Sampling Distance: Measure, with a tape or calibrated range finder, the length in meters of reach sampled.

Sampling Area: Multiply the length or reach sampled by the average width sampled. Express in meters squared.

Crew: Indicate crew chief and crew members.

Habitat Complexity/Quality: Circle the descriptor that best describes subjective evaluation of the physicochemical habitat.

Weather: Record air temperature, estimated wind velocity, percent cloud cover, and precipitation.

Flow: Circle most appropriate descriptor.

Gear Used: Specify type, model, and number of electrofisher, mesh size and length of seine, or concentration of fish toxicant.

Gear/Crew Performance: Indicate effectiveness of crew in sampling the site. Note problems with equipment, staff, or site obstacles, such as extensive cover, high velocity current, excessive turbidity, floating debris, deep muck or pools, or weather conditions.

Comments: Record any additional qualitative site data: sketch map or photographs, presence of springs, evidence of fishing activity, any potential or current impacts, weather conditions (such as evidence of recent high flows or unusually hot or cold weather immediately preceding the survey), biota observed (insect hatches, potential vertebrate predators, fish nesting and grazing sites, fish reproductive condition, fish seen but not captured).

Fish (preserved): Indicate if specimens were preserved for permanent collection or further examination.

Number of Individuals; Number of Anomalies: Give total numbers of fish and anomalies for the sample.

Genus/Species: Enter scientific name or unique standard abbreviation for each species captured.

Adults (Number, Weight): Enter the number of adults of each species and their total weight in grams. Weigh individually or by batch, depending on the species' size and abundance. Species weight can also be determined by weighing a subsample of individuals (20-30 fish spanning the size range collected) and extrapolating for the total number of that species.

Juveniles (Number, Weight): Record the number of juveniles of each species and their total weight as above. Juveniles and adults are distinguished subjectively by coloration and size; the objective is to determine whether both age classes are present.

Anomalies (Number): Indicate the number of fish by individual or species, that are diseased, deformed, damaged, or heavily parasitized. These are determined through careful external examination by a field-trained fish biologist.

A.7 GUIDANCE FOR DATA SUMMARY SHEET FOR FISH RBP V (Figure 7.2-5)

Station Number: Indicate station number.

Station Location: Record brief description of sampling site relative to established landmarks (i.e., roads, bridges).

Metrics: List metrics used to conduct IBI calculations. Use Karr's original metrics or published (or well supported) substitutes. Precede metric selection with analysis of reference site data or a high quality historical database from a representative, large river basin.

Scoring Criteria: List published scoring criteria or use substitutes where necessary. Analyze reference site data or historical data from a representative large river basin before selecting criteria.

Metric Value: Record metric values (number or percent) for the station. Metric values are obtained by comparing the collection data (Figure 7.2-3) with the tolerance and trophic guilds previously listed (e.g., Appendix D). For taxonomic metrics numbers of species are added. Total number of individuals is recorded from the field collection data sheet. Proportional metrics are determined by adding the number of individuals in each category and then dividing by the total number of individuals.

Metric Score: Score each metric by comparing the metric value for the station with the previously chosen scoring criteria. Marginal values can be given a plus or minus (see IBI score below).

Scorer: Enter scorer's name.

IBI Score: The metric scores (and pluses and minuses if used) are added to give the IBI score. Three pluses or three minuses may increase or decrease the IBI score by two points.

Comments: Metrics producing contrary results or suggestions for improvement are entered here.

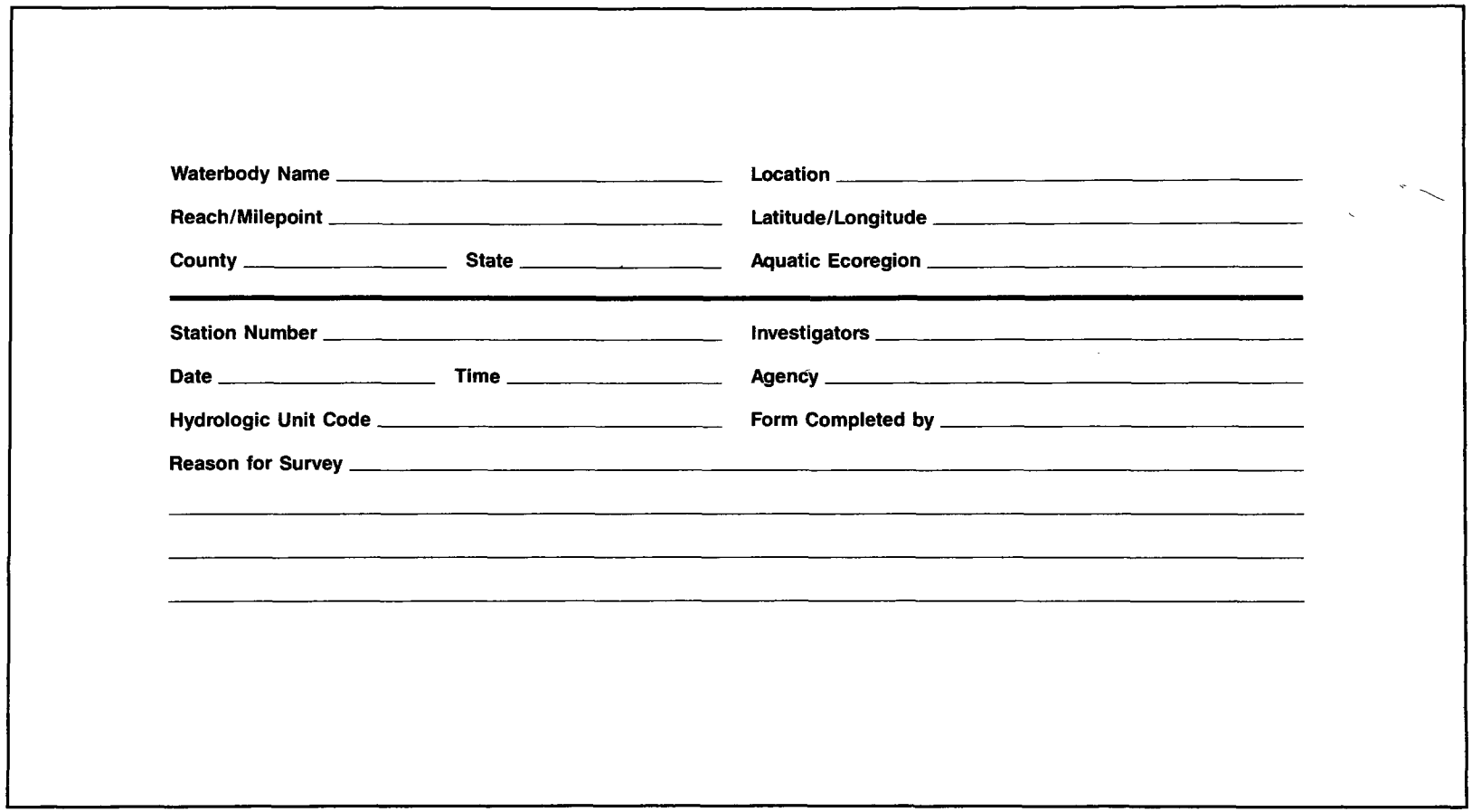

Figure 2.6-1. Header information used for documentation and identification for sampling stations.

Rapid Bioassessment Protocol I

Biosurvey Field Data Sheet

RELATIVE ABUNDANCE OF AQUATIC BIOTA

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

0 = Absent/Not Observed 1 = Rare 2 = Common 3 = Abundant 4 = Dominant

MACROBENTHOS QUALITATIVE SAMPLE LIST (Indicate Relative Abundance R = Rare, C = Common, A = Abundant, D = Dominant)

Porifera	Anisoptera	Chironomidae
Hydrozoa	Zygoptera	Plecoptera
Platyhelminthes	Hemiptera	Ephemeroptera
Turbellaria	Coleoptera	Trichoptera
Hirudinea	Lepidoptera	Other
Oligochaeta	Sialidae	
Isopoda	Corydalidae	
Amphipoda	Tipulidae	
Decapoda	Empididae	
Gastropoda	Simuliidae	
Bivalvia	Tabanidae	
	Culicidae	

Rare < 3 Common 3–9 Abundant > 10 Dominant > 50 (Estimate)

Observations

Figure 6.1-1. Biosurvey Field Data Sheet for use with Rapid Bioassessment Protocol I.

Rapid Bioassessment Protocol II

Biosurvey Field Data Sheet

RELATIVE ABUNDANCE OF AQUATIC BIOTA

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

0 = Absent/Not Observed 1 = Rare 2 = Common 3 = Abundant 4 = Dominant

MACROBENTHOS QUALITATIVE SAMPLE LIST		List Families Present/Indicate Abundance
Oligochaeta		
	Anisoptera	
Gastropoda		Coleoptera
	Zygoptera	
Bivalvia		Diptera
	Plecoptera	
Ephemeroptera		
	Trichoptera	

Other

RIFFLE SAMPLE
FUNCTIONAL FEEDING GROUPS (Indicate No. of Individuals Representing Group)

Scrapers	Filtering Collectors

CPOM SAMPLE FUNCTIONAL FEEDING GROUPS (Indicate No. of Individuals Representing Group)

Shredders	Total Org. in Sample

Observations

Figure 6.2-1. Biosurvey Field Data Sheet for use with Rapid Bioassessment Protocol II.

Rapid Bioassessment Protocol III

Biosurvey Field Data Sheet

RELATIVE ABUNDANCE OF AQUATIC BIOTA

Periphyton	0	1	2	3	4	Slimes	0	1	2	3	4
Filamentous Algae	0	1	2	3	4	Macroinvertebrates	0	1	2	3	4
Macrophytes	0	1	2	3	4	Fish	0	1	2	3	4

0 = Absent/Not Observed 1 = Rare 2 = Common 3 = Abundant 4 = Dominant

MACROBENTHOS QUALITATIVE SAMPLE LIST (Indicate Relative Abundance R = Rare, C = Common, A = Abundant, D = Dominant)

Porifera	Anisoptera	Chironomidae
Hydrozoa	Zygoptera	Plecoptera
Platyhelminthes	Hemiptera	Ephemeroptera
Turbellaria	Coleoptera	Trichoptera
Hirudinea	Lepidoptera	Other
Oligochaeta	Sialidae	
Isopoda	Corydalidae	
Amphipoda	Tipulidae	
Decapoda	Empididae	
Gastropoda	Simuliidae	
Bivalvia	Tabanidae	
	Culicidae	

Rare < 3 Common 3–9 Abundant > 10 Dominant > 50 (Estimate)

CPOM SAMPLE FUNCTIONAL FEEDING GROUPS (Indicate No. of Individuals Representing Group)

Shredders	Total Org. in Sample

Observations

Figure 6.3-1. Biosurvey Field Data Sheet for use with Rapid Bioassessment Protocol III.

IMPAIRMENT ASSESSMENT SHEET

1. Detection of impairment: Impairment detected (Complete items 2-6) No impairment detected (Stop here)

2. Biological impairment indicator:

Benthic macroinvertebrates	Other aquatic communities
____ absence of EPT taxa	____ Periphyton
____ dominance of tolerant groups	____ filamentous
____ low benthic abundance	____ other
____ low taxa richness	____ Macrophytes
____ other	____ Slimes
	____ Fish

3. Brief description of problem: _____

Year and date of previous surveys: _____

Survey data available in: _____

4. Cause: (indicate major cause) organic enrichment toxicants flow habitat limitations other _____

5. Estimated areal extent of problem (m^2) and length of stream reach affected (m), where applicable: _____

6. Suspected source(s) of problem:

____ point source discharge (name, type of facility, location)
____ construction site runoff
____ combined sewer outfall
____ silviculture runoff
____ animal feedlot
____ agricultural runoff
____ urban runoff
____ ground water
____ other
____ unknown

Briefly explain:

Figure 6.1-2. Impairment Assessment Sheet for use with macroinvertebrate Rapid Bioassessment Protocols.

IMPAIRMENT ASSESSMENT SHEET

1. Detection of impairment: Impairment detected (Complete Items 2-6) No impairment detected (Stop here)

2. Biological impairment indicator:

Fish	Other aquatic communities
___ sensitive species reduced/absent	___ Macroinvertebrates
___ dominance of tolerant species	___ Periphyton
___ skewed trophic structure	___ Macrophytes
___ abundance reduced/unusually high	
___ biomass reduced/unusually high	
___ hybrid or exotic abundance unusually high	
___ poor size class representation	
___ high incidence of anomalies	

3. Brief description of problem: _____

Year and date of previous surveys: _____

Survey data available in: _____

4. Cause (indicate major cause): organic enrichment toxicants flow

sediment temperature poor habitat

other _____

5. Estimated areal extent of problem (m^2) and length of stream reach affected (m) where applicable: _____

6. Suspected source(s) of problem

___ point source	___ mine
___ urban runoff	___ dam or diversion
___ agricultural runoff	___ channelization or snagging
___ silvicultural runoff	___ natural
___ livestock	___ other
___ landfill	___ unknown

Comments: _____

_____

Figure 7.2-1. Impairment Assessment Sheet for use with fish Rapid Bioassessment Protocol V.

DATA SUMMARY SHEET

Station No.								
Station Location								
Taxa Richness								
FBI (modified)								
Functional Feeding Groups								
Riffle Community								
Scrapers/Filt. Collect.								
CPOM Community								
Shredders/Total								
EPT/Chironomidae								
% Contribution (dom. family)								
EPT Index								
Community Similarity Index								
Comments:								

Figure 6.2-2. Data Summary Sheet suggested for use in recording benthic data utilized in Rapid Bioassessment Protocol II.

DATA SUMMARY SHEET

Station No.								
Station Location								
Taxa Richness								
FBI (modified)								
Functional Feeding Groups								
Riffle Community								
Scrapers/Filt. Collect.								
CPOM Community								
Shredders/Total								
EPT/Chironomidae								
% Contribution (dom. family)								
EPT Index								
Community Similarity Index								
Comments:								

Figure 6.3-3. Data Summary Sheet suggested for use in recording benthic data utilized in Rapid Bioassessment Protocol III.

LABORATORY BENCH SHEET

	Number of Organisms			
Station Number				
Station Location				
Species Name				
Total Organisms				
Number of Taxa				

Figure 6.3-2. Laboratory Bench Sheet suggested for use in recording benthic data utilized in Rapid Bioassessment Protocol III.

page___ of___

FISH FIELD COLLECTION DATA SHEET

Drainage _____ Date _____
Sampling Duration (min) _____
Sampling Distance (m) _____ Sampling Area (m^2) _____ Crew _____
Habitat Complexity/Quality (excellent good fair poor very poor)
Weather _____ Flow (flood bankfull moderate low)
Gear Used _____ Gear/Crew Performance _____
Comments _____
Fish (preserved) Number of Individuals ____ Number of Anomalies _____

Genus/Species	Adults		Juveniles		Anomalies(*)
	No.	Wt.	No.	Wt.	No.

(*) Discoloration, deformities, eroded fins, excessive mucus, excessive external parasites, fungus, poor condition, reddening, tumors, and ulcers

Figure 7.2-3. Fish Field Collection Data Sheet for use with Rapid Bioassessment Protocol V.

Station No. _____

Site _____

Metrics[a]	Scoring Criteria[b] 5 (%)	3 (%)	1 (%)	Metric Value	Metric Score
1. Number of Native Fish Species	>67	33-67	<33		
2. Number of Darter or Benthic Species	>67	33-67	<33		
3. Number of Sunfish or Pool Species	>67	33-67	<33		
4. Number of Sucker or Long-Lived Species	>67	33-67	<33		
5. Number of Intolerant Species	>67	33-67	<33		
6. % Green Sunfish or Tolerant Individuals	<10	10-25	>25		
7. % Omnivores	<20	20-45	>45		
8. % Insectivores or Invertivores	>45	20-45	<20		
9. % Top Carnivores	>5	1-5	<1		
10. Total Number of Individuals	>67	33-67	<33		
11. % Hybrids or Exotics	0	0-1	>1		
12. % Anomalies	<1	1-5	>5		

Scorer _____ IBI Score _____

Comments: _____

_____

_____

(a) Karr's original metrics or commonly used substitutes. See text and Table 7.2-1 for other possibilities.

(b) Karr's original scoring criteria or commonly used substitutes. These may require refinement in other ecoregions.

Figure 7.2-5. Data Summary Sheet for Rapid Bioassessment Protocol V.

APPENDIX B

RAPID BIOASSESSMENT SUBSAMPLING METHODS FOR BENTHIC PROTOCOLS II AND III (100-Organism Count Technique)

APPENDIX B
RAPID BIOASSESSMENT SUBSAMPLING METHODS FOR BENTHIC PROTOCOLS II AND III
(100-Organism Count Technique)

B.1 RAPID BIOASSESSMENT SUBSAMPLING METHODS FOR PROTOCOL II

1. Thoroughly rinse sample in a (500-micron) screen or the sampling net to remove fine sediments. Any large organic material (whole leaves, twigs, algal or macrophyte mats) should be rinsed, visually inspected, and discarded.

2. Place sample contents in a large, flat pan with a light-colored (preferably white) bottom. The bottom of the pan should be marked with a numbered grid pattern, each block in the grid measuring 5 cm × 5 cm. (Sorting using a gridded pan is only feasible if the organism movement in the sample can be slowed by the addition of club soda or tobacco to the sample. If the organisms are not anesthetized, 100 organisms should be removed from the pan as randomly as possible.) A 30 × 45 cm pan is generally adequate, although pan size ultimately depends on sample size. Larger pans allow debris to be spread more thinly, but they are unwieldy. Samples too large to be effectively sorted in a single pan may be thoroughly mixed in a container with some water, and half of the homogenized sample placed in each of two gridded pans. Each half of the sample must be composed of the same kinds and quantity of debris and an equal number of grids must be sorted from each pan, in order to ensure a representative subsample.

3. Add just enough water to allow complete dispersion of the sample within the pan; an excessive amount of water will allow sample material to shift within the grid during sorting. Distribute sample material evenly within the grid.

4. Use a random numbers table to select a number corresponding to a square within the gridded pan. Remove all organisms from within that square and proceed with the process of selecting squares and removing organisms until the total number sorted from the sample is within 10 percent of 100. Any organism which is lying over a line separating two squares is considered to be in the square containing its head. In those instances where it is not possible to determine the location of the head (worms for instances), the organism is considered to be in the square containing the largest portion of its body. Any square sorted must be sorted in its entirety, even after the 100 count has been reached. In order to lessen sampling bias, the investigator should attempt to pick smaller, cryptic organisms as well as the larger, more obvious organisms.

Source: Modified from Hilsenhoff 1987b.

B.2 RAPID BIOASSESSMENT SUBSAMPLING METHODS FOR PROTOCOL III

1. Thoroughly rinse sample in a No. 35 mesh (500-micron) screen to remove preservative. Any large organic material (whole leaves, twigs, algal or macrophyte mats) not removed in the field should be rinsed, visually inspected, and discarded. If the samples have been preserved in alcohol, it will be necessary to soak the sample contents in water for about 15 minutes to hydrate the benthic organisms, preventing them from floating on the water surface during sorting.

2. Place sample contents in a large, flat pan with a light-colored (preferably white) bottom. The bottom of the pan should be marked with a numbered grid pattern, each block in the grid measuring 5 cm × 5 cm. A 30 × 45 cm pan is generally adequate, although pan size ultimately depends on sample size. Larger pans allow debris to be spread more

thinly, but they are unwieldy. Samples too large to be effectively sorted in a single pan may be thoroughly mixed in a container with some water, and half of the homogenized sample placed in each of two gridded pans. Each half of the sample must be composed of the same kinds and quantity of debris and an equal number of grids must be sorted from each pan, in order to ensure a representative subsample.

3. Add just enough water to allow complete dispersion of the sample within the pan; an excessive amount of water will allow sample material to shift within the grid during sorting. Distribute sample material evenly within the grid.

4. Use a random numbers table to select a number corresponding to a square within the gridded pan. Remove all organisms from within that square and proceed with the process of selecting squares and removing organisms until the total number sorted from the sample is within 10 percent of 100. Any organism which is lying over a line separating two squares is considered to be in the square containing its head. In those instances where it is not possible to determine the location of the head (worms for instances), the organism is considered to be in the square containing the largest portion of its body. Any square sorted must be sorted in its entirety, even after the 100 count has been reached. If many of the organisms are very small and it appears that the potential for missing individuals is great, an illuminated 5X magnifier will facilitate the sorting procedure.

Source: Modified from Hilsenhoff 1987b.

APPENDIX C

FAMILY AND SPECIES-LEVEL MACROINVERTEBRATE TOLERANCE CLASSIFICATIONS

APPENDIX C
FAMILY AND SPECIES-LEVEL MACROINVERTEBRATE TOLERANCE CLASSIFICATION

C.1 FAMILY-LEVEL TOLERANCE CLASSIFICATION

RBP II is based on family-level identifications. The adequate assessment of biological condition for RBP II requires the use of a tolerance classification for differentiating among responses of the benthic community to pollutants. Hilsenhoff's Family Biotic Index (FBI) is used as a basis for the family-level tolerance classification presented in this document.

A brief description of the FBI is taken from Hilsenhoff's paper entitled "Rapid Field Assessment of Organic Pollution with a Family Biotic Index" (Hilsenhoff 1988). The family-level tolerance values assigned for western Great Lakes region stream arthropods are presented in Table C-1.

A special symposium on rapid biological assessment at the 1986 meeting of the North American Benthological Society stressed the need for rapid field-based assessment approaches. It was recognized that in order to save time, a degree of accuracy would be sacrificed. Consequently, I adapted the biotic index (BI) of organic pollution (Hilsenhoff 1987b) for rapid evaluation by providing tolerance values for families (Table C-1) to allow a family-level biotic index (FBI) to be calculated in the field. The FBI is an average of tolerance values of all arthropod families in a sample. It is not intended as a replacement for the BI and can be effectively used in the field only by biologists who are familiar enough with arthropods to be able to identify families without using keys.

Using the same method and more than 2,000 stream samples from throughout Wisconsin that were used to revise tolerance values for species and genera (Hilsenhoff 1987b) family-level tolerance values were established by comparing occurrence of each family with the average BI of streams in which they occurred in the greatest numbers. Thus, family-level tolerance values tend to be a weighted average of tolerance values of species and genera within each family based on their relative abundance in Wisconsin.

C.2 GENUS/SPECIES-LEVEL TOLERANCE CLASSIFICATION

The tolerance classification used in RBP III is based on Hilsenhoff (1987b). Because Hilsenhoff's tolerance classification is restricted to arthropods, nonarthropod tolerance designations have been taken from Bode (1988). Some of these tolerance values for macroinvertebrates not listed in Hilsenhoff (1982, 1987b) are presented in Table C-2.

C.3 REFERENCES FOR DETERMINING FAMILY AND SPECIES-LEVEL TOLERANCE CLASSIFICATIONS

Beck, W.M., Jr. 1977. Environmental Requirements and Pollution Tolerance of Common Freshwater Chironomidae. Environmental Monitoring and Support Laboratory, Report No. EPA-600/4-77-024. U.S. EPA, Cincinnati.

Bode, R.W. 1988. Quality Assurance Workplan for Biological Stream Monitoring in New York State. New York State Department of Environmental Conservation, Albany, New York.

Dawson, C.L. and R.A. Hellenthal. 1986. A Computerized System for the Evaluation of Aquatic Habitats Based on Environmental Requirements and Pollution Tolerance Association of Resident Organisms. Report No. EPA-600/S3-86/019.

Harris, T.L. and T.M. Lawrence. 1978. Environmental

TABLE C-1 TOLERANCE VALUES FOR FAMILIES OF STREAM ARTHROPODS IN THE WESTERN GREAT LAKES REGION (FROM HILSENHOFF 1988)

Plecoptera	Capniidae 1, Chloroperlidae 1, Leuctridae 0, Nemouridae 2, Perlidae 1, Perlodidae 2, Pteronarcyidae 0, Taeniopterygidae 2
Ephemeroptera	Baetidae 4, Baetiscidae 3, Caenidae 7, Ephemerellidae 1, Ephemeridae 4, Heptageniidae 4, Leptophlebiidae 2, Metretopodidae 2, Oligoneuriidae 2, Polymitarcyidae 2, Potomanthidae 4, Siphlonuridae 7, Tricorythidae 4
Odonata	Aeshnidae 3, Calopterygidae 5, Coenagrionidae 9, Cordulegastridae 3, Corduliidae 5, Gomphidae 1, Lestidae 9, Libellulidae 9, Macromiidae 3
Trichoptera	Brachycentridae 1, Glossosomatidae 0, Helicopsychidae 3, Hydropsychidae 4, Hydroptilidae 4, Lepidostomatidae 1, Leptoceridae 4, Limnephilidae 4, Molannidae 6, Odontoceridae 0, Philopotamidae 3, Phryganeidae 4, Polycentropodidae 6, Psychomyiidae 2, Rhyacophilidae 0, Sericostomatidae 3
Megaloptera	Corydalidae 0, Sialidae 4
Lepidoptera	Pyralidae 5
Coleoptera	Dryopidae 5, Elmidae 4, Psephenidae 4
Diptera	Athericidae 2, Blephariceridae 0, Ceratopogonidae 6, Blood-red Chironomidae (Chironomini) 8, other (including pink) Chironomidae 6, Dolochopodidae 4, Empididae 6, Ephydridae 6, Psychodidae 10, Simuliidae 6, Muscidae 6, Syrphidae 10, Tabanidae 6, Tipulidae 3
Amphipoda	Gammaridae 4, Talitridae 8
Isopoda	Asellidae 8

TABLE C-2 TOLERANCE VALUES FOR SOME MACROINVERTEBRATES NOT INCLUDED IN HILSENHOFF (1982, 1987b)[a]; FROM BODE (1988)

Acariformes	4
Decapoda	6
Gastropoda	
Amnicola	8
Bithynia	8
Ferrissia	6
Gyraulus	8
Helisoma	6
Lymnaea	6
Physa	8
Sphaeriidae	8
Oligochaeta	
Chaetogaster	6
Dero	10
Nais barbata	8
Nais behningi	6
Nais bretscheri	6
Nais communis	8
Nais elinguis	10
Nais pardalis	8
Nais simplex	6
Nais variabilis	10
Pristina	8
Stylaria	8
Tubificidae	
Aulodrilus	8
Limnodrilus	10
Hirudinea	
Helobdella	10
Turbellaria	4

(a) These values are for use with the biotic index scale of 0-10. Additional tolerance values are available in Bode (1988).

Requirements and Pollution Tolerance of Trichoptera. Report No. EPA-600/4-78-063. U.S. EPA, Washington.

Hilsenhoff, W.L. 1982. Using a Biotic Index to Evaluate Water Quality in Streams. Technical Bulletin No. 132. Department of Natural Resources, Madison, Wisconsin.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist 20:31-39.

Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. J. N. Am. Benthol. Soc. 7(1):65-68.

Hubbard, M.D. and W.L. Peters. 1978. Environmental Requirements and Pollution Tolerance of Ephemeroptera. Report No. EPA-600/4-78-061. U.S. EPA, Washington.

Shackleford, B. 1988. Rapid Bioassessment of Lotic Macroinvertebrate Communities: Biocriteria Development. Arkansas Department of Pollution Control and Ecology, Little Rock, Arkansas.

Surdick, R.F. and A.R. Gaufin. 1978. Environmental Requirements and Pollution Tolerance of Plecoptera. Report No. EPA-600/4-78-062. U.S. EPA, Cincinnati.

U.S. Department of Agriculture. 1985. Fisheries Survey Handbook, Aquatic Ecosystem Inventory, Chapter 5 Aquatic Macroinvertebrate Surveys. Document No. R-4 FSH 2609.23. U.S. Department of Agriculture, Forest Service, Ogden, Utah.

Weber, C.I. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. Report No. EPA-670/4-73-001. U.S. EPA, Cincinnati.

Winget, R.N. and F.A. Mangum. 1979. Biotic Condition Index: Integrated Biological, Physical, and Chemical Stream Parameters for Management. U.S. Department of Agriculture, Forest Service, Ogden, Utah.

C.4 A PARTIAL LISTING OF AGENCIES THAT HAVE DEVELOPED TOLERANCE CLASSIFICATIONS AND/OR BIOTIC INDICES

Florida Department of Environmental Regulation
Illinois EPA
New York Department of Environmental Conservation
North Carolina Department of Environmental Management
Ohio EPA
U.S. Department of Agriculture, Forest Service, Intermountain Region
U.S. EPA Region V
Vermont Department of Environmental Conservation

APPENDIX D

TOLERANCE, TROPHIC GUILDS, AND ORIGINS OF SELECTED FISH SPECIES

APPENDIX D
TOLERANCE, TROPHIC GUILDS, AND ORIGINS OF SELECTED FISH SPECIES

D.1 SPECIES-LEVEL FISH TOLERANCE, TROPHIC, AND ORIGIN CLASSIFICATIONS

Calculation of the IBI and assessment of biotic integrity requires classification of fish species tolerances to an array of stressors and/or the species' characteristic trophic guilds (Table D-1). Classifications for the Willamette River, Oregon were derived from Wydoski and Whitney (1979), Moyle (1976), Scott and Crossman (1973), Simpson and Wallace (1982), Dimick and Merryfield (1945), and C.E. Bond (1988, personal communication). Those for midwestern fishes were taken from Karr et al. (1986) and Ohio EPA (1987b). Classifications for other species and regions can be developed using Lee et al. (1980), the regional fish texts listed in Section D.2, and various journal manuscripts, theses, and grey literature.

Agencies contemplating the development of regional IBIs may wish to contact the author of this protocol, authors of the IBI papers cited in Section 7, or the State agencies now using the IBI (Section D.3) for further guidance.

TABLE D-1 TOLERANCE, TROPHIC GUILDS, AND ORIGINS OF SELECTED FISH SPECIES[a]

WILLAMETTE SPECIES	Trophic Level	Tolerance	Origin
Salmonidae			
Chinook salmon	piscivore	intolerant	native
Cutthroat trout	insectivore	intolerant	native
Mountain whitefish	insectivore	intolerant	native
Rainbow trout	insectivore	intolerant	native
Cyprinidae			
Chiselmouth	herbivore	intermediate	native
Common carp	omnivore	tolerant	exotic
Goldfish	omnivore	tolerant	exotic
Leopard dace	insectivore	intermediate	native
Longnose dace	insectivore	intermediate	native
Northern squawfish	piscivore	tolerant	native
Peamouth	insectivore	intermediate	native
Redside shiner	insectivore	intermediate	native
Speckled dace	insectivore	intermediate	native
Catostomidae			
Largescale sucker	omnivore	tolerant	native
Mountain sucker	herbivore	intermediate	native

(a) Not necessarily the final designations; designations may vary for different regions.

TABLE D-1 (Cont.)

	Trophic Level	Tolerance	Origin
Ictaluridae			
Brown bullhead	insectivore	tolerant	exotic
Yellow bullhead	insectivore	tolerant	exotic
Percopsidae			
Sand roller	insectivore	intermediate	native
Gasterosteidae			
Threespine stickleback	insectivore	intermediate	native
Centrarchidae			
Bluegill	insectivore	tolerant	exotic
Largemouth bass	piscivore	tolerant	exotic
Smallmouth bass	piscivore	intermediate	exotic
White crappie	insectivore	tolerant	exotic
Percidae			
Yellow perch	insectivore	intermediate	exotic
Cottidae			
Paiute sculpin	insectivore	intolerant	native
Prickly sculpin	insectivore	intermediate	native
Reticulate sculpin	insectivore	tolerant	native
Torrent sculpin	insectivore	intolerant	native
MIDWEST SPECIES			
Petromyzontidae			
Silver lamprey	piscivore	intermediate	native
Northern brook lamprey	filterer	intolerant	native
Mountain brook lamprey	filterer	intolerant	native
Ohio lamprey	piscivore	intolerant	native
Least brook lamprey	filterer	intermediate	native
Sea lamprey	piscivore	intermediate	exotic
Polyodontidae			
Paddlefish	filterer	intolerant	native
Acipenseridae			
Lake sturgeon	invertivore	intermediate	native
Shovelnose sturgeon	insectivore	intermediate	native
Lepisosteidae			
Alligator gar	piscivore	intermediate	native
Shortnose gar	piscivore	intermediate	native
Spotted gar	piscivore	intermediate	native
Longnose gar	piscivore	intermediate	native
Amiidae			
Bowfin	piscivore	intermediate	native
Hiodontidae			
Goldeye	insectivore	intolerant	native
Mooneye	insectivore	intolerant	native
Clupeidae			
Skipjack herring	piscivore	intermediate	native
Alewife	invertivore	intermediate	exotic
Gizzard shad	omnivore	intermediate	native
Threadfin shad	omnivore	intermediate	native
Salmonidae			
Brown trout	insectivore	intermediate	exotic

TABLE D-1 (Cont.)

	Trophic Level	Tolerance	Origin
Rainbow trout	insectivore	intermediate	exotic
Brook trout	insectivore	intermediate	native
Lake trout	piscivore	intermediate	native
Coho salmon	piscivore	intermediate	exotic
Chinook salmon	piscivore	intermediate	exotic
Lake herring	piscivore	intermediate	native
Lake whitefish	piscivore	intermediate	native
Osmeridae			
Rainbow smelt	invertivore	intermediate	exotic
Umbridae			
Central mudminnow	insectivore	tolerant	native
Esocidae			
Grass pickerel	piscivore	intermediate	native
Chain pickerel	piscivore	intermediate	native
Northern pike	piscivore	intermediate	native
Muskellunge	piscivore	intermediate	native
Cyprinidae			
Common carp	omnivore	tolerant	exotic
Goldfish	omnivore	tolerant	exotic
Golden shiner	omnivore	tolerant	native
Horneyhead chub	insectivore	intolerant	native
River chub	insectivore	intolerant	native
Silver chub	insectivore	intermediate	native
Bigeye chub	insectivore	intolerant	native
Streamline chub	insectivore	intolerant	native
Gravel chub	insectivore	intermediate	native
Speckled chub	insectivore	intolerant	native
Blacknose dace	generalist	tolerant	native
Longnose dace	insectivore	intolerant	native
Creek chub	generalist	tolerant	native
Tonguetied minnow	insectivore	intolerant	native
Suckermouth minnow	insectivore	intermediate	native
Southern redbelly dace	herbivore	intermediate	native
Redside dace	insectivore	intolerant	native
Pugnose minnow	insectivore	intolerant	native
Emerald shiner	insectivore	intermediate	native
Silver shiner	insectivore	intolerant	native
Rosyface shiner	insectivore	intolerant	native
Redfin shiner	insectivore	intermediate	native
Rosefin shiner	insectivore	intermediate	native
Striped shiner	insectivore	intermediate	native
Common shiner	insectivore	intermediate	native
River shiner	insectivore	intermediate	native
Spottail shiner	insectivore	intermediate	native
Blackchin shiner	insectivore	intolerant	native
Bigeye shiner	insectivore	intolerant	native
Steelcolor shiner	insectivore	intermediate	native
Spotfin shiner	insectivore	intermediate	native
Bigmouth shiner	insectivore	intermediate	native
Sand shiner	insectivore	intermediate	native

TABLE D-1 (Cont.)

	Trophic Level	Tolerance	Origin
Mimic shiner	insectivore	intolerant	native
Ghost shiner	insectivore	intermediate	native
Blacknose shiner	insectivore	intolerant	native
Pugnose shiner	insectivore	intolerant	native
Silverjaw minnow	insectivore	intermediate	native
Mississippi silvery minnow	herbivore	intermediate	native
Bullhead minnow	omnivore	intermediate	native
Bluntnose minnow	omnivore	tolerant	native
Fathead minnow	omnivore	tolerant	native
Central stoneroller	herbivore	intermediate	native
Popeye shiner	insectivore	intolerant	native
Grass carp	herbivore	intermediate	exotic
Red shiner	omnivore	intermediate	native
Brassy minnow	omnivore	intermediate	native
Central silvery minnow	herbivore	intolerant	native
Catostomidae			
Blue sucker	insectivore	intolerant	native
Bigmouth buffalo	insectivore	intermediate	native
Black buffalo	insectivore	intermediate	native
Smallmouth buffalo	insectivore	intermediate	native
Quillback	omnivore	intermediate	native
River carpsucker	omnivore	intermediate	native
Highfin carpsucker	omnivore	intermediate	native
Silver redhorse	insectivore	intermediate	native
Black redhorse	insectivore	intolerant	native
Golden redhorse	insectivore	intermediate	native
Shorthead redhorse	insectivore	intermediate	native
Greater redhorse	insectivore	intolerant	native
River redhorse	insectivore	intolerant	native
Harelip sucker	invertivore	intolerant	native
Northern hog sucker	insectivore	intolerant	native
White sucker	omnivore	tolerant	native
Longnose sucker	insectivore	intermediate	native
Spotted sucker	insectivore	intermediate	native
Lake chubsucker	insectivore	intermediate	native
Creek chubsucker	insectivore	intermediate	native
Ictaluridae			
Blue catfish	piscivore	intermediate	native
Channel catfish	generalist	intermediate	native
White catfish	insectivore	intermediate	native
Yellow bullhead	insectivore	tolerant	native
Brown bullhead	insectivore	tolerant	native
Black bullhead	insectivore	intermediate	native
Flathead catfish	piscivore	intermediate	native
Stonecat	insectivore	intolerant	native
Mountain madtom	insectivore	intolerant	native
Slender madtom	insectivore	intolerant	native
Freckled madtom	insectivore	intermediate	native
Northern madtom	insectivore	intolerant	native
Scioto madtom	insectivore	intolerant	native

TABLE D-1 (Cont.)

	Trophic Level	Tolerance	Origin
Brindled madtom	insectivore	intolerant	native
Tadpole madtom	insectivore	intermediate	native
Anguillidae			
American eel	piscivore	intermediate	native
Cyprinodontidae			
Western banded killifish	insectivore	intolerant	native
Eastern banded killifish	insectivore	tolerant	native
Blackstripe topminnow	insectivore	intermediate	native
Poeciliidae			
Mosquitofish	insectivore	intermediate	exotic
Gadidae			
Burbot	piscivore	intermediate	native
Percopsidae			
Trout-perch	insectivore	intermediate	native
Aphredoderidae			
Pirate perch	insectivore	intermediate	native
Atherinidae			
Brook silverside	insectivore	intermediate	native
Percichthyidae			
White bass	piscivore	intermediate	native
Striped bass	piscivore	intermediate	exotic
White perch	piscivore	intermediate	exotic
Yellow bass	piscivore	intermediate	native
Centrarchidae			
White crappie	invertivore	intermediate	native
Black crappie	invertivore	intermediate	native
Rock bass	piscivore	intermediate	native
Smallmouth bass	piscivore	intermediate	native
Spotted bass	piscivore	intermediate	native
Largemouth bass	piscivore	intermediate	native
Warmouth	invertivore	intermediate	native
Green Sunfish	invertivore	tolerant	native
Bluegill	insectivore	intermediate	native
Orangespotted sunfish	insectivore	intermediate	native
Longear sunfish	insectivore	intolerant	native
Redear sunfish	insectivore	intermediate	native
Pumpkinseed	insectivore	intermediate	native
Percidae			
Sauger	piscivore	intermediate	native
Walleye	piscivore	intermediate	native
Yellow perch	piscivore	intermediate	native
Dusky darter	insectivore	intermediate	native
Blackside darter	insectivore	intermediate	native
Longhead darter	insectivore	intolerant	native
Slenderhead darter	insectivore	intolerant	native
River darter	insectivore	intermediate	native
Channel darter	insectivore	intolerant	native
Gilt darter	insectivore	intolerant	native
Logperch	insectivore	intermediate	native

TABLE D-1 (Cont.)

	Trophic Level	Tolerance	Origin
Crystal darter	insectivore	intolerant	native
Eastern sand darter	insectivore	intolerant	native
Western sand darter	insectivore	intolerant	native
Johnny darter	insectivore	intermediate	native
Greenside darter	insectivore	intermediate	native
Banded darter	insectivore	intolerant	native
Variegate darter	insectivore	intolerant	native
Spotted darter	insectivore	intolerant	native
Bluebreast darter	insectivore	intolerant	native
Tippecanoe darter	insectivore	intolerant	native
Iowa darter	insectivore	intermediate	native
Rainbow darter	insectivore	intermediate	native
Orangethroat darter	insectivore	intermediate	native
Fantail darter	insectivore	intermediate	native
Least darter	insectivore	intermediate	native
Slough darter	insectivore	intermediate	native
Sciaenidae			
Freshwater drum	invertivore	intermediate	native
Cottidae			
Spoonhead sculpin	insectivore	intermediate	native
Mottled sculpin	insectivore	intermediate	native
Slimy sculpin	insectivore	intermediate	native
Deepwater sculpin	insectivore	intermediate	native
Gasterosteidae			
Brook stickleback	insectivore	intermediate	native

D.2 SELECTED REFERENCES FOR DETERMINING FISH TOLERANCE, TROPHIC, REPRODUCTIVE, AND ORIGIN CLASSIFICATIONS

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D.3 AGENCIES CURRENTLY USING OR EVALUATING USE OF THE IBI FOR WATER QUALITY INVESTIGATIONS

Alabama Geological Survey (Scott Mettee)

Illinois Environmental Protection Agency (Bob Hite)

Iowa Conservation Commission (Vaughn Paragamian)

Kansas Department of Wildlife and Parks (L. Zuckerman)

Kansas Department of Health and Environment (S. Haslover)

Kentucky Cabinet for Natural Resources and Environmental Protection (Mike Mills)

Nebraska Department of Environmental Control (Terry Maret)

North Carolina Division of Environmental Management (Vince Schneider)

Ohio Environmental Protection Agency (Ed Rankin)

Oklahoma State Department of Health (Jimmy Pigg)

Tennessee Valley Authority (Neil Carriker)

U.S. EPA Region II (Jim Kurtenbach)

U.S. EPA Region I (Jim Luey)

Vermont Department of Environmental Conservation (Rich Langdon)

Wisconsin Department of Natural Resources (Steve Lyons)

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