3 ELEMENTS OF BIOMONITORING

3.1 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 (RBPs), are best used for detecting aquatic life impairments and assessing their relative severity. Once an impairment is detected, however, additional ecological data, such as chemical and biological (toxicity) testing is helpful to identify the causative agent, its source, and to implement appropriate mitigation (USEPA 1991c). Integrating information from these data types as well as from habitat assessments, hydrological investigations, and knowledge of land use is helpful to provide a comprehensive diagnostic assessment of impacts from the 5 principal factors (see Karr et al. 1986, Karr 1991, Gibson et al. 1996 for description of water quality, habitat structure, energy source, flow regime, and biotic interaction factors). 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 and rehabilitation activities. Some of the advantages of using biosurveys for this type of monitoring are:

- ! 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 (CWA).
- ! Biological communities integrate the effects of different stressors and thus provide a broad measure of their aggregate impact.
- ! Communities integrate the stresses over time and provide an ecological measure of fluctuating environmental conditions.
- ! 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 1987).
- ! The status of biological communities is of direct interest to the public as a measure of a pollution free environment.
- ! 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, i.e., identifying causes and limiting sources, require integrating information of various types—chemical, physical, toxicological, and/or biosurvey data. These data are needed to:

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 or ambient toxicity). For situations where habitat degradation is prevalent, a combination of biosurvey and physical habitat assessment is most useful (Barbour and Stribling 1991).

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 helpful to confirm the point sources and develop appropriate discharge limits. Impacts due to factors other than chemical contamination will require different ecological data.

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 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. Improvement of the ecosystem both from restoration or rehabilitation activities are best monitored by biosurvey techniques.

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.

3.2 USE OF DIFFERENT ASSEMBLAGES IN BIOSURVEYS

The techniques presented in this document focus on the evaluation of water quality (physicochemical constituents), habitat parameters, and analysis of the periphyton, benthic macroinvertebrate, and fish assemblages. 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 8 in this document. The fish survey protocol is based largely on Karr's Index of Biotic Integrity (IBI) (Karr 1981, Karr et al. 1986, Miller et al. 1988), which uses the structure of the fish assemblage 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 rapid bioassessment approaches.

The periphyton assemblage (primarily algae) is also useful for water quality monitoring, but has not been incorporated widely in monitoring programs. They represent the primary producer 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 assemblages, integration of structural/compositional and functional characteristics provides the best means of assessing impairment (Rodgers et al. 1979).

In selecting the aquatic assemblage appropriate for a particular biomonitoring situation, the advantages of using each assemblage must be considered along with the objectives of the program. Some of the advantages of using periphyton, benthic macroinvertebrates, and fish in a biomonitoring program are presented in this section. References for this list are Cairns and Dickson (1971), American Public Health Association et al. (1971), Patrick (1973), Rodgers et al. (1979), Weitzel (1979), Karr (1981), USEPA (1983), Hughes et al. (1982), and Plafkin et al. (1989).

3.2.1 Advantages of Using Periphyton

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

3.2.2 Advantages of Using Benthic Macroinvertebrates

- ! Macroinvertebrate assemblages 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 (upstreamdownstream studies).
- ! Macroinvertebrates integrate the effects of short-term environmental variations. Most species have a complex life cycle of approximately one year or more. Sensitive life stages will respond quickly to stress; the overall community will respond more slowly.
- ! Degraded conditions can often be detected by an experienced biologist with only a cursory examination of the benthic macroinvertebrate assemblage. Macroinvertebrates are relatively easy to identify to family; many "intolerant" taxa can be identified to lower taxonomic levels with ease.
- ! Benthic macroinvertebrate assemblages are made up of species that constitute a broad range of trophic levels and pollution tolerances, thus providing strong information for interpreting cumulative effects.
- ! Sampling is relatively easy, requires few people and inexpensive gear, and has minimal detrimental effect on the resident biota.
- ! Benthic macroinvertebrates serve as a primary food source for fish, including many recreationally and commercially important species.
- ! 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.
- ! Most state water quality agencies that routinely collect biosurvey data focus on macroinvertebrates (Southerland and Stribling 1995). Many states already have background macroinvertebrate data. Most state water quality agencies have more expertise with invertebrates than fish.

3.2.3 Advantages of Using Fish

- ! 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).
- ! Fish assemblages 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 assemblage structure is reflective of integrated environmental health.
- ! Fish are at the top of the aquatic food web and are consumed by humans, making them important for assessing contamination.
- ! Fish are relatively easy to collect and identify to the species level. Most specimens can be sorted and identified in the field by experienced fisheries professionals, and subsequently released unharmed.
- ! Environmental requirements of most fish are comparatively well known. Life history information is extensive for many species, and information on fish distributions is commonly available.
- ! Aquatic life uses (water quality standards) are typically characterized in terms of fisheries (coldwater, coolwater, warmwater, sport, forage). Monitoring fish provides direct evaluation of "fishability" and "fish propagation", which emphasizes the importance of fish to anglers and commercial fishermen.
- ! Fish account for nearly half of the endangered vertebrate species and subspecies in the United States (Warren and Burr 1994).

3.3 IMPORTANCE OF HABITAT ASSESSMENT

The procedure for assessing physical habitat quality presented in this document (Chapter 5) 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, particularly the catchment of the site under investigation. All of the habitat parameters evaluated are related to overall aquatic life use and are a potential source of limitation to the aquatic biota.

The alteration of the physical structure of the habitat is one of 5 major factors from human activities described by Karr (Karr et al. 1986, Karr 1991) that degrade aquatic resources. Habitat, as structured by instream and surrounding topographical features, is a major determinant of aquatic community potential (Southwood 1977, Plafkin et al. 1989, and Barbour and Stribling 1991). Both the quality and quantity of available habitat affect the structure and composition of resident biological communities. Effects of such features on biological assessment results 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 physical habitat quality at a test site is similar to that of a reference, detected impacts can be attributed to water quality factors (i.e., chemical contamination) or other stressors. However, where habitat quality differs substantially from reference conditions, the question of appropriate aquatic life use designation 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.

3.4 THE REGIONAL REFERENCE CONCEPT

The issue of reference conditions is critical to the interpretation of biological surveys. Barbour et al. (1996a) describe 2 types of reference conditions that are currently used in biological surveys: sitespecific and regional reference. The former typically consists of measurements of conditions upstream of a point source discharge or from a "paired" watershed. Regional reference conditions, on the other hand, consist of measurements from a population of relatively unimpaired sites within a relatively homogeneous region and habitat type, and therefore are not site-specific.

The reference condition establishes the basis for making comparisons and for detecting use impairment; it should be applicable to an individual waterbody, such as a stream segment, but also to similar waterbodies on a regional scale (Gibson et al. 1996).

Although both site-specific and ecoregional references represent conditions without the influence of a particular discharge, the 2 types of references may not yield equivalent measurements (Barbour et al. 1996a). While site-specific reference conditions represented by the upstream, downstream, or pairedsite approach are desirable, they are limited in their usefulness. Hughes (1995) points out three problems with site-specific reference conditions: (1) because they typically lack any broad study design, site-specific reference conditions possess limited capacity for extrapolation— they have only site-specific value; (2) usually site-specific reference conditions allow limited variance estimates; there are too few sites for robust variance evaluations because each site of concern is typically represented by one-to-three reference sites; the result could be an incorrect assessment if the upstream site has especially good or especially poor habitat or chemical quality; and (3) they involve a substantial assessment effort when considered on a statewide basis.

The advantages of measuring upstream reference conditions are these: (1) if carefully selected, the habitat quality is often similar to that measured downstream of a discharge, thereby reducing complications in interpretation arising from habitat differences, and (2) impairments due to upstream influences from other point and nonpoint sources are already factored into the reference condition (Barbour et al. 1996a). New York DEC has found that an upstream-downstream approach aids in diagnosing cause-and-effect to specific discharges and increase precision (Bode and Novak 1995).

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. In significantly altered systems (i.e., channelized or heavily urbanized streams), suitable reference sites are usually not available (Gibson et al. 1996). In these cases, historical data or simple ecological models may be necessary to establish reference conditions. See Gibson et al. (1996) for more detail.

Innate regional differences exist in forests, lands with high agricultural potential, wetlands, and waterbodies. These regional differences have been mapped by Bailey (1976), U.S. Department of Agriculture (USDA) Soil Conservation Service (1981), Energy, Mines and Resources Canada (1986), and Omernik (1987). Waterbodies reflect the lands they drain (Omernik 1987, Hunsaker and Levine 1995) and it is assumed that similar lands should produce similar waterbodies. This ecoregional approach provides robust and ecologically-meaningful regional maps that are based on an examination of several mapped land variables. 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.

Omernik (1987) provided 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. Geographic patterns of similarity among ecosystems can be grouped into ecoregions or subecoregions. 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 efficient management of aquatic ecosystems and their components (Hughes 1985, Hughes et al. 1986, 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 approximate ecoregional boundaries 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 1987).

However, some programs, such as EMAP (Klemm and Lazorchak 1994) and the Maryland Biological Stream Survey (MBSS) (Volstad et al. 1995) have found that a surrogate measure of stream size (catchment size) is useful in partitioning the variability of stream segments for assessment. Hydrologic regime can include flow regulation, water withdrawal, and whether a stream is considered intermittent or perennial. Elevation has been found to be an important classification variable when using the benthic macroinvertebrate assemblage (Barbour et al. 1992, Barbour et al. 1994, Spindler 1996). In addition, descriptors at a smaller scale may be needed to characterize streams within regions or classes. For example, even though a given stream segment is classified within a subecoregion or other type of stream class, it may be wooded (deciduous or coniferous) or open within a perennial or intermittent flow regime, and represent one of several orders of stream size.

Individual descriptors *will not apply to all regional reference streams,* nor will all conditions (i.e., deciduous, coniferous, open) be present in all streams. Those streams or stream segments that represent characteristics atypical for that particular ecoregion should be excluded from the regional aggregate of sites and treated as a special situation. For example, Ohio EPA (1987) considered aquatic systems with unique (i.e., unusual for the ecoregion) natural characteristics to be a separate aquatic life use designation (exceptional warmwater aquatic life use) on a statewide basis.

Although the final rapid bioassessment guidance should be generally applicable to all regions of the United States, 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 regional reference concept versus the site-specific control approach will need to be examined. When Rapid Bioassessment Protocols (RBPs) are used to assess impact sources (upstream-downstream studies), regional reference criteria may not be as important if an unimpacted site-specific control station can be sampled. However, when a synoptic ("snapshot") or trend monitoring survey is being conducted in a watershed or river basin, use of regional criteria may be the only means of discerning use impairment or assessing impact. Additional investigation will be needed to: delineate areas (classes of streams)that differ significantly in their innate biological potential; locate reference sites within each stream class that fully support aquatic life uses; develop biological criteria (e.g., define optimal values for the metrics) using data generated from each of the assemblages.

3.5 STATION SITING

Site selection for assessment and monitoring can either be "targeted", i.e., relevant to special studies that focus on potential problems, or "probabilistic", which provides information of the overall status or condition of the watershed, basin, or region. In a probabilistic or random sampling regime, stream characteristics may be highly dissimilar among the sites, but will provide a more accurate assessment of biological condition throughout the area than a targeted design. Selecting sites randomly provides an unbiased assessment of the condition of the waterbody at a scale above the individual site or stream. Thus, an agency can address questions at multiple scales. Studies for 305(b) status and trends assessments are best done with a probabilistic design.

Most studies conducted by state water quality agencies for identification of problems and sensitive waters are done with a targeted design. In this case, sampling sites are selected based on known existing problems, knowledge of upcoming events that will adversely affect the waterbody such as a development or deforestation; or installation of BMPs or habitat restoration that are intended to improve waterbody quality. This method provides assessments of individual sites or stream reaches. Studies for aquatic life use determination and those related to TMDLs can be done with a random (watershed or higher level) or targeted (site-specific) design.

To meaningfully evaluate biological condition in a targeted design, sampling locations must be similar enough to have similar biological expectations, which, in turn, provides a basis for comparison of impairment. If the goal of an assessment is to evaluate the effects of water chemistry degradation, comparable physical habitat should be sampled at all stations, otherwise, the differences in the biology attributable to a degraded habitat will be difficult to separate from those resulting from chemical pollution water quality degradation. Availability of appropriate habitat at each sampling location can be established during preliminary reconnaissance. 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 sample stations.

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 because these areas will have habitat more typical of the larger waterbody (Karr et al. 1986).

For bioassessment activities where the concern is non-chemical stressors, e.g., the effects of habitat degradation or flow alteration, or cumulative impacts, a different approach to station selection is used. Physical habitat differences between sites can be substantial for two reasons: (1) one or a set of sites is more degraded (physically) than another, or (2) is unique for the stream class or region due to the essential natural structure resulting from geological characteristics. Because of these situations, the more critical part of the siting process comes from the recognition of the habitat features that are representative of the region or stream class. In basin-wide or watershed studies, sample locations should not be avoided due to habitat degradation or to physical features that are well-represented in the stream class.

3.6 DATA MANAGEMENT AND ANALYSIS

USEPA is developing a biological data management system linked to STORET, which provides a centralized system for storage of biological data and associated analytical tools for data analysis. The field survey file component of STORET 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 STORET 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 STORET field survey file using header information presented on the field data forms (Appendix A) to identify sampling stations.

Habitat and physical characterization information may also be stored in the field survey file with organism 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 include stream depth, velocity, and substrate characteristics, as well as many other parameters. STORET also allows storage of other pertinent station or sample information in the comments section.

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 Quality Assurance/Quality Control (QA/QC) plan. An agency conducting rapid bioassessment programs can choose an existing system within their agency or utilize the STORET system developed as a national database system.

Data collected as part of state bioassessment programs are usually entered, stored and analyzed in easily obtainable spreadsheet programs. This method of data management becomes cumbersome as the database grows in volume. An alternative to spreadsheet programs is a multiuser relational database management system (RDMS). Most relational database software is designed for the Windows operating system and offer menu driven interfaces and ranges of toolbars that provide quick access to many routine database tasks. Automated tools help users quickly create forms for data input and lookup, tables, reports, and complex queries about the data. The USEPA is developing a multiuser relational database management system that can transfer sampling data to STORET. This relational database management system is EDAS (Ecological Data Application System) and allows the user to input, compile, and analyze complex ecological data to make assessments of ecosystem condition. EDAS includes tools to format sampling data so it may be loaded into STORET as a batch file. These batch files are formatted as flat ASCII text and can be loaded (transferred) electronically to STORET. This will eliminate the need to key sample data into STORET.

By using tables and queries as established in EDAS, a user can enter, manipulate, and print data. The metrics used in most bioassessments can be calculated with simple queries that have already been created for the user. New queries may be created so additional metrics can be calculated at the click of the mouse each time data are updated or changed. If an operation on the data is too complex for one of the many default functions then the function can be written in code (e.g., visual basic access) and stored in a module for use in any query. Repetitive steps can be handled with macros. As the user develops the database other database elements such as forms and reports can be added.

Figure 3-1. Example of the relationship of data tables in a typical relational database.

Table design is the foundation of the relational database, such as EDAS (Figure 3-1), because they function as data containers. Tables are related through the use of a unique identifier or index. In the example database "StationId" links the tables "ChemSamps", "HabSamps", and "BenSamps" to the "Stations" table. The chemical parameters and habitat parameters table act as reference tables and contain descriptive data (e.g., measurement units, detection limits). This method of storing data is more efficient than spreadsheets, because it eliminates a lot of redundant data. Master Taxa tables are created for the biological data to contain all relevant information about each taxon. This information does not have to be repeated each time a taxon is entered into the database.

Input or lookup forms (Figure 3-2) are screens that are designed to aid in entering or retrieving data. Forms are linked to tables so data go to the right cell in the right table. Because of the relationships among the tables, data can be updated across all the tables that are linked to the form. Reports can be generated in a variety of styles, and data can be exported to other databases or spreadsheet programs.

3.7 TECHNICAL ISSUES FOR SAMPLING THE PERIPHYTON ASSEMBLAGE

3.7.1 Seasonality

Stream periphyton have distinct seasonal cycles, with peak abundance and diversity typically occurring

in late summer or early fall (Bahls 1993). High flows may scour and sweep away periphyton. For these reasons, the index period for periphyton sampling is usually late summer or early fall, when stream flow is relatively stable (Kentucky DEP 1993, Bahls 1993).

Algae are light limited, and may be sparse in heavily shaded streams. Early spring, before leafout, may be a better sampling index period in shaded streams.

Finally, since algae have short generation times (one to several days), they respond rapidly to environmental changes. Samples of the algal community are "snapshots" in time, and do not integrate environmental effects over entire seasons or years.

3.7.2 Sampling Methodology

Artificial substrates (periphytometers) have long been used in algal investigations, typically using glass slides as the substrate, but also with glass rods, plastic plates, ceramic tiles and other substances. However, many agencies are sampling periphyton from natural substrates to characterize

Figure 3-2. Example input or lookup form in a typical relational database.

the natural community. Advantages of artificial and natural substrates are summarized below (Cairns 1982, Bahls 1993).

Advantages of Artificial Substrates:

- ! 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).
- ! 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.
- ! 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.
- ! Sampling variability is decreased due to a reduction in microhabitat patchiness, improving the potential for spatial and temporal similarity among samples.
- ! Sample collection using artificial substrates may require less skill and training than direct sampling of natural substrates.

Disadvantages of Artificial Substrates:

- ! Artificial substrates require a return trip; this may be a significant consideration in large states or those with limited technical resources.
- ! Artificial substrates are prone to loss, natural damage or vandalism.
- ! The material of the substrate will influence the composition and structure of the community; solid artificial substrates will favor attached forms over motile forms and compromise the usefulness of the siltation index.
- ! Orientation and length of exposure of the substrate will influence the composition and structure of the community.

3.8 TECHNICAL ISSUES FOR SAMPLING THE BENTHIC MACROINVERTEBRATE ASSEMBLAGE

3.8.1 Seasonality for Benthic Collections (adapted from Gibson et al. 1996)

The ideal sampling procedure is to survey the biological community with each change of season, then select the appropriate sampling periods that accommodate seasonal variation. Such indexing makes the best use of the biological data. However, resident assemblages integrate stress effects over the course of the year, and their seasonal cycles of abundance and taxa composition are fairly predictable within the limits of interannual variability.

Many programs have found that a single index period provides a strong database that allows all of their management objectives to be addressed. However, if one goal of a program is to understand seasonal variability, then establishing index periods during multiple seasons is necessary. Although a single index period would not likely be adequate for assessing the effects of catastrophic events, such as spill, those assessments should be viewed as special studies requiring sampling of reference sites during the same time period.

Ultimately, selection of the appropriate sampling period should be based on 3 factors that reflect efforts to:

- 1. minimize year-to-year variability resulting from natural events,
- 2. maximize gear efficiency, and
- 3. maximize accessibility of targeted assemblage.

Sampling and comparisons of data from the same seasons (or index periods) as the previous year's sampling provides some correction and minimization of annual variability. The season of the year during which sampling gear is most effective is an important consideration for selecting an index period. For example, low flow or freezing conditions may hamper an agency's ability to sample with its selected gear. Seasons where those conditions are prevalent should be avoided. The targeted assemblage(s) should be accessible and not be inhabiting hard-to-reach portions of the sampling area. For example, if benthos are primarily deep in the substrate in winter, beyond normal sampling depth, that period should be avoided and another index period chosen. If high flows are typical of spring runoff periods, and sampling cannot occur, the index period should be established during typical or low flow periods.

3.8.2 Benthic Sampling Methodology

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. The Science Advisory Board (SAB 1993) cautioned that the only appropriate type of artificial substrates to be used for assessment are those that are "introduced substrates", i.e., substrates that are representative of the natural substrate of the stream system, such as rock-filled baskets in cobble- or gravel-bottomed streams. Ohio EPA and Maine DEP, are examples of states that use artificial substrates for their water resource investigations (Davis et al. 1996).

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

Advantages of Artificial Substrates:

! 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).

- ! 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.
- ! 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 second bullet under Disadvantages below). Most artificial substrates, by design, select for the Scraper and Filterer components of the benthic assemblages or for Collectors if accumulation of debris has occured in the substrates.
- ! Sampling variability is decreased due to a reduction in microhabitat patchiness, improving the potential for spatial and temporal similarity among samples.
- ! Sample collection using artificial substrates may 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 Artificial Substrates:

- ! 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.
- ! Samples may not be fully representative of the benthic assemblage 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.
- ! 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.
- ! 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.

3.9 TECHNICAL ISSUES FOR THE SURVEY OF THE FISH ASSEMBLAGE

3.9.1 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 (1987) stated 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 meters 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 substantial population fluctuations are not likely to occur in response to purely natural environmental phenomena. However, comparison of data collected during different seasons is discouraged, as are data collected during or immediately after major flow changes.

3.9.2 Fish Sampling Methodology

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

3.9.2.1 Advantages and Disadvantages of Electrofishing

Advantages of Electrofishing:

- ! Electrofishing allows greater standardization of catch per unit of effort.
- ! Electrofishing requires less time and a reduced level of effort than some sampling methods (e.g., use of ichthyocides) (Hendricks et al. 1980).
- ! Electrofishing is less selective than seining (although it is selective towards size and species) (Hendricks et al. 1980). (See second bullet under Disadvantages below).
- ! If properly used, adverse effects on fish are minimized.
- ! Electrofishing is appropriate in a variety of habitats.

Disadvantages of Electrofishing:

- ! Sampling efficiency is affected by turbidity and conductivity.
- ! 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).
- ! Electrofishing is a hazardous operation that can injure field personnel if proper safety procedures are ignored.

3.9.2.2 Advantages and Disadvantages of Seining

Advantages of Seining:

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

Disadvantages of Seining:

- ! 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).
- ! Sample effort and results for seining are more variable than sampling with electrofishing.
- ! Use of seines is generally restricted to slower water with smooth bottoms, and is most effective in small streams or pools with little cover.
- ! Standardization of unit of effort to ensure data comparability is difficult.

3.10 SAMPLING REPRESENTATIVE HABITAT

Effort should be made when sampling to avoid regionally unique natural habitat. Samples from such situations, when compared to those from sites lacking the unique habitat, will appear different, i.e., assess as in either better or worse condition, than those not having the unique habitat. This is due to the usually high habitat specificity that different taxa have to their range of habitat conditions; unique habitat will have unique taxa. Thus, all RBP sampling is focused on sampling of representative habitat.

Composite sampling is the norm for RBP investigations to characterize the reach, rather than individual small replicates. However, a major source of variance can result from taking too few samples for a

composite. Therefore, each of the protocols (i.e., for periphyton, benthos, fish) advocate compositing several samples or efforts throughout the stream reach. Replication is strongly encouraged for precision evaluation of the methods.

When sampling wadeable streams, rivers, or waterbodies with complex habitats, a complete inventory of the entire reach is not necessary for bioassessment. However, the sampling area should be representative of the reach, incorporating riffles, runs, and pools if these habitats are typical of the stream in question. Midchannel 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. Although some deep water or wetland species may be undersampled, the data should be adequate for the objective of bioassessment.