Summary of State and National Biological Assessment Methods, Physical Habitat Assessment Methods, and Biological Criteria

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OVERVIEW

The primary purpose of this review and summary of State and National biological assessment methods, physical habitat assessment methods, and biological criteria is to aid the Heartland Network of National Park Service in the development of a biological monitoring methodology. In addition to the individual reviews of identified methods, a number of tabular summaries were constructed to facilitate ready comparisons of elements and factors common to all or most reviewed methods. It was envisioned that assessments of past efforts of scientists and environmental management organizations involved in monitoring aquatic resources would yield the necessary information to construct general park monitoring plans including the primary indicator group and measurement metrics. Prior Heartland discussions suggested that methods probably existed that could be adopted and modified for use by individuals NPS facilities. This work summarizes the specific methods used by all regulatory entities within states where Heartland Network facilities are located and thus would provide a logical monitoring framework upon which parks could build. Certainly from a water quality perspective water bodies located on NPS facilities must meet established state water criteria and biological criteria for the state(s) in which the facility occurs. Specific monitoring and biological assessment documents produced by or for National entities such as US EPA, USGS, and USFS were also reviewed as these agencies have long standing monitoring programs and years of associated aquatic data that could be used in comparisons by NPS facilities to evaluate existing water and biological quality. In addition, a number of existing NPS monitoring documents prepared for individual NPS facilities were reviewed for potential direct NPS application to these areas.

Much of this review focuses on lotic ecosystems (*e.g.,* streams and rivers) because these are the most common or prominent aquatic ecosystems of these facilities, and documentation of standard bioassessment methods for lentic ecosystems (*e.g.,* ponds, lakes, wetlands) are few. The biological quality of lakes and ponds is often associated with chlorophyll *a* concentrations with persistently high levels (> 10µg/L) being indicative of cultural eutrophication. We have covered some biological methods for lakes and wetlands when these aquatic ecosystems were included in state determined methodologies. This work was produced under the cooperative agreement between the Center of Research of University of Kansas and the National Park Service (Coop. Agreement # H6067B10031).

NATIONAL TRENDS IN BIOLOGICAL ASSESSMENT AND BIOLOGICAL CRITERIA

Monitoring the quality of water resources is best accomplished using an integrated ecological approach. The quality or integrity of an aquatic resource is determined by physical, chemical, and biological factors both instream and in the surrounding watershed. Traditionally water chemistry measurements have been heavily relied upon for monitoring the quality of waters. However, measurements of water column chemical constituents only contain information on the conditions at the time the samples were taken (USEPA 1994). Chemical measurements alone also fail to incorporate the long-term effects of instream, riparian, and watershed-wide habitat degradation. For these reasons the assessment of aquatic biological communities has become a common and effective means to supplement physical and chemical water resource data. The use of macroinvertebrates as biological indicators of water quality and ecological integrity began in the early part of the 20th century (USEPA 1990). Since then the use of biological assessment methods has become a standard tool of the scientific community and regulatory agencies. This is a result of the abundance of research and development that has been devoted to the development of biological monitoring field methods and data analysis methods by various universities, federal agencies, and state agencies. The methods used today are scientifically defensible, applicable in nearly every region of the country, and have become indispensable in monitoring the health of the nation's aquatic ecosystems. Aquatic organisms provide an integrated view of ecological condition because they are often long-lived and sensitive to watershed-wide land use practices and management. For these reasons USEPA has required all states to assess, protect and if necessary restore the biological integrity of their stream resources. In addition EPA requires states to define aquatic life uses, utilize biological monitoring to determine attainment or non-attainment of those designated uses, and define biological criteria in support of those uses.

All 50 states currently have biological assessment programs in place. Fish, macroinvertebrates, and periphyton comprise the major aquatic assemblages utilized by state biological monitoring programs. The most common assemblage used is macroinvertebrates. All 50 states with the exception of Hawaii utilize this group of organisms for biological monitoring. However many states are benefiting from assessing more than one assemblage. USEPA (2002)found that using only one assemblage is only 80-85% effective for identifying attainment or non-attainment of designated uses, and thus recommends using more than one biological assemblage. As of 2001, 41 states were employing more than one assemblage and of those 20 were using at least three (USEPA 2002).

The major difficulty with biological monitoring is determining from biological data whether or not a sample site is degraded or not, and if it is to what extent. The concept of reference sites or reference conditions have proven very useful in determining the degree of impact a sample site may be experiencing. Reference sites are commonly chosen based on their level of human disturbance. Reference sites are most commonly defined as being the least-disturbed sites within an ecoregion. Reference conditions are determined statistically from an aggregate of data obtained from several least-disturbed sites within a region. For the reference site or condition concept to be effective, control or sample stations must have physical and chemical habitat characteristics similar to the reference site or reference condition. Reference sites or conditions provide a convenient and scientifically defensible yardstick from which to compare the biological condition of control stations. Currently the ecoregion reference condition concept is taking precedence over the use of site-specific reference conditions. In 1995 only 15 states had integrated ecoregional reference conditions into their biological monitoring programs, but by 2001, 39 states were utilizing this concept (USEPA 2002).

State and Federal biological monitoring programs commonly employ two basic methods of data analysis; these are the multimetric and multivariate approaches. Multimetric and multivariate analysis approaches are used to reduce large amounts of environmental and biological data into numeric values associated with biological condition. Biological metrics are indices that are expected to increase or decrease in value in response to increases or decreases in environmental perturbation or in response to specific environmental stressors. Commonly several metrics are combined to form an additive multimetric index of biological or ecological integrity. Multivariate approaches are statistical techniques, which determine the relationships of several variables simultaneously. Of 54 state and tribal entities surveyed by (USEPA 2002), 41 employed the multimetric approach. Biological multimetric indices and multivariate approaches are useless without integrating habitat condition data. Most states utilize a visual based habitat assessment method to support biological data. Methods such as Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) and USEPA's Rapid Bioassessment Protocol (RBP) are commonly employed by state agencies because they have been proven useful in nearly all regions of the country and are scientifically sound. In addition to the visual based assessment most entities also use quantitative measurements that characterize the stream channel under study and provide a measurable basis for detecting changes that may be associated with biological condition change.

Finally, the Clean Water Act (Federal Water Pollution Control Act of 1972, Clean Water Act of 1977, and the Water Quality Act of 1987) requires States to develop biological criteria; sections 303 and 304 provide the basis for development of biological criteria. Most states have developed and implemented narrative biological criteria, often in an antidegradation statement in their water quality standards. Some have developed biological criteria with specific reference to biological conditions and most have some form of specific criteria under development. Few states have developed numerical criteria based on a multimetric or multivariate biological assessment approach. Of the state entities presented in this report only Ohio has developed and implemented numeric biological criteria.

SUMMARY OF STATE BIOLOGICAL ASSESSMENT METHODS

I. STATE OF ARKANSAS

The Arkansas Department of Environmental Quality biological monitoring program utilizes macroinvertebrate and fish communities. Fish communities are evaluated following methods contained in USEPA's Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analysis (USEPA 1983) with an emphasis on the management of game fish. Macroinvertebrate communities are evaluated following USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Plafkin, Barbour et al. 1989). Other details of methodology are outlined in state documents (7/1/1994; Arkansas Game and Fish Commission and USDA Forest Service 1994).

SAMPLING REACH CONSIDERATIONS

No related material.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

A D-frame net with a width of 0.3 meters and a mesh size of 500 microns is used for the collection of macroinvertebrates. Because Arkansas' topography results in two very different general habitat conditions for streams, Ozark mountain high gradient streams and lowland low gradient streams, a different method is utilized for each type of stream. Macroinvertebrates are collected in both during the spring (April through mid-June) and fall (September through October). It appears that a single composite sample is collected at each site and consists of 20 one-meter kick samples.

Mountain Regions –

Traveling kick method. Five-minute kick samples along diagonal transect. Two riffles sampled at each site.

Low Gradient Streams –

Twenty one-meter long kick samples taken to equal approximately 6 square meters of sampled substrate. Habitats are sampled in proportion to their area in sample reach.

Specimens preserved in the field with 70% ETOH

LABORATORY METHODS

Subsampling

4-inch diameter ring randomly tossed into tray containing samples. Invertebrates collected from ring. Method repeated until a minimum of 95 organisms has been sampled.

Level of Taxonomic Identification

No related material.

DATA ANALYSIS AND INTERPRETATION

Table 1. Metrics used by Arkansas Department of Environmental Quality for the interpretation of macroinvertebrate community data.

Data Category	Metric	Expected metric response to increasing perturbation
Taxa Richness	Number of of taxa	Decrease
	Number of of EPT taxa	Decrease
	Number of of Ephemeroptera taxa	Decrease
	Number of of Plecoptera taxa	Decrease
	Number of of Trichoptera taxa	Decrease
	Number of of Coleoptera taxa	Decrease
	Number of of Diptera Taxa	Decrease
Community Composition Measures	Shannon-Weiner Index	Decrease
	% dominant taxon	Increase
	% EPT	Decrease
	% Ephemeroptera	Decrease
	% Plecoptera	Decrease
	% Trichoptera	Decrease
	% Diptera	Increase
	% Chironomidae	Increase
	% Amphipoda	Decrease
	% Isopoda	Increase
Tolerance Measures	Hilsenhoff Biotic Index	Increase
	Number of of Intolerant taxa	Decrease
	% Tolerant taxa	Decrease
Trophic Measures	% Shredders	Decrease
	% Collectors	Variable
	% Filterers	Decrease
	% Scrapers	Decrease
	% Predator	Variable

FISH COMMUNITY ASSESSMENT

ADEQ uses the methods and protocols presented in USEPA's Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analysis (USEPA 1983).

Arkansas Game and Fish Commission uses a variety of fish sampling techniques utilizing both quantitative and qualitative sampling. Electrofishing is conducted with either a backpack unit or an electrofishing boat unit. Depletion, mark-recapture, and catch per unit effort (CPUE) data are collected depending on the management goals of the sampling effort. Rotenone sampling is conducted primarily in areas such as bayous where current is slow and water is very turbid. Explosives are sometimes used because of its effectiveness in collecting large fish. Seines, trawls, and gill, trammel, hoop, and trap nets are used to collect qualitative, mark-recapture, and CPUE data.

PHYSICAL HABITAT ASSESSMENT

The physical habitat assessment methods used by ADEQ are taken from USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Plafkin, Barbour et al. 1989). It consists of subjectively scoring several categories of instream and riparian habitat parameters on a 0 to 20 scale. Habitat quality is rated according to four qualitative condition categories defined as poor, marginal, suboptimal, and optimal.

REFERENCE SITE SELECTION

No related material.

BIOLOGICAL CRITERIA

Narrative

Biological Integrity - All waters with specifically designated Fisheries uses must demonstrate aquatic life communities, which are similar in variety and abundance to least-disturbed waters within the same ecoregion and with similar hydrologic conditions. Measurements of biological integrity should include fish community structure and other associated aquatic life *e.g.*, macroinvertebrates, periphyton, plankton, etc. Measurements should be extensive and timely in order to compensate for the seasonal and natural variability of aquatic life communities. A distinguishable alteration of the abundance or variety of the aquatic life community constitutes a violation of these water quality standards.

Numeric

None

II. STATE OF INDIANA

Sampling protocols are presented in a variety of state documents (Indiana Department of Environmental Management 2001; Indiana Department of Environmental Management 2001; Dofour Consulting No date given).

SAMPLING REACH CONSIDERATIONS

Avoid areas of obvious degradation, bridges provide convenient access. For some studies sample sites are chosen randomly and are sampled regardless of condition.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Indiana Department of Environmental Management only indicates that several USEPA Rapid Bioassessment Protocol methods are used (Indiana Department of Environmental Management 2001; 2001). The sampling period for macroinvertebrates extends from July to October. The number of macroinvertebrate samples collected at each site was not specified but each site is visited on a rotational basis such that sampling occurs no more than once ever other year.

LABORATORY METHODS

Subsampling

100 organism subsample

Level of Taxonomic Identification

Family

DATA ANALYSIS AND INTERPRETATION

Average multi-metric score compared to reference conditions. Metrics used not listed.

FISH COMMUNITY ASSESSMENT

FIELD METHODS

No related material except that fish are sampled once per site and sampling is done on a rotational watershed basis such that sites are visited once every two to five years or longer.

LABORATORY METHODS

No related material.

DATA ANALYSIS AND INTERPRETATION

Fish data are analyzed using the Index of Biological Integrity (IBI). The IBI is plotted against Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) to determine relationships between habitat and fish community characteristics.

Table 2. Fish Index of Biological Integrity metrics used by the Indiana Department of Environmental Management.

Fish IBI metrics used by IDEM	
Total Number of Species	Percent omnivore species
Number of darter, madtom, and sculpin species	Percent insectivore species
Number of darter species	Percent pioneer species
Percent headwater species	Percent carnivore species
Number of sunfish species	Catch per unit effort/number of individuals
Number of minnow species	Percent simple lithophilic species
Number of sucker species	Percent DELT anomalies
Number of sensitive species	Percent tolerant species
Number of salmonid species	Percent large river species
Number round-bodied sucker species	Number of centrarchid species
Catch per unit effort/number of individuals – gizzard shad	

PHYSICAL HABITAT ASSESSMENT

Follows Ohio EPA's Qualitative Habitat Evaluation Index (QHEI).

REFERENCE SITE SELECTION

Reference conditions determined using regional site aggregation methods and professional judgment. Deviations from central tendency in multimetric biological indices and Ohio EPA's QHEI are taken into consideration.

BIOLOGICAL CRITERIA

Under Development.

III. STATE OF IOWA

Protocols are presented in a variety of state documents (Iowa Department of Natural Resources 1994; Iowa Department of Natural Resources 1999).

SAMPLING REACH CONSIDERATIONS

Sample reaches for streams vary from 150 meters to 500 meters depending on stream size and habitat type frequency. Guidelines suggest that the reach should include 3 distinct pool and riffle habitats. In streams where pool and riffle sequences are unavailable then the reach should include three well-defined channel bends.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

Iowa Department of Natural Resources utilizes both semi-quantitative and qualitative sampling of macroinvertebrates to assess the biological integrity of streams. A macroinvertebrate index of biological integrity (MIBI) is used for data analysis and interpretation, however metrics for the MIBI were not provided. Three replicate samples are taken at each site and are analyzed as separate samples.

FIELD METHODS AND INDEX PERIOD

Semi-Quantitative:

> Modified-Hess samplers or Surber samplers are used in streams and rivers with riffles and runs less than 1.5 feet deep. The samples are not composited.
>
> Modified Hester-Dendy samplers are used in streams where riffle habitat is absent. Three modified artificial substrates consisting of eight 1/8" × 3" × 3" wood plates and twelve 1" square wooden spacers are placed at a sampling site. The total area of artificial substrate available for colonization at each sample site is 145.6 square inches. The samplers are deployed in water 1 to 2 feet deep having a velocity of 0.5 to 1.5 feet per second. The colonization period is a minimum of 4 weeks and a maximum of 6 weeks. The samples are not composited.

Multi-habitat Sampling - Qualitative

> Qualitative sampling is performed to supplement the semi-quantitative sampling by provide better representation of taxa throughout the sample reach. This is performed the same day artificial substrates are retrieved. The mesh size of sieves, wash-buckets, and kick nets should be 500 to 600 microns. Sampling is performed from all available natural habitats concentrating on those that provide the highest amount of diversity, *e.g.*, riffles and woody debris snags. The qualitative samples are composited in the field. Stream sampling for macroinvertebrates is conducted annually between July 15 and October 15.

Field Preservation

> Samples are preserved in the field with a 10% formalin solution buffered with 3 grams of borax to each liter of formalin solution.

LABORATORY METHODS

Laboratory Preservation

> Prior to identification and enumeration of the samples the organisms are transferred from the 10% buffered formalin solution to 85% ethanol solution.

Subsampling

Random 100-organism subsamples. Each of the triplicate Modified Hess samples are subsampled individually. The qualitative composite sample is subsampled.

Level of Taxonomic Identification

Lowest practical level depending on the abundance of difficult to identify organisms such as chironomids and oligochaetes, time constraints, and availability of taxonomic keys. Dipteran midge larvae (Chironomidae) are not currently identified beyond family.

DATA ANALYSIS AND INTERPRETATION

Iowa is in the process of finalizing the development and adoption of a multimetric index for use in assessing macroinvertebrate community health for Iowa's streams and rivers. They evaluated about 39 potential metrics and produced a final index consisting of 12 (Table 3).

Table 3. Summary of Iowa Department of Natural Resources' macroinvertebrate metrics and some of their characteristics. Multi-habitat (MH) and single habitat (SH) derived metrics are indicated in the table. All percents are based on number of organisms not number of taxa.

Data Metric	Metric variability (sampling error)	Impacted Site Discriminatory Power
MH – taxa richness	low	moderate
MH – EPT richness	low	high
MH – sensitive taxa	moderate	high
SH – taxa richness	low	moderate
SH – EPT richness	low	high
Percent of 3 dominant taxa	low	high
Biotic index	low	high
Percent EPT	low	low
Percent Chironomidae	high	high
Percent Ephemeroptera	moderate	moderate
Percent scrapers	moderate	moderate
Percent dominant functional feeding group	low	moderate

A metric score procedure was established so that each metric scoring range was from 0 –10 and the macroinvertebrate index (BM-IBI) has a possible range from 0 to 100.

FISH COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Single pass electrofishing. Second pass may be performed if results of first pass are unsatisfactory. Fish are captured using ¼" mesh nets. Time sampled in seconds and length and average width of the stream reach sampled are recorded to provide CPUE data. Fish are preserved in 10% formalin solution. Fish sampling is most often accomplished from spring through fall (*e.g.,* October), and one sample is collected per site.

LABORATORY METHODS

Fish are identified to species.

DATA ANALYSIS AND INTERPRETATION

Elements analyzed include: species composition, number of species, relative abundance of species, catch per unit effort, proportion of fish sampled with external abnormalities such as parasites, lesions, eroded fins, or deformities.

PHYSICAL HABITAT ASSESSMENT

The habitat assessment is performed in conjunction with the macroinvertebrate and fish sampling. Two forms are used. One is used for assessment of the habitat in which the semi-quantitative macroinvertebrate sampling devices were deployed and the other for the assessment of habitats sampled during the course of the qualitative multi-habitat macroinvertebrate samples. Together the assessment includes subjective estimates of periphyton growth, dominant type of periphyton, amount of sedimentation and a ranking of the five most abundant types of benthic substrates in the sample reach.

REFERENCE SITE SELECTION

The documentation of a reference conditions and site selection process is ongoing.

BIOLOGICAL CRITERIA

Under Development.

IV. STATE OF KANSAS

SAMPLING REACH CONSIDERATIONS

Kansas Department of Wildlife and Parks (KDWP) conducts fish and macroinvertebrate sampling as part of USEPA Regional Environmental Monitoring and Assessment Program (REMAP) projects and utilizes the protocols designed for that program, including sample reach definitions and considerations (USEPA 1998). Kansas Department of Health and Environment (KDHE) conducts macroinvertebrate sampling in support of their water quality programs. The KDHE protocols used are presented below.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

KDHE field collections are made by two people sampling over a period of 30 minutes for a total of one person/hour sample effort at a site. All available macrohabitats are sampled using D-frame invertebrate nets with 500-micron mesh nets. Kick sampling; sweep sampling, and sieving fine sediments through the net are viable collection techniques used with the D-frame net. Directly picking invertebrates from large substrates is also used. The total sample should contain 200 or more total organisms, with no more than 50 organisms being obtained from any single microhabitat. Each person collects and stores their samples in their own individual sample container. Samples are collected in the spring of the first year, the summer of the next, and the fall of the next, the cycle is repeated every three years. The typical index period is May through September but can extend into October.

Field Preservation

70-80% ethyl alcohol (ETOH).

LABORATORY METHODS

Laboratory Preservation

70-80% ETOH with 5% glycerin. Samples are retained for two years.

Subsampling

No subsampling in the laboratory. The samples obtained individually by the two field collectors are considered as two subsamples.

Level of Taxonomic Identification

Lowest possible level.

DATA ANALYSIS AND INTERPRETATION

The results of the two 30 minute sample efforts are compared as an indication of sampling precision. KDHE uses number of taxa; EPT ratio; KBI using tolerance values for nutrient and oxygen-demanding substances (see Huggins and Moffett 1988); and the MBI as metrics for quantifying macroinvertebrate communities. The MBI is a family/order level biotic index based on the Hilsenhoff Biotic Index (Hilsenhoff 1987). Percent mussel taxa loss is also used and typically all metrics are given equal weight in 305(b) evaluations.

FISH COMMUNITY ASSESSMENT

No related material for KDHE. Kansas Department of Wildlife and Parks as part of the EPA Region 7 REMAP program use USEPA REMAP protocols for the evaluation of stream fish communities.

PHYSICAL HABITAT ASSESSMENT

KDHE utilizes a simple subjective habitat development index (HDI). Macrohabitat types are given a score of 3 if present or a score of 0 if not present. Average depths of the macrohabitats are scored from 0 to 2 according to a list of categories on the HDI form. Scores are generated for categories of riffle substrate size and level of embeddedness. The type and quantity of organic debris are scored on a scale of 0 to 3. Filamentous algal masses are scored 0 for absence or 1 for presence, with limited epiphytic biological growths scored as 0 as they provide little physical shelter. Macrophytes are scored from 0 to 2 depending on presence, absence, and quantity. Bank vegetation is scored from 0 to 2 depending presence, absence, and the quantity adjacent to each sampled macrohabitat. The total score on the form is tabulated to derive a sample score.

Kansas Department of Wildlife and Parks uses an HDI that derives a total stream quality score based on subjective ratings of several variables within each of four basic habitat quality components. The first component is physical habitat including ratings for flow, substrate types, substrate quality, instream cover, macrohabitat presence/absence, and bank erosion. The second component is riparian/floodplain quality including estimates of riparian vegetation, canopy cover, and adjacent land use categories. A biological component is included which requires data on fish, macroinvertebrates, mussels, and amphibians. The final component is pollution as indicated by Secchi depth, evidence of pollution other than silt, and whether chemical variables measured in the field are considered limiting or non-limiting to the aquatic community.

REFERENCE SITE SELECTION

The Kansas Department of Health and Environment KDHE (1995) defines a reference site as one that "represents an unusually pristine location, suitable for use as a long-term ecoregional reference location." Their intended use is to "identify the variation in community structure and species abundance associated with relatively unperturbed streams in a given land use setting, geological or geographical area, or ecoregion." Reference site data in Kansas are compared to invertebrate community data from sample sites to determine if the community is degraded and affected by water quality problems.

BIOLOGICAL CRITERIA

Under Development.

V. STATE OF MINNESOTA

The macroinvertebrate biological assessment SOPs of Minnesota Pollution Control Agency concentrate on wetlands. The wetlands SOPs also include assessment and calculation of indices based on vegetative sampling. Stream macroinvertebrate protocols are Under Development.and not currently available. However, fish SOPs are detailed and data analysis of fish data in the form of IBIs is separated into two protocols developed for cool-water streams of the upper Mississippi River basin and cool-water streams of the St. Croix River basin. Methodologies are documented in a variety of references (1994; Niemela and Feist 2000; Gernes and Helgen 2002; Niemela and Feist 2002; Genet and Chirhart 2004; Minnesota Pollution Control Agency No date given; Minnesota Pollution Control Agency No date given; Minnesota Pollution Control Agency No date given).

SAMPLING REACH CONSIDERATIONS

Sampling reach for fish should be 35 times the mean stream width.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Standard No. 30 sieve.

D-frame net with 600-micron mesh. Two samples are taken within same general location of nearshore emergent vegetation by sweeping action of the net 3-5 times. The two samples are composited.

Activity trap samplers are used to sample invertebrates that are active swimmers or night-active predators. This is a device constructed from clear 2-liter beverage containers by cutting the top of the container off and inverting it into the body of the container. Ten activity traps are deployed in 5 pairs 3-4 meters apart in wetlands near shoreline emergent vegetation and left for two nights. The samples are composited. A sampling period of June to early July is preferred, but sampling could be performed earlier if spring temperatures permit.

Field Preservation

80% ETOH

LABORATORY METHODS

Level of Taxonomic Identification

Typically lowest level, but most commonly genus level.

Laboratory Preservation

80% ETOH

DATA ANALYSIS AND INTERPRETATION

Stream macroinvertebrate IBI, are in development and may be similar to those proposed for the Upper Mississippi River basin in Minnesota Table 4.

Wetland macroinvertebrate IBI metrics are listed in Table 5.

Table 4. Scoring criteria for the three separate M-IBIs developed for the Upper Mississippi River Basin in Minnesota (modified from Genet and Chirhart 2004).

Metric	Range	Response to disturbance	Score 0	Score 2	Score 4
Riffle/Run, < 500 mi^2					
# Trichoptera Taxa	1–15	decrease	0–4	5–8	>8
# Ephemeroptera + Plecoptera Taxa	1–9	decrease	0–4	5–6	>6
# Diptera Taxa	4–24	decrease	0–10	11–16	>16
# Orthocladiinae +Tanytarsini Taxa	1–11	decrease	0–4	5–7	>7
# Intolerant Taxa	0–14	decrease	0	1–4	>4
# Scraper Taxa	0–13	decrease	0–4	5–7	>7
# Collector–Gatherer Taxa	3–19	decrease	0–10	11–14	>14
% Trichoptera (excluding Hydropsychidae)	0–22.2	decrease	0	>0–3.3 >3.3	
% Non–Insect	2.8–76.2	increase	>42.6	>22.7–42 .	6 0–22.7
HBI	4.77–7.67	increase	>6.70	>5.74–6.70	<5.74
Glide/Pool, < 40 mi^2					
POET	1–16	decrease	0–6	7–11	>11
# Clinger Taxa	0–11	decrease	0–4	5–7	>7
# Collector–Filterer Taxa	1–8	decrease	0–3	4–6	>6
# Intolerant Taxa	0–5	decrease	0–2	3	>3
% Dominant Taxon	12.8–65.4	increase	>47.8	>30.3–47.8	<30.3
% Ephemeroptera	0–50.3	decrease	0–5.9	>5.9–22.8	>22.8
% Intolerant	0–32.1	decrease	0–1	>1–3.3	>3.3
% Tolerant	28.2–95.1	increase	>72.8	>50.5–72.8	0–50.5
% Trichoptera (excluding Hydropsychidae)	0–8.4	decrease	0	>0–1	>1
HBI	4.85–8.65	increase	>7.38	>6.11–7.38	<6.11
Glide/Pool, > 40 mi^2					
% Coleoptera + Hemiptera	0–38.4	increase	>16.5	>8.2–16.5	0–8.2
# Gastropoda Taxa	1–6	decrease	0–2	3–4	>4
# Non–Insect Taxa	4–10	decrease	0–6	7–8	>8
% Caenidae	0–43.2	increase	>7	>0–7	0
% Oligochaeta	0–10.6	increase	>2.3	>1.1–2.3	0–1.1
% Crustacea + Mollusca	0.6–94.6	decrease	0–26.2	>26.2–51.7	>51.7
# Odonata + Trichoptera Taxa	2–17	decrease	0–7	8–12	>12

Table 5. Wetland invertebrate IBI metrics with criteria and score for Minnesota large depressional wetlands.

Metric	Criteria	Score
Total invertebrate taxa	>51-77	5
	>36-51	3
	<21-36	1
Odonata taxa	>4	5
	3-4	3
Chironomid genera taxa	14->21	5
	7-13	3
	0-6	1

Leech taxa	5-9	5
	3-4	3
	0-2	1
Snail taxa	7-9	5
	4-6	3
	0-3	1
ETSD Metric: number of mayfly genera, number of caddisfly genera, presence of fingernail clams, presence of dragonflies	>6-10	5
	>3-6	3
	0-3	1
Number of intolerant taxa	5-7	5
	3-4	3
	0-2	1
Proportion of tolerant taxa	16-42%	5
	>42-69%	3
	>69%	1
Proportion of dominant 3 taxa	<34-54%	5
	>54-74%	3
	>74-94%	1
Proportion of Corixidae as beetles and bugs in activity traps	<33%	5
	33-67%	3
	>67%	1

FISH COMMUNITY ASSESSMENT

FIELD METHODS

Backpack electrofishing units used in streams with less than 8 meters mean stream width and watersheds less than 50 square miles. A single run is conducted in an upstream direction.

Towed electrofishing units used in streams with a mean stream width greater than 8 meters and a watershed size of 50-500 square miles. A single run is conducted in an upstream direction.

Mini-boom electrofishing unit is used in non-wadeable streams where accessibility for large electrofishing boats is not feasible or the stream is too small for such craft. The mini-boom is an electrofishing unit set up in a small "jon" style boat. A single run is conducted in a downstream direction.

Boom-shocker is used in large accessible rivers. This is a large stable boat capable of carrying a three-person electrofishing crew. Three runs are conducted in a downstream direction; one run on each bank of the river and one in the mid-channel.

Voucher specimens are retained. Fish less than 25 mm in total length are not included in data. Total length and minimum and maximum lengths are recorded for each species and the collective weight of each species measured obtained to the nearest 0.5 gram.

Field Preservation

10% formalin solution

LABORATORY METHODS

No related material.

DATA ANALYSIS AND INTERPRETATION

Minnesota has developed several fish IBIs for the upper Mississippi River basin and the St. Croix River basin. Various IBI metrics are applied in each basin depending on drainage size, creating tailored IBIs for these two major basins and for various drainage sizes within each basin. A major deviation in the Minnesota Pollution Control Agency's IBIs from most is that the scoring criteria for the metrics have a wider range of values, using a scoring system ranging from 0 to 10 instead of the usual 1, 3, and 5. The total IBI score range is 0 to 100. The tables on the following pages show generally how the MPCA fish IBIs metrics are scored and interpreted.

Table 6. Interpretation of MPCA fish IBI metrics total score.

Total IBI Score	Biological Integrity Rating	Interpretation
100-80	Excellent	Comparable to minimal disturbance.
79-60	Good	Somewhat disturbed.
59-40	Fair	Decreased species richness, some signs of ecological imbalance.
39-20	Poor	Decreased species richness, growth and condition of fish depressed.
19-0	Very Poor	Fish community severely modified by human disturbance.
no score		Few or no fish, impossible to calculate score.

Table 7. Scoring criteria for the 7 metrics used to calculate the IBI for very small streams (< 5 mi^2 drainage area) in the Upper Mississippi River Basin of Minnesota*.

	Scoring criteria				
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	9 or more	7 or 8	5 or 6	3 or 4	0 - 2
Number of wetland species**	2 or more		1		0
Percent tolerant species***	0-80	81-85	86 - 90	90-95	96-100
Percent dominant two species***	0-60	61-70	71 - 80	81-90	91-100
Trophic metrics					
Number of invertivore species**	2 or more		1		0
Fish abundance and condition metrics					
Number of fish per 100 meters	2		5 or more		0-4
Percent DELT anomalies***	0-1		2 or 3		4 or more

* For very small streams the sum of the 7 metrics must be multiplied by 1.43 to obtain a final IBI score.
The metrics for number of wetland species, number of invertivore species, and number of fish per 100 meters do not include tolerant species.*Round all percent metrics to the nearest 1 percent.

Table 8. Scoring criteria for the 10 metrics used to calculate the IBI for small streams (5 to 35 mi^2 drainage area) in the Upper Mississippi River Basin of Minnesota.

	Scoring Criteria				
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	14 or more	11-13	8-10	5-7	0-4
Number of wetland species*	3 or more		1 or 2		0
Number of minnow species*	5 or more	4	2 or 3	1	0
Number of intolerant species	2 or more		1		0

Percent tolerant species**	0-40	41-55	56-70	71-85	86-100
Percent dominant two species**	0-52	53-64	65-76	77-88	89-100
Trophic and reproductive function metrics					
Number of invertivore species*	5 or more	4	2 or 3	1	0
Percent simple lithophils**	49-100	37-48	25-36	13-24	0-12
Fish abundance and condition metrics					
Number of fish per 100 meters*	5 or more		0–4		
Percent DELT anomalies**	0-1		2 or 3	4 or more	

* Number of wetland species, number of minnow species, number of invertivore species, and number of fish per 100 meters metrics do not include tolerant species.
** Round all percent metrics to the nearest 1 percent.

Table 9. Scoring criteria for the 10 metrics used to calculate the IBI for moderate size streams (35 to 200 mi^2 drainage area) in the Upper Mississippi River Basin of Minnesota.

	Scoring Criteria				
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	20 or more	16-19	12-15	8-11	0-7
Number of darter, sculpin, and madtom species	4 or more	3	2	1	0
Number of wetland species*	3 or more		1 or 2		0
Number of intolerant species	4 or more	3	2	1	0
Percent tolerant species**	0-35	36-50	51-65	66-80	81-100
Trophic and reproductive function metrics					
Number of invertivore species*	8 or more	6 or 7	4 or 5	2 or 3	0-1
Number of piscivore species	5 or more	4	2 or 3	1	0
Percent simple lithophils**	61-100	46-60	31-45	16-30	0-15
Fish abundance and condition metrics					
Number of fish per 100 meters*	5 or more				0
Percent DELT anomalies**	0-1		2 or 3		4 or more

*Number of wetland species, number of invertivore species, and number of fish per 100 meters metrics do not include tolerant species
**Round all percent metrics to the nearest 1 percent.

Table 10. Scoring criteria for the 10 metrics used to calculate the IBI for rivers (> 200 mi^2 drainage area) in the Upper Mississippi River Basin of Minnesota.

Scoring Criteria					
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	28 or more	23-27	18-22	13-17	0-12
Number of darter, sculpin, and madtom species	4 or more	3	2	1	0
Number of intolerant species	4 or more	3	2	1	0
Percent tolerant species**	0-15	16-30	31-45	46-60	61-100
Trophic and reproductive function metrics					
Number of invertivore species*	14 or more	11-13	8-10	5-7	0-4
Percent omnivore species**	0-10	11-20	21-30	31-40	41 or more
Number of piscivore species	7 or more	6	4 or 5	3	0-2
Percent simple lithophils**	81-100	61-80	41-60	21-40	0-20
Fish abundance and condition metrics					
Number of fish per 100 meters	1		5 or more		0
Percent DELT anomalies**	0-1		2 or 3		4 or more

*Number of invertivore species and number of fish per 100 meters metrics do not include tolerant species.
**Round all percent metrics to the nearest 1 percent.

Table 11. Scoring criteria for the nine metrics used to calculate the IBI for very small streams (< 20 mi^2 drainage area) in the St. Croix River Basin of Minnesota*.

	Scoring Criteria				
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	10 or more	8 or 9	6 or 7	4 or 5	0 - 3
Number of headwater species**	3 or more		1 or 2		0
Number of minnow species**	5 or more	4	2 or 3	1	0
Percent tolerant species***	0-60	61-70	71 - 80	81-90	91-100
Percent dominant two species***	0-52	53-64	65 - 76	77-88	89-100
Trophic composition and reproductive function metrics					
Number of invertivore species**	5 or more	4	2 or 3	1	0
Percent simple lithophils***	49-100	37-48	25-36	13-24	0-12
Fish abundance and condition metrics					
Number of fish per 100 meters**	11 or more				0-10
Percent DELT anomalies***	0-1		2 or 3		4 or more

*The sum of the nine metrics for headwater streams must be multiplied by 1.11 to obtain the final IBI score.
**Number of headwater species, number of minnow species, number of invertivore species, and number of fish per 100 meters metrics do not include tolerant species.
***Round all percent metrics to the nearest 1 percent.

Table 12. Scoring criteria for the nine metrics used to calculate the IBI for small streams (20 to 54 mi^2 drainage area) in the St. Croix River Basin of Minnesota*.

	Scoring Criteria				
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	15 or more	12-14	9-11	6-8	0-5
Number of intolerant species	4 or more	3	2	1	0
Number of minnow species**	6 or more	5	3 or 4	2	0 or 1
Percent tolerant species***	0-40	41-55	56-70	71-85	86-100
Percent dominant two species***	0-44	45-58	59-72	73-86	87-100
Trophic composition and reproductive function metrics					
Number of benthic invertivore species	4 or more	3	2	1	0
Percent simple lithophils3***	49-100	37-48	25-36	13-24	0-12
Fish abundance and condition metrics					
Number of fish per 100 meters**	11 or more				0–10
Percent DELT anomalies***	0-1		2 or 3		4 or more

*The sum of the 9 metrics for headwater streams must be multiplied by 1.11 to obtain the final IBI score.
**Number of minnow species, and number of fish per 100 meters metrics do not include tolerant species.
***Round all percent metrics to the nearest 1 percent.

Table 13. Scoring criteria for the ten metrics used to calculate the IBI for moderate size streams (55 to 270 mi^2 drainage area) in the St. Croix River Basin and rivers (>270 mi^2 drainage area) in the Northern Lakes and Forests ecoregion portion of the St. Croix River Basin in Minnesota.

	Scoring Criteria				
Metric	**10**	**7**	**5**	**2**	**0**

Species richness and composition metrics					
Total number of species	23 or more	20-22	17-19	14-16	0-13
Number of darter species	5 or more	4	3	2	0 or 1
Number of intolerant species	8 or more	7	4-6	3	0-2
Percent tolerant species*	0-20	21-40	41-60	61-80	81-100
Trophic composition and reproductive function metrics					
Number of benthic invertivore species	9 or more	7 or 8	5 or 6	3 or 4	0-2
Number of omnivore species	0 or 1	2	3	4	5 or more
Percent piscivore species*	25-100	19-24	13-18	7-12	0-6
Percent simple lithophils*	61-100	46-60	31-45	16-30	0-15
Fish abundance and condition metrics					
Number of fish per 100 meters**	11 or more				0–10
Percent DELT anomalies*	0-1	2 or 3	4 or more		

*Round all percent metrics to the nearest 1 percent.
**Number of fish per 100 meters metrics does not include tolerant species.

Table 14. Scoring criteria for the ten metrics used to calculate the IBI for rivers (>270 mi^2 drainage area) in the North Central Hardwood Forests ecoregion portion of the St. Croix basin of Minnesota.

	Scoring Criteria				
Metric	**10**	**7**	**5**	**2**	**0**
Species richness and composition metrics					
Total number of species	29 or more	24-28	19-23	14-18	0-13
Number of darter species	5 or more	4	3	2	0 or 1
Number of intolerant species	8 or more	7	6	5	0-4
Percent tolerant species*	0-20	21-40	41-60	61-80	81-100
Trophic composition and reproductive function metrics					
Number of benthic invertivore species	11 or more	9 or 10	7 or 8	5 or 6	0-4
Number of omnivore species	0 or 1	2	3	4	5 or more
Percent piscivore species*	25-100	19-24	13-18	7-12	0-6
Percent simple lithophils*	61-100	46-60	31-45	16-30	0-15
Fish abundance and condition metrics					
Number of fish per 100 meters**	11 or more				0–10
Percent DELT anomalies*	0-1		2 or 3		4 or more

*Round all percent metrics to the nearest 1 percent.
**Number of fish per 100 meters metric does not include tolerant species.

PHYSICAL HABITAT ASSESSMENT

Habitat assessment is conducted using 13 transects evenly spaced along the length of the sampling station. At each transect five visual estimates or measurements are taken of habitat variables. Measurements are made of water depth, depth of fine sediments and water. Visual estimates are made of embeddedness of coarse substrates, and dominant substrate type. Five 0.3 × 0.3 meter quadrats are established across the transect from which percent of the quadrat containing algae and the percent of the quadrat containing emergent or submergent macrophytes are estimated. Cover and land use characteristics are estimated along the length of each transect including percent of the transect that contains various classes of fish cover, bank erosion, riparian land use, riparian buffer width, and canopy density. Channel characteristics are measured or estimated including channel condition, mean distance between bends, mean distance between riffles, total length of pools, runs, and riffles, and total number of pools, runs and riffles.

REFERENCE SITE SELECTION

No related material.

BIOLOGICAL CRITERIA

Narrative

The biological quality of any given surface water body shall be assessed by comparison to the biological integrity of a reference condition or conditions which best represents the most natural condition for that surface water body type within a geographic region. The biological quality shall be determined by reliable measures of indicative communities of fauna and flora.

Numeric

None

VI. STATE OF MISSOURI

Methodology is detailed in a number of state documents (Missouri Department of Natural Resources - Division of Environmental Quality 1994; Missouri Department of Natural Resources 1998; Missouri Department of Natural Resources 1998; Missouri Department of Natural Resources 1998).

SAMPLING REACH CONSIDERATIONS

Macroinvertebrate bioassessment methods are intended for use in streams with an average depth of less than 1.5 meters. Only habitats that are commonly found in the study reaches are sampled, uncommon habitats are not sampled. Sampling reaches are defined as twenty times the average stream width. Two comparable reaches within three stream miles are sampled for community characterization.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Missouri utilizes methods obtained from EPA RPB and the North Carolina Division of Environmental Quality. These methods provide a multi-habitat semi-quantitative approach. The following sample types are taken at a sample site. Macroinvertebrates are collected between September 15 and October 15, which coincides with stable baseflow conditions.

Flowing water – coarse substrates
- Kick samples using D-frame net with 500-micron mesh
- Approximately one square meter each sample
- Six-sample composite
- Variety of microhabitats sampled

Non-flowing water
- Kick samples using D-frame net with 500-micron mesh
- Approximately one square meter each sample
- Six-sample composite
- Variety of microhabitats sampled

Vegetation
- Kick samples using D-frame net with 500-micron mesh
- Approximately one square meter each sample
- Six-sample composite
- Variety of microhabitats sampled

Flowing water – fine substrates
- Kick samples using D-frame net with 500-micron mesh
- Approximately one square meter each sample
- Twelve-sample composite
- Variety of microhabitats sampled

Leaf packs
- Six handfuls are collected and elutriated through a brine shrimp net
- Variety of flow conditions are sampled
- No mention of compositing samples in document

Snags
- Twelve-sample composite
- 400 × 600 square centimeters each sample
- Organisms are brushed off of snag material into a 500-micron mesh bag
- Variety of flow conditions and log deterioration states are sampled

Root mats and undercut banks
- Kick samples using D-frame net with 500-micron mesh
- Approximately one-meter strip of shoreline for each sample

Six-sample composite
Variety of microhabitats sampled

Field Preservation

10% Formalin solution

LABORATORY METHODS

Level of Taxonomic Identification

Lowest possible level.

Laboratory Preservation

80% ETOH is used during subsampling and identification.
70% ETOH is used for permanent storage of reference specimens.

Subsampling

600-organism subsample for coarse substrate samples taken in flowing water..
300-organism subsample for all other habitat samples.

Organisms are subsampled from composited samples using a grid system in large plastic trays. Grids are chosen using a random number generator.

DATA ANALYSIS AND INTERPRETATION

Large/rare species are only used in the calculation of Taxa Richness and EPT Taxa Index values; they are omitted from the calculation of all other metrics.

Stream Condition Index (SCI) is a multimetric approach used to quantify the biological condition at sample sites. Usually only the four primary metrics listed are used (see Table 15), only in special circumstances are secondary metrics used. A minimum of six reference site samples is used to form a distribution of metric scores. The range of these score distributions is divided into four quartiles, with the upper bound of the lower quartile defining an approximation of reference condition. Metrics calculated from study sites that score above the reference condition assigned a value of 5, the range of values below the reference condition are divided in half and assigned values of 3 and 1. The sample site metric scores are totaled for the final SCI site score. Interpretation of the final score is currently under development.

Quality control is achieved by collecting duplicate samples at 10% of the sample sites and they are analyzed for similarity with the regular samples.

Table 15. Macroinvertebrate metrics used in the Missouri Department of Natural Resources Stream Condition Index.

Primary Metrics	**Response to Increasing Water Quality**
Taxa Richness	Increase
EPT Taxa Index	Increase
Biotic Index	Decrease
Shannon's Diversity Index	Increase
Secondary Metrics	**Response to Increasing Water Quality**

Quantitative Similarity Index for Taxa (QSI-T)	Increase
Pinkham and Pearson Similarity Index (PPSI)	Compared to Reference Site Data
Percent Dominant Taxa	Decrease
Dominants in Common	Compared to Reference Site Data
Percent Scrapers	Decrease
Quantitative Similarity Index for Functional Feeding Groups (QSI-FFG)	Compared to Reference Site Data

FISH COMMUNITY ASSESSMENT

No related material.

PHYSICAL HABITAT ASSESSMENT

Missouri's Department of Natural Resources habitat assessment is a modification of EPA RPBs. Three main categories of habitat parameters are measured or estimated. Primary parameters characterize microhabitat scale features and are scored on a scale of 0-19. Secondary parameters characterize macrohabitat scale features and are scored on a scale of 0-15. Tertiary parameters characterize riparian and bank features and are scored on a scale of 0-11. This scoring scheme weighs primary and secondary parameters above tertiary parameters. The final score of a sample site is compared to nearby or regional reference stream data and interpreted as comparable to reference conditions or three lower levels of supporting conditions. For low gradient streams, two primary parameters and one secondary parameter are substituted.

Table 16. List of Primary, Secondary, and Tertiary parameters for use in high gradient riffle/run prevalent streams and substitution parameters for use in glide/pool prevalent low gradient streams utilized by the Missouri Department of Natural Resources.

Riffle/Run Prevalent High Gradient	**Glide/Pool Prevalent Low Gradient Parameter Substitutions**
Primary Parameters	**Primary Parameters**
Bottom Substrate/Instream Cover	
Embeddedness	Pool Substrate Characterization
Stream Flow or Velocity/Depth Regime	Pool Variability
Canopy cover	
Secondary Parameters	**Secondary Parameters**
Island and Point Bar Growth	
Bottom Scouring and Deposition	
Riffle to Riffle/Width Ratio	Channel Sinuosity
Lower Bank Channel Capacity	
Tertiary Parameters	
Upper Bank Stability	
Bank Vegetative Stability/Grazing Pressure	
Streamside Cover	
Riparian Vegetative Zone Width	

Table 17. Missouri Department of Natural Resources habitat assessment score interpretation. The score is interpreted by its percent similarity to local or regional reference conditions.

Percent Similarity to Reference Condition	**Interpretation**
$\geq$ 90%	Comparable to Reference
75-89%	Supporting
60-74%	Partially Supporting

<59%	Non-Supporting

REFERENCE SITE SELECTION

Definition

Reference sites are defined as having as little human disturbance as possible.

Identification

Potential reference sites are selected if the habitat quality of the potential site is comparable to established reference conditions. The total score from the physical habitat assessment must be greater than or equal to 90% similar to established reference conditions to be considered a reference site.

BIOLOGICAL CRITERIA

Narrative

The biological integrity of waters, as measured by lists or numeric diversity indices of benthic invertebrates, fish, algae, or other appropriate indicators shall not be significantly different from reference waters. Waters shall be compared with reference waters of similar size within an ecoregion.

Numeric

None

VII. STATE OF NEBRASKA

Methodologies are detailed in a number of state documents (Nebraska Department of Environmental Quality 1992; 1993; Nebraska Department of Environmental Quality 1997; Nebraska Department of Environmental Quality 1999).

SAMPLING REACH CONSIDERATIONS

Sampling reach should contain all available habitats needed to obtain a representative sample of fish and macroinvertebrates.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Both qualitative and quantitative methods are used dependent upon monitoring objectives. While no specific collection period was noted, inspection of their data suggests that nearly all macroinvertebrate samples were collected from May through September. The number of macroinvertebrate samples collected during routine monitoring efforts appears to be limited to a single sample.

Qualitative Sampling

> Sampling equipment includes No. 30 mesh screen or D-frame net. Sampling is conducted for 15 to 30 minutes in all available habitat types. Hand picking of larger substrates such as rocks and woody debris is also recommended.

Semi-Quantitative Sampling

> Nebraska Department of Environmental Quality (NDEQ) uses EPA Rapid Bioassessment Protocols (Barbour, Gerritsen et al. 1999).
>
> Sampling equipment includes No. 30 mesh screen or D-frame net. All available habitats sampled for a known length or area.
>
> Ponar grab sampler, Surber sampler, Hester-Dendy multiple plate artificial substrate, Peterson grab sampler. D-frame kick nets are used to collect from a known area.

Field Preservation

> 10% Formalin or 70 ETOH

LABORATORY METHODS

Laboratory preservation

> Samples are stained with rose Bengal (100g/L) in either 10% formalin or 70% ETOH. Samples are retained for three years after collection.
>
> No. 30 mesh screen sieve for washing samples.
>
> Sugar may be added to 70% ETOH or 10% formalin solution to aid in floating organisms free of debris.

Chironomidae, Naididae, and Tubificidae are mounted with CMC-10 mounting media on a glass slide with a cover slip.

Subsampling

Field samples are washed in No. 30 (500 micron) mesh screens. Subsampling is only conducted in cases where excessive amounts of specimens are contained in the sample.

Subsamples are obtained by mixing a field sample in a container rapidly, and then the sample is poured into a dish with an evenly marked grid. From this grid a percentage of the sample is removed at the discretion of the analyst. The unused portion is then examined for new and unusual specimens not included in the subsample. Each subsample should contain no fewer than 100 organisms.

In samples dominated by very large number of individuals from a single taxonomic group, all organisms are separated and counted. 70 organisms are then removed from the total sample and the ratio of the various taxa is then calculated to determine the approximate number of each taxon in the total sample.

Biomass

Biomass per unit area is calculated for samples taken with semi-quantitative sampling equipment. Dry weight and ash-free dry weight are measured.

Level of Taxonomic Identification

Lowest possible level.

DATA ANALYSIS AND INTERPRETATION

The following indices are calculated:

Shannon-Weaver diversity index.
Dry weight and ash-free dry weight are reported as grams per square meter.
Hilsenhoff Biotic Index
Jaccard Coefficient of community Similarity
Invertebrate Community Index (ICI) (Plafkin, Barbour et al. 1989; Barbour, Gerritsen et al. 1999).

Table 18. Invertebrate Community Index metrics used by Nebraska Department of Environmental Quality.

Taxa Richness	Shredder/Total Number Individuals Ratio
EPT Taxa Richness	EPT/Total Taxa Index
Chironomid Taxa Richness	EPT/Chironomidae Abundance
Hisenhoff Biotic Index	Community similarity Indices
EPT/Chironomid + EPT Ratio	Community Loss Indices
Percent Dominant Taxa	Index of Similarity Between Two Samples
Scraper to collector + Filterer Ratio	Pinkham and Person Community Index
Jaccard Index	Other Similarity Indices
Scraper/Filterer + Scraper Ratio	Presence/Absence of Specific Indicator Organisms

Data Quality Control

Complete samples are re-identified by a different analyst. The difference in the numbers of individual taxa and the relative abundance of the taxa must fall within 20 percent of the original

identification effort. All debris present during the first identification will be retained for the second identification to ensure replicability.

FISH COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Semi-quantitative sampling methods are employed where all habitat types are sampled in a reach of sufficient length and for a time adequate to obtain a representative sample from the reach. Collection of fish samples for bioassessment purposes is most desirable between late May and early September.

Seining

No guidelines provided for seine size, mesh size, or number of passes. Electric seines are cited as being more efficient than seines and backpack electrofishing units.

Electrofishing

Boat electrofishing rigs, boat mounted (tote barge), and backpack units using either DC or AC current. Document favors DC. Sampling in a downstream direction is recommended for electrofishing from a boat in a stream or river.

Passive sampling equipment

Gill nets, hoop nets, and fyke nets. Gill nets are recommended for shallow areas with no current. Hoop nets are recommended for sampling rivers with strong current. Fyke nets are recommended for sampling shallow areas in ponds and lakes.

Block nets are recommended for isolating the stream fish communities.

Field Preservation

10% formalin is used for preservation in the field.

Field Fish Processing

Fish that are readily identifiable to species are identified in the field and released. Total lengths and weights are recorded for game species. Fish that are not readily identifiable in the field are identified in the laboratory.

LABORATORY METHODS

Preservation

70% ETOH is used for laboratory preservation.

Level of taxonomic identification

Species level.

DATA ANALYSIS AND INTERPRETATION

Catch per unit time and stream length sampled are calculated.

The Index of Biological Integrity (IBI) as defined and refined by Karr *et al.* (1986) is used by NDEQ. The IBI is scored based on comparisons between the individual metric values at control

sites to those expected from reference conditions given a stream of similar size in a similar geographic region. A value of (5) is given if the metric approaches that expected at a reference site, (3) if it deviates somewhat, and (1) if it deviates strongly from values expected at a reference site. The following values may be calculated for analysis of fish community data.

Table 19. Fish Index of Biological Integrity metrics used by the Nebraska Department of Environmental Quality.

Community Diversity Indices	
Shannon-Weaver index	Simpson Values for Nonrandom Samples
Margalef Diversity	Shannon Diversity and Evenness
Menhinick Diversity	Brillouin Diversity and Evenness
Simpson Dominance	Heip Evenness
Simpson Diversity and Evenness	Sheldon Evenness
Inverse Simpson Dominance and Evenness	
Community Similarity Indices	
Jaccard Coefficient	Sorensen Coefficient
Percent Similarity	Morisita Index
Dissimilarity Index	Horn Index

Population Estimation

Peterson Method – This is a mark-recapture method, where the fish captured at a sampling event are marked in some way, usually a fin clip, then the population is sampled again and marked individuals are counted as recaptures. A population estimate can then be calculated from the proportion of marked individuals to unmarked individuals.

Schnabel Method – This is mark-recapture method using the multiple-census method where fish are marked and recaptured several times within the same population. This method is best used on large bodies of water for increasing the confidence interval of the samples.

Leslie Method – This is a depletion sampling method where the population, *e.g.*, in a small pond or blocked section of stream are sampled to point where the catch per unit effort (CPUE) is diminished. Linear regression is then used on the subsequent sampling passes to obtain a population estimate.

PHYSICAL HABITAT ASSESSMENT

Physical habitat characteristics are estimated subjectively and measured directly depending on the variable. The observations include flow, channel alteration, bank stability, habitat degradation rating, substrate composition, stream width, stream depth, pool description, land use, grazing damage, riffle and run occurrence, stream cover, stream stage, water clarity, runoff influence, aquatic vegetation composition, vegetation stability, and stream order.

Simple subjective ranked assessment of stream quality and a simple inventory of the habitat in support of fish.

NDEQ also conducts a "Biological Network Reconnaissance and Habitat Survey" with several subjective ratings of 1) watershed conditions such as topography, land use, point sources, and land treatments, 2) riparian conditions including general and stream bank, 3) general stream conditions including flow and channel conditions, water quality, and instream habitat measurements taken at three transects.

A "Biological Network Aquatic Vegetation Survey" is conducted where species of emergent and submergent vegetation present in the sample reach are recorded.

Additional measurements or subjective observations are vegetative riparian zone width, bank stability, degradation rating, instream substrate size, stream width, stream depth, stream stage, vegetation occurrence, grazing damage, riffle/run occurrence, pool description, sand bars, land use, and stream cover.

REFERENCE SITE SELECTION

Definition

Reference sites as defined by NDEQ should meet the following conditions: 1) least impacted site typical of ecoregion and stream type conditions, 2) unimpacted by point sources, 3) unimpacted by nonpoint source activities, and 4) habitat conditions represent the best that is presently achievable.

Identification

Reference sites are determined in the field using a standardized evaluation form consisting of 7 subjective threshold yes/no questions, any of which answered as “no” will disqualify the site. It also contains 7 subjective ratings of various instream, watershed, and riparian factors, which are scored on a scale of 1-7. An index value is determined by adding all rating scores and a site is rejected if the index score is below a specific threshold value.

BIOLOGICAL CRITERIA

Narrative

Any human activity that would significantly impact or displace an identified "key species" shall not be allowed.

DEFINITIONS

Key species are identified endangered, threatened, sensitive, or recreationally important aquatic species. Key species are designated by stream segment.

Numeric

None

VIII. STATE OF OHIO

Methodologies and regulations are detailed in a variety of references (Ohio EPA 1987; Ohio EPA 1987; Ohio EPA 1987; Rankin 1989; 1990; DeShon 1995).

SAMPLING REACH CONSIDERATIONS

Selection of fish community sampling sites used by Ohio EPA are based upon the following factors. 1) Location of point source discharges. 2) Stream use designation evaluation issues. 3) Location of physical habitat features. 4) Location of non-point sources of pollution. 5) Variations in habitat. Sample sites should contain typical and representative habitat to that of the segment under study. Sites downstream of pollution sources are usually supplemented with data from control sites upstream of the pollution source. If no control sites are available upstream of the study area, then control sites may be chosen from adjacent streams with similar physical characteristics. Ecoregional reference sites may also be used.

Sample stations have a length of 150 to 200 meters for wadeable and headwater stream sites, and 500 meters for non-wadeable stream sites.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Quantitative Sampling

> The primary sampling equipment used by Ohio EPA is the modified Hester-Dendy multi-plate artificial substrate sampler. The surface area of the sampler totals 145.6 square inches. Hester-Dendy samplers are placed in runs whenever this habitat type is available. Colonization time is six weeks. Three or five samplers are placed per sample site. Surber square foot samplers are also used. Quantitative samples from deep water are taken with Ekman, Peterson, or Ponar samplers. Five samples are taken with the respective deepwater gear. Ohio EPA samples macroinvertebrates from June 15 to September 30. Sampling that occurs outside this window needs to be scrutinized since reference values are calibrated to sites sampled within the indexing period.

Qualitative Sampling

> D-frame kick nets, Ekman, Peterson, and Ponar are used for qualitative sampling. Sampling takes place near the artificial substrate quantitative sampling devices. All available habitats are sampled until no new taxa appear in subsequent samples. When qualitative samples are taken without quantitative sampling the sampling effort is increased substantially. Qualitative only samples are taken for a minimum of 30 minutes and may continue longer than 30 minutes if further sampling generates new taxa.

Field Preservation

> 70% ETOH

LABORATORY METHODS

Level of Taxonomic Identification

> Lowest possible level.

Laboratory Preservation

70% ETOH
10% KOH used for clearing Chironomidae species.

CMCP 9/9AF or Euparal mounting mediums used for mounting Chironomidae species.

Subsampling

Quantitative samples are either composited or used as replicates, depending on study objectives, *e.g.*,routine monitoring or litigation purposes. Organisms and material are sieved through a standard number 30 then standard number 40 standard testing sieves. Folsom sample splitters are used for all subsampling after sorting and removing large/rare taxa. Very large sampled may be split before sorting. After sorting an entire sample to the family level, families that have unmanageable numbers of individuals may be subsampled. At least 250 organisms must be contained in a subsample, including 50-100 midges, 70 caddisflies, and 70 mayflies.

DATA ANALYSIS AND INTERPRETATION

Ohio EPA uses several indices and coefficients for describing in the analysis of benthic macroinvertebrate communities. The community measures are then related to environmental variables measured or estimated during the course of a site visit. Community composition metrics include Shannon's diversity index, Lloyd and Ghelardi's expression of the Equitability Index, Van Horn's Community Similarity index. Spearman's Rank Correlation Coefficient is used to describe the relationship between measured biological, chemical, or physical data. Coefficient of Variation is calculated for replicate samples.

Ohio EPA's Invertebrate Community Index (ICI) is the principal measure of overall community condition used by its staff. Ten metrics are scored and assigned points according to one of four numeric scoring categories; 6 points if a metric has a value comparable to that of an exceptional stream community; 4 points for values typical of a good stream community; 2 points for values deviating slightly from values typical of a good stream community; and 0 points for values deviating strongly from values typical of a good stream community.

Table 20. Ohio EPA's Invertebrate Community Index metrics and scoring criteria.

	Scoring Criteria			
Metric	**0**	**2**	**4**	**6**
Total Number of Taxa		Varies With Drainage Area		
Total Number of Mayfly Taxa		Varies With Drainage Area		
Total Number of Caddisfly Taxa		Varies With Drainage Area		
Total Number of Dipteran Taxa		Varies With Drainage Area		
Percent Mayflies	0	0-10	10-25	>25
Percent Caddisflies		Varies With Drainage Area		
Percent Tribe Tanytarsini Midges	0	0-10	10-25	>25
Percent Other Dipterans and Non-insects		Varies With Drainage Area		
Percent Tolerant Organisms		Varies With Drainage Area		
Total Number of Qualitative EPT Taxa		Varies With Drainage Area		

FISH COMMUNITY ASSESSMENT

FIELD METHODS

Pulsed DC electrofishing is the primary fish collection method used by Ohio EPA, however eleven methods are described as valid for use in their biological assessment program. Non-wadeable streams are sampled using a boat-mounted electrofishing unit. Backpack electrofishing units, Sportyak-generator units

(*e.g.,* towed barge unit), longline generator units, gill nets, Fyke nets, hoop nets, trap nets, and seines are also used.

Sample reaches for boat electrofishing are at least 0.5 kilometer and fished in a downstream direction. A single pass is made; subsequent passes are made in five to six week intervals.
Fish samples are weighed collectively, by species, or individually.

Field Preservation

10% formalin solution buffered with one teaspoon of borax per ½ gallon.

LABORATORY METHODS

Laboratory Preservation

Fish are fixed in a buffered 10% formalin solution for 2 to 3 weeks. Formalin is drained from the fish for ½ hour before being placed in 35% ETOH for another 2 to 3 weeks. Fish are then transferred to 50% ETOH for another 2 to 3 weeks before being placed in 70% ETOH for permanent storage.

DATA ANALYSIS AND INTERPRETATION

Relative abundance data is generated in terms of numbers per species per unit distance or weight per species per unit distance for active sampling gear, numbers per unit time for passive gear. Measures of community composition and characteristics include total number of species per sample, cumulative number of species per sampling location, Shannon' Diversity based on numbers of individuals and weight of individuals, modified Index of Well-Being, and the Index of Biological Integrity.

PHYSICAL HABITAT ASSESSMENT

Ohio EPA uses a habitat assessment called the Qualitative Habitat Evaluation Index (QHEI) (Rankin 1989). This index provides a measure of macrohabitat quality factors that affect the quality of habitat available for fish communities and also those habitat characteristics that generally important to groups of aquatic invertebrates.

The QHEI consist of 18 parameters contained in six broad categories. The scoring range for QHEI is from 0 to 100. Table 21 shows the parameters their respective scoring ranges.

Table 21. Ohio EPA's QHEI main parameter categories, parameters score ranges, and total potential points per category.

Parameter Category	Subcategory	Potential Points/Subcategory	Parameter Score Range
Substrate			**20**
	Type	0 - 20	
	Quality	-5 - 3	
Instream Cover			**20**
	Type	0 - 9	
	Amount	1 - 11	
Channel Quality			**20**
	Sinuosity	1 - 4	
	Development	1 - 7	
	Channelization	1 - 6	
	Stability	1 - 3	
Riparian/Erosion			**10**

	Width	0 - 4	
	Floodplain Quality	0 - 3	
	Bank Erosion	1 - 3	
Pool/Riffle			**20**
	Maximum Depth	0 - 6	
	Current Available	-2 - 4	
	Pool Morphology	0 - 2	
	Riffle/Run Depth	0 - 4	
	Riffle Substrate Stability	0 - 2	
	Riffle Embeddedness	-1 - 2	
Gradient		0 - 10	**10**

REFERENCE SITE SELECTION

Identification

Map detailing human population density, number and size of point discharges, and current and past land use patterns are used to determine least-impacted watersheds within an ecoregion. Candidate sites within these watersheds are examined aerially for desirable and representative features as well as examined on the ground. Ground examination includes biological and physical habitat assessment.

BIOLOGICAL CRITERIA

Narrative

Biological criteria presented in table 7-17 (see numeric criteria section) to this rule provide a direct measure of the attainment of the warm water habitat, exceptional warm water habitat and modified warm water habitat aquatic life uses. Biological criteria and the exceptions to chemical-specific or whole-effluent criteria allowed by this paragraph do not apply to any other use designations.
(a) Demonstrated attainment of the applicable biological criteria in a water body will take precedence over the application of chemical-specific or whole-effluent criteria associated with these uses when the director, upon considering appropriately detailed chemical, physical and biological data, finds that one or more chemical-specific or whole-effluent criteria are inappropriate. In such cases the options that exist include:
(a)(i) The director may develop, or a discharger may provide for the director's approval, a justification for a site-specific water quality criterion according to methods described in "Water Quality Standards handbook, 1983, U.S. EPA Office of Water";
(a)(ii) The director may proceed with establishing water quality based effluent limits consistent with attainment of the designated use.
(b) Demonstrated nonattainment of the applicable biological criteria in a water body with concomitant evidence that the associated chemical-specific criteria and whole-effluent criteria are met will cause the director to seek and establish, if possible, the cause of the nonattainment of the designated use. The director shall evaluate the existing designated use and, where not attainable, propose to change the designated use. If the designated use is deemed attainable, the director shall, whenever possible and reasonable, implement regulatory controls or make other recommendations regarding water resource management to restore the designated use.

Definitions-

"Warmwater"- these are waters capable of supporting and maintaining a balanced, integrated, adaptive community of warmwater aquatic organisms having a species composition, diversity, and functional organization comparable to the twenty-fifth percentile of the identified reference sites within each of the following ecoregions: the interior plateau ecoregion, the Erie/Ontario lake plains ecoregion, the western Allegheny plateau ecoregion and eastern corn belt plains ecoregion. For the Huron/Erie lake plains ecoregion, the comparable species composition, diversity and functional organization are based upon the

ninetieth percentile of all sites within the ecoregion. For all ecoregions, the attributes of species composition, diversity and functional organization will be measured using the index of biotic integrity, the modified index of well-being and the invertebrate community index as defined in "Biological Criteria for the Protection of Aquatic Life: volume II, Users Manual for Biological field Assessment of Ohio Surface Waters," ... Attainment of this use designation is based on the criteria in table 7-17 to this rule. A temporary variance to the criteria associated with this use designation may be granted as described in paragraph (G) of rule 3745-1-01 of the Administrative Code.

"Exceptional Warmwater" - these are waters capable of supporting and maintaining an exceptional or unusual community of warmwater aquatic organisms having a species composition, diversity, and functional organization comparable to the seventy-fifth percentile of the identified reference sites on a statewide basis. The attributes of species composition, diversity and functional organization will be measured using the index of biotic integrity, the modified index of well-being and the invertebrate community index as defined in "Biological Criteria for the Protection of Aquatic Life: volume II, Users Manual for Biological field Assessment of Ohio Surface Waters,". In addition to those stream segments designated in rules 3745-1-08 to 3745-1-32 of the Administrative Code, all lakes and reservoirs, except upground storage reservoirs, are designated exceptional warmwater habitats. Attainment of this use designation (except for lakes and reservoirs) is based on the criteria in table 7-17 to this rule. A temporary variance to the criteria associated with this use designation may be granted as described in paragraph (G) of rule 3745-1-01 of the Administrative Code.

"Modified Warmwater" - these are waters that have been the subject of a use attainability analysis and have been found to be incapable of supporting and maintaining a balanced, integrated, adaptive community of warmwater organisms due to irretrievable modifications of the physical habitat. Such modifications are of a long-lasting duration (*i.e.*, twenty years or longer) and may include the following examples: extensive stream channel modification activities permitted under sections 401 and 404 of the act or Chapter 6131 of the Revised Code, extensive sedimentation resulting from abandoned mine land runoff, and extensive permanent impoundment of free-flowing water bodies. The attributes of species composition The attributes of species composition, diversity and functional organization will be measured using the index of biotic integrity, the modified index of well-being and the invertebrate community index as defined in "Biological Criteria for the Protection of Aquatic Life: volume II, Users Manual for Biological field Assessment of Ohio Surface Waters,". Attainment of this use designation is based on the criteria in table 7-17 to this rule. Each water body designated modified warmwater habitat will be listed in the appropriate use designation rule (rules 3745-1-08 to 3745-1-32 of the Administrative Code) and will be identified by ecoregion and type of physical habitat modification as listed in table 7-17 to this rule. The modified warmwater habitat designation can be applied only to those waters that do not attain the warmwater habitat designation that do not attain the warmwater habitat biological criteria in table 7-17 to this rule because of irretrievable modifications of the physical habitat. All stream segments designated modified warmwater habitat will be reviewed on a triennial basis (or sooner) to determine whether the use designation should be changed. A temporary variance to the criteria associated with this use designation may be granted as described. in paragraph (G) of rule 3745-1-01 of the Administrative Code.

Numeric

Table 22. State of Ohio Numeric Biological Criteria for waters of the state.

Ohio Numeric Biocriteria Biological criteria for Warm water, Exceptional Warm water, and Modified Warm water Habitats. Description and derivation of indices and ecoregions are contained in "Biological Criteria for the Protection of Aquatic Life: Volume II. Users Manual for Biological Field Assessment of Ohio Surface Waters" cited in paragraph (B) of Rule 3745-1-03 of the Administrative Code. These criteria do not apply to the Ohio River, lakes or Lake Erie river mouths.					
	Modified Warm Water Habitat				
Index Sampling Site Ecoregion[1]	**Channel Modification**	**Mine Affected**	**Impounded**	**Warm water**	**Exceptional Warm water**

				Habitat	Habitat
I. Index of Biotic Integrity (Fish)					
A. Wading Sites[2]					
HELP	22	--	--	32	50
IP	24	--	--	40	50
EOLP	24	--	--	38	50
WAP	24	24	--	44	50
ECBP	24	--	--	40	50
B. Boat Sites[2]					
HELP	20	--	22	34	48
IP	24	--	30	38	48
EOLP	24	--	30	40	48
WAP	24	24	30	40	48
ECBP	24	--	30	42	48
C. Headwater Sites[3]					
HELP	20	20	--	28	50
IP	24	24	--	40	50
EOLP	24	24	--	40	50
WAP	24	24	24	44	50
ECBP	24	24	--	40	50
II. Modified Index of Well-Being (Fish)[4]					
A. Wading Sites[2]					
HELP	5.6	--	--	7.3	9.4
IP	6.2	--	--	8.1	9.4
EOLP	6.2	--	--	7.9	9.4
WAP	6.2	5.5	--	8.4	9.4
ECBP	6.2	--	--	8.3	9.4
B. Boat Sites[2]					
HELP	5.7	--	5.7	8.6	9.6
IP	5.8	--	6.6	8.7	9.6
EOLP	5.8	--	6.6	8.7	9.6
WAP	5.8	5.4	6.6	8.6	9.6
ECBP	5.8	--	6.6	8.5	9.6
III. Invertebrate Community Index (Macroinvertebrates)					
A. Artificial Substrate Samplers[2]					
HELP	22	--	--	34	46
IP	22	--	--	30	46
EOLP	22	--	--	34	46
WAP	22	30	--	36	46
ECBP	22	--	--	36	46

[1] HELP = Huron/Erie Lake Plain Ecoregion. IP = Interior Plateau Ecoregion. EOLP = Erie/Ontario Lake Plain Ecoregion. WAP = Western Allegheny Plateau Ecoregion. ECPB = Eastern Corn Belt Plains Ecoregion

[2] Sampling methods descriptions are found in the "Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices," cited in paragraph (B) of Rule 3745-1-03 of the Administrative Code.

[3] Modification of the IBI that applies to sites with drainage areas less than 20 square miles.

[4] Does not apply to sites with drainage areas less than 20 square miles.

(Effective February 14, 1978; April 4, 1985; August 19, 1985; April 30, 1987; May 1, 1990)

SUMMARY OF NATIONAL BIOLOGICAL ASSESSMENT METHODS

I. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY: ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM (EMAP) METHODS.

The EMAP methodology (USEPA 1998) was designed to provide a protocol that may be used to conduct a one-site per day assessment with a field crew of four people. These methods were used in USEPA's EMAP from 1993 to 1998 and are used in modified versions for current state, regional, and national projects.

SAMPLING REACH CONSIDERATIONS

Sample reaches are defined as 40 times the average wetted width or 150 m, whichever is greater. Eleven cross-sectional transects divide the reach into ten equal portions and the transect lines provide points of measurement for assessment activities. Sample reaches are not moved to avoid man-made stream changes such as bridges, culverts, rip-rap, or channelized sections.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Sample points are located at the nine interior transect lines starting at the downstream-most interior transect line. It is determined randomly whether the first sample will be taken from the left , right, or center of the transect. Left, center, and right sample points correspond to 25%, 50%, and 75% distance across the wetted width. Once this is determined subsequent samples are taken at points following the left, center, and right sequence moving up the reach until the last of the nine transects is completed. Riffle/run and pool/glide samples are composited respectively for the entire reach and stored in a single container.

Each sample is a 20 second kick sample from a quadrant one net width wide and two net widths long giving a sampled area of approximately 0.5 m^2. A D-frame kick net with a net width of 0.5 m and having 600-micron mesh is used for sampling, the width of this net is not the standard 0.3-meter width that is common. A U.S. standard number 30 sieve is used for cleaning the field composited samples.

No guidelines presented for index period.

Field Preservation

70% ETOH.

LABORATORY METHODS

No guidelines presented.

DATA ANALYSIS AND INTERPRETATION

No guidelines presented.

FISH COMMUNITY ASSESSMENT

FIELD METHODS

The sample reaches are isolated with block nets. The reach is electrofished first using a backpack electrofishing unit. Seining is conducted after electrofishing. In streams having high habitat complexity and/or very large wadeable streams, intertransect sampling efforts are proportional to the mean intertransect

zone width. The total amount of effort expended on fish sampling should be no less than 45 minutes in small streams and no more than 3 hours in the largest streams.

Field Preservation

10% Formalin solution.

LABORATORY METHODS

No guidelines presented.

DATA ANALYSIS AND INTERPRETATION

No guidelines presented.

PERIPHYTON COMMUNITY ASSESSMENT

FIELD METHODS

Periphyton include algae, fungi, bacteria, and protozoa and are employed as indicators of ecological condition in the EMAP program. Periphyton samples are collected at the nine interior transects of the sampling reach. Two basic types of samples are collected from erosional and depositional habitats. Erosional habitat samples are collected from a large piece of hard substrate such as a rock or piece of wood. An area delimiter is used for periphyton sample collection. An area delimiter is a ring with an inside area of 12 cm^2. This device is placed on the substrate and the inside area is scrubbed with a toothbrush to dislodge the periphyton. The dislodged material is then funneled into a 500 ml sample bottle. In depositional habitats with fine substrates such as sand or silt, the area delimiter confines an area, then the top 1 cm of sediment is withdrawn from the delimiter using a 60 ml syringe. The contents of the syringe are then deposited into a 500 ml sample bottle.

LABORATORY METHODS

Both erosional habitat and depositional habitat samples are composited into one sample. Four further sample types are then created from each of the composited habitat type samples. 1) ID/enumeration samples which are used to determine taxonomic composition and the relative abundances of each taxa, 2) a sample for analysis of chlorophyll a, 3) a biomass sample analyzed as ash-free dry weight, and 4) an acid/alkaline phosphatase activity (APA) sample.

Laboratory preservation

Periphyton samples used for ID/enumeration analyses are preserved with 10% formalin solution.

PHYSICAL HABITAT ASSESSMENT

The following seven general habitat attributes are measured or estimated. Channel dimensions, channel gradient, channel substrate size and type, habitat complexity and cover, riparian vegetation cover and structure, anthropogenic alterations, and channel-riparian interaction.

The EMAP procedure for measuring stream channel characteristics is data intensive and thorough. 100 to 150 thalweg measurements are made at evenly spaced intervals along the stream reach. Wetted width is measured at each of the 11 transects and at a point in between each transect for a total of 21 measurements. Slope and backsite bearing are measured between each pair of transects.

Large woody debris such as tree branches, root wads, and logs are tallied in according to defined length and diameter classes at each of the 10 intertransect zones. Separate woody debris tallies are made in these zones for woody debris found below and above the bankfull height.

Channel and riparian characterization measurements are made at each of the 11 transects. Measurements include bank height, bank undercut distance, bank angle, bar width, riparian canopy density, substrate size class, substrate embeddedness, areal cover class and type, mid-layer and ground cover, areal cover class of fish concealment features, aquatic macrophytes, filamentous algae, human disturbances, and discharge.

Following the completion of all field activities at a sample site, including the habitat assessment generalized above, a rapid habitat and visual stream assessment utilizing EPA's RPB protocol is conducted to provide further documentary support to the overall assessment.

REFERENCE SITE SELECTION

EMAP methods are designed to provide data to help define reference conditions, but no specific guidance is given as to how these definitions should be developed.

II. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY: RAPID BIOASSESSMENT PROTOCOLS (RBPs)

EPA Rapid Bioassessment Protocols (RBPs) were designed as an inexpensive tool for determining whether streams are supporting or not supporting designated aquatic life use (Barbour, Gerritsen et al. 1999). However, they are also useful for characterizing stream impairment and determining the causes of impairment. They may also provide data in support of control and mitigation efforts, use attainability, cumulative impact studies, and characterizing regional reference conditions.

SAMPLING REACH CONSIDERATIONS

A 100-meter reach length is recommended for both single and multihabitat sampling. Alternatively the EMAP definition of a sample reach, *i.e.,* 40 times the average stream width may be used. It is also recommended that sample reaches should be at least 100 meters upstream of any anthropogenic disturbances such as bridges, low water dams, or rip-rap piles. The biological sampling methods employed in a given stream should be based on the habitat availability in reference streams in the same region (ecoregion), thus ensuring that sampling methods are uniform.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

USEPA's macroinvertebrate rapid bioassessment protocols are the most widely used set of protocols utilized by state agencies responsible for monitoring aquatic resources. The various methods presented in the RBP's are suitable for the effective sampling of streams having a wide range of habitat structure and complexity, gradients, and flow regimes.

FIELD METHODS AND INDEX PERIOD

Single Habitat Approach

> Riffle and run habitats, if available, should be the target sampling areas of the single habitat approach. A 1 square meter kick net with 500 micron mesh attached to two poles, analogous to a small fish seine, is used. One square meter of substrate is disturbed upstream of the kick net. Alternatively, if a D-frame invertebrate net is used several kicks are composited for each sample location. No set number of samples is recommended, however it is recommended that at least 2 to 3 samples are taken at various points in a riffle or run based on variations in velocity/depth characteristics or from a series of riffles or runs.

Multiple Habitat Approach

> The multihabitat approach should be used in streams with highly variable habitat characteristics especially when comparing streams in regions with highly variable streams in terms of habitat structure and flow regimes, and gradient. A D-frame net with 500-micron mesh is used to sample all available habitat types. 20 kick samples or jab samples are taken from all major habitat types. The 20 samples should be distributed proportional to the percentage of available major habitats. The total area in square meters sampled should be approximately 3.1. This approach should target the most productive major habitat types from which the highest diversity of macroinvertebrates is likely to be found. These habitat types include cobble and gravel, snags, vegetated banks, submerged macrophytes. In many low gradient streams it may be necessary to sample soft/fine sediments. The samples are composited into a single sample in the field.

Index Period

> No guidelines presented.

Field Preservation

95% ETOH diluted by water in sample.

LABORATORY METHODS

Level of Taxonomic Identification

Lowest practical level.

Laboratory Preservation

70% ETOH.

Subsampling

Subsamples consist of at least 200 organisms. A pan marked with a grid system is subsampled using random numbers until the desired number of organisms is achieved.

DATA ANALYSIS AND INTERPRETATION

Table 23. EPA's RBP best candidate benthic macroinvertebrate metrics and predicted responses to increasing stream perturbation.

Metric Category	Metric	Predicted response to increasing perturbation
Richness Measures		
	Total no. of Taxa	Decrease
	Number of EPT Taxa	Decrease
	Number of Ephemeroptera Taxa	Decrease
	Number of Plecoptera Taxa	Decrease
	Number of Trichoptera Taxa	Decrease
Composition Measures		
	% EPT Taxa	Decrease
	% Ephemeroptera	Decrease
Tolerance/Intolerance Measures		
	Number of Intolerant Taxa	Decrease
	% Tolerant Organisms	Increase
	% Dominant Taxon	Increase
Feeding Measures		
	% Filterers	Variable
	% Grazers and Scrapers	Decrease
Habitat Measures		
	Number of Clinger Taxa	Decrease
	% Clingers	Decrease

Table 24. EPA's RBP potential benthic macroinvertebrate metrics and predicted responses to increasing stream perturbation.

Metric Category	Metric	Predicted Response to increasing perturbation
Richness Measures		
	Number of Pteronarcys species	Decrease

	Number of Diptera Taxa	Decrease
	Number of Chironomidae Taxa	Decrease
Composition Measures		
	% Plecoptera	Decrease
	% Trichoptera	Decrease
	% Diptera	Increase
	% Chironomidae	Increase
	% Tribe Tanytarsini	Decrease
	% Other Diptera and non-insects	Increase
	% Corbicula	Increase
	% Oligochaeta	Variable
Tolerance/Intolerance Measures		
	Number of Intolerant Snail and Mussel Species	Decrease
	% Sediment Tolerant Organisms	Increase
	Hilsenhoff Biotic Index	Increase
	Florida Index	Decrease
	% Hydropsychidae or Trichoptera	Increase
Feeding Measures		
	% Omnivores and Scavengers	Increase
	% Collector Feeders of Coarse Particulate Organic Matter and Fine Particulate Organic Matter.	Variable
	% Gatherers	Variable
	% Predators	Variable
	% Shredders	Decrease
Life Cycle Measures	% Multivoltine	Increase
	% Univoltine	Decrease

FISH COMMUNITY ASSESSMENT

FIELD METHODS

Sample reaches should be consistently selected based on a fixed-distance designation or a proportional-distance designation at least 100 meters upstream from human disturbances and major tributary influences.

Block nets should be used to isolate the fish community to be sampled.

Fish less than 20 millimeters in length should not be included in the sample because of seasonal skewing effects on data, unreliable capture of such small individuals, age/size class ecological function differences, and difficulty of identification.

Backpack electrofishing units, tote barge electrofishing units, and seines are recommended sampling equipment. Electrofishing is conducted in an upstream direction and seining is conducted in a downstream direction.

All rare, threatened, endangered or other species in need of conservation should be identified and released in the field. Photographs may be taken to aid in identification of these species.

Field Preservation

10% Buffered Formalin Solution

LABORATORY METHODS

Laboratory Preservation

10% Buffered Formalin Solution

Level of Taxonomic Identification

Species

DATA ANALYSIS AND INTERPRETATION

The Index of Biological Integrity (IBI) is recommended for analysis of fish communities. Figure 1generalizes the development of a fish IBI adapted for regional or local fish assemblages. The original metrics used in the IBI of Karr et al. (1986), and various potential other metrics, are listed in Table 25.

Figure 1. Sequential process of IBI development, calculation, and interpretation of the fish Index of Biological Integrity. Taken from EPA RPB second edition (Barbour, Gerritsen et al. 1999).

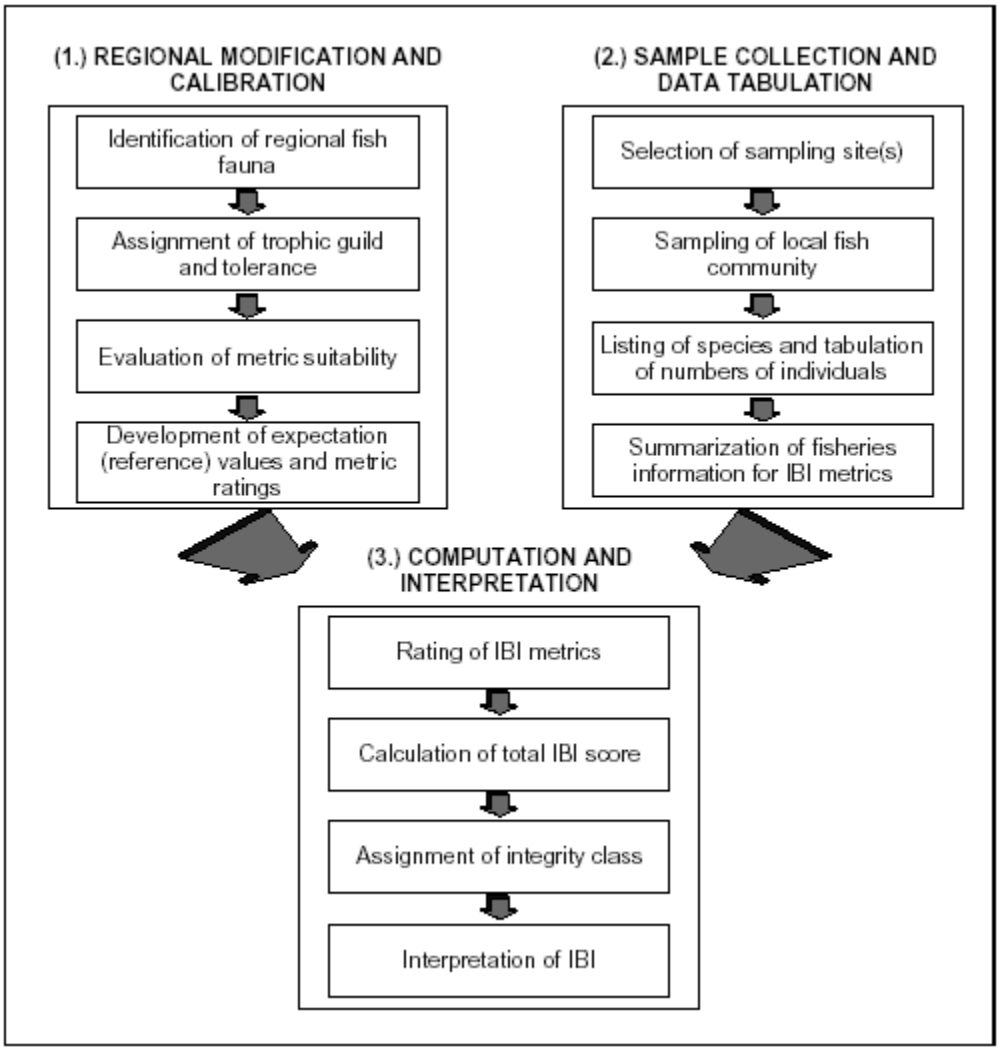


Table 25. Fish Index of Biological Integrity original metrics and alternative metrics developed for various regions of North America. Taken from EPA RPB second edition (Barbour, Gerritsen et al. 1999).

Alternative IBI Metrics	Midwestern United States	Central Appalachians	Sacramento-San Joaquin	Colorado Front Range	Western Oregon	Ohio Headwater Sites	Northeastern United States	Ontario	Central Corn Belt Plain	Wisconsin-Warmwater	Wisconsin-Coldwater	Maryland Coastal Plain	Maryland Non-Tidal
1. Total Number of Species	X	X	X	X				X		X		X	X
#native fish species					X	X	X		X		X		
# salmonid age classes[b]				X	X								
2. Number of Darter Species	X	X		X		X				X	X		
# sculpin species					X								
# benthic insectivore species								X					
# darter and sculpin species							X						
# darter, sculpin, and madtom species											X		
# salmonid juveniles (individuals)[b]			X		X			X					
% round-bodied suckers						X[c]							
# sculpins (individuals)			X										
# benthic species												X	X
3. Number of Sunfish Species	X			X		X				X	X		
# cyprinid species					X								
# water column species								X					
# sunfish and trout species									X				
# salmonid species			X							X			
# headwater species							X						
% headwater species							X			X			
4. Number of Sucker Species	X				X	X		X		X	X		
# adult trout species[b]			X		X								
# minnow species				X			X			X			
# sucker and catfish species									X				
5. Number of Intolerant Species	X			X	X	X		X			X	X	X	X
# sensitive species							X			X				
# amphibian species			X											
presence of brook trout									X					
% stenothermal cool and cold water species												X		
% of salmonid ind. as brook trout												X		
6. % Green Sunfish	X													
% common carp					X									
% white sucker				X				X						
% tolerant species						X	X			X	X	X	X	X
% creek chub		X												
% dace species									X					
% eastern mudminnow													X	

Table 26. Continuation of Table 25.

Alternative IBI Metrics	Midwestern United States	Central Appalachians	Sacramento-San Joaquin	Colorado Front Range	Western Oregon Ohio	Ohio Headwater Sites	Northeastern United States	Ontario	Central Corn Belt Plain	Wisconsin-Warmwater	Wisconsin-Coldwater	Maryland Coastal Plain	Maryland Non-Tidal
7. % Omnivores	X			X		X	X	X	X	X	X		
% generalist feeders		X											
% generalists, omnivores, and invertivores													X
8. % Insectivorous Cyprinids	X											X	
% insectivores					X			X		X	X	X	X[e]
% specialized insectivores		X		X									
# juvenile trout			X										
% insectivorous species						X	X						
9. % Top Carnivores	X					X		X	X	X	X	X	
% catchable salmonids					X								
% catchable trout			X										
% pioneering species							X			X		X	
Density catchable wild trout			X										
10. Number of Individuals (or catch per effort)	X	X	X	X	X	X[d]	X[d]		X	X	X[d]	X	
Density of individuals								X					X
% abundance of dominant species												X	X
Biomass (per m^2)													X[f]
11. % Hybrids	X							X					
% introduced species				X	X								
% simple lithophills						X				X	X		X
# simple lithophills species							X						
% native species			X										
% native wild individuals			X										
% silt-intolerant spawners												X	
12. % Diseased Individuals (deformities, eroded fins, lesions, and tumors)	X	X		X	X	X	X	X	X	X	X	X	X

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

PERIPHYTON

Two protocols are presented in EPA's RBP. The first is a species composition and biomass method performed in a laboratory, and the second is a field-based rapid survey without the need of a high level of taxonomic expertise. The two methods may be used in conjunction.

STANDARD LABORATORY-BASED APPROACH

Field Methods

Multihabitat sampling or single habitat sampling may be used. Multihabitat sampling is recommended for characterizing a single stream reach. Single habitat sampling is described as being the preferred method for comparison of different stream reaches. The single habitat approach should still be based on a stream reach, not a single location in a stream. Artificial substrates may be used as conditions dictate, such as in non-wadeable streams.

Multihabitat Field Sampling

Algae are collected from all available habitat types. A single composite sample is collected to characterize the algae assemblage at a reach. 5-milliliter subsamples from each habitat may be used for the reach composite sample. Sampling consists of placing substrates, including woody debris, into a bucket and scrubbing the organisms free of the substrate. For large substrates that cannot be scrubbed in a bucket, a piece of PVC pipe with a neoprene collar fitted to one end is placed on the substrate and the area inside is scrubbed with a brush. Water is then removed from the PVC ring with a pipette and placed into a sample container.

Single Habitat Field Sampling

Algae should be collected from riffles and runs with cobble substrate if possible, if not samples should be taken from any hard substrates available. Phytoplankton may be a reasonable alternative in low gradient streams lacking solid substrates. Several subsamples are collected from the single habitat type and composited.

Artificial Substrate Field Sampling

Floating or benthic periphytometers may be used with substrates consisting of glass slides, glass rods, clay tiles, Plexiglas plates or similar substrates. A minimum of 3 replicates should be placed at each sample reach. Two to four weeks should be allowed for periphyton to colonize the substrates. Samples may be composited or analyzed individually.

Level of Taxonomic Identification

Lowest possible level and generic.

Field Preservation

Lugol's (IKI) solution

FIELD-BASED RAPID PERIPHYTON SURVEY

This approach is a semi-quantitative rapid periphyton survey of algal biomass and taxonomic composition. Three transects are established across a habitat, usually riffle or run with water clarity such that algal accumulation can be observed readily. Three locations along each transect, *e.g.*,right bank, middle, and left bank, are sampled. At each sample location a viewing bucket (≥0.5 m diameter) containing a grid of 50

dots, is immersed into the water. First, the number of dots that occur over macroalgae where no substrate can be seen are counted. Second, the type of algae at each dot is also recorded. The number of dots under which suitable substrata available for macroaglal accumulation is recorded and the thickness (density) of macroalgal accumulation is recorded according to a scale ranging from 0 to 5.

LABORATORY METHODS

Non-Diatom Algae Relative Abundance and Taxa Richness.

Samples are homogenized using tissue homogenizer or blender. Pipettes are used for sampling the homogenized samples for identification. 300 algal cells units are identified under 400× magnification.

Diatom Relative Abundance and Taxa Richness

Subsamples of at least 5-10 concentrated preserved samples are identified under oil immersion at 1000× magnification. A count of 600 valves equaling 300 cells should be made until 10 valves each of 10 species have been identified at minimum.

Periphyton Biomass

Chlorophyll *a*, ash-free dry weight, cell densities per cm^2, and biovolume per cm^2 may be used for rapid bioassessment of algal communities.

Laboratory Preservation

Lugol's (IKI) solution

Diatoms should be cleared using concentrated acid oxidation using nitric or sulfuric acid, or hydrogen dioxide.

Subsampling

In both non-diatom and diatom relative abundance and taxa richness, 300 cells should be counted.

DATA ANALYSIS AND INTERPRETATION

Algal Metrics used in Rapid Bioassessment

Metrics of Biotic Integrity

1. Species richness – diatoms, soft algae, or both
2. Total Number of Genera – diatoms, soft algae, or both
3. Total Number of Divisions
4. Shannon's Diversity Index
5. Percent Community Similarity
6. Pollution Tolerance Index for Diatoms
7. Percent Sensitive Diatoms
8. Percent *Achnanthes minutissima*
9. Percent Live Diatoms

Diagnostic Metrics that Infer Ecological Conditions

1. Percent Aberrant Diatoms
2. Percent Motile Diatoms, *e.g.*, *Navicula* + *Nitzchia* + *Surirella*
3. Simple Diagnostic Metrics - % relative abundance of species that have environmental optima in extreme environmental conditions.

% acidobiontic + % acidophilic

% alkalibiontic + % alkaliphilic
% halophilic
% mesosaprobic + % oligosaprobic + % saprophilic
% eutrophic

4. Inferred Ecological Conditions with Simple Autecological Indices (SAI)
PH Spectrum
Nutrient (N and P) Spectrum
Halobion Spectrum – based on chloride concentrations or conductivity
Saprobien System –based on organic pollution

5. Inferred Ecological Conditions with Weighted Average Indices – based on specific ecological optima for algae

6. Impairment of Ecological Conditions – inferred from by calculating the deviation between inferred environmental conditions at a test site and at a reference site.

Algal Biomass

Repeated measures of algal biomass allow for the mean and maximum benthic chlorophyll *a* to be used in defining the trophic status of a stream, *e.g.*, oligotrophic, eutrophic, etc.

Information from the viewing bucket rapid assessment method should be expressed as the mean density of each type of macroalgae on suitable substrates and the maximum density of each type of microalgae on suitable substrates.

PHYSICAL HABITAT ASSESSMENT

The EPA RBP habitat assessment is a visually based estimate of the quality of ten basic and important habitat features important to the ecological function of a stream. Taking into account the fundamental differences in the structure of high and low gradient streams several of the parameters are scored differently based on high or low gradient status of the stream. The parameters are scored subjectively on either a scale of 0 to 20 or 0 to 10 with the scores divided into four classes defined as optimal, suboptimal, marginal, or poor. Listed below are 13 parameters whose quality is visually estimated.

1) Epifaunal Substrate/Available cover
2) Substrate Embeddedness
3) Pool Substrate Characterization
4) Velocity/Depth Combinations
5) Pool Variability
6) Sediment Deposition
7) Channel Flow Status
8) Channel Alteration
9) Frequency of Riffles or Bends
10) Channel Sinuosity
11) Bank Stability
12) Bank Vegetative Protection
13) Riparian Vegetative Zone Width

In some instances it may be desired to include some quantitative measurements and it is recommended in EPA RBP's to utilize EPA's EMAP protocols for quantitative habitat assessments.

REFERENCE SITE SELECTION

Reference conditions should be selected based on the goal of the monitoring effort. For some monitoring objectives (e.g. short-term impact studies), site specific or paired watershed reference conditions may be appropriate. For others, a regional approach may be more appropriate. No additional guidance given.

III. UNITED STATES GEOLOGICAL SURVEY: NATIONAL WATER-QUALITY ASSESSMENT PROGRAM (NAWQA).

The NAWQA program (Fitzpatrick, Waite et al. 1998; Moulton, Kennen et al. 2002)was implemented in 1991 as a national effort to provide data in support of national, regional, and local policy and management of water quality. The protocols utilized by NAWQA are supportive of biological assessment and long-term trend monitoring of aquatic resources affected by the quality of water.

SAMPLING REACH CONSIDERATIONS

The recommended reach length for wadeable streams is 150 to 300 meters depending on stream width, depth, and habitat disturbances. For non-wadeable streams and rivers, sampling reach lengths between 500 and 1000 meters are recommended.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Semi-Quantitative Sampling

A series of discrete semi-quantitative samples are taken from Richest-Targeted Habitats (RTH). RTHs are microhabitats of high macroinvertebrate abundance and taxa richness such as cobble riffles, woody debris snags, or the bases of emergent macrophytes. The RTH samples are combined into a single composite sample for the reach. No guidelines are given in the protocols for the number of samples to be taken at a particular site or habitat.

Equipment that may be used, depending on the characteristics of the sample reach is:

Slack Sampler with 500-micron screen, and 0.25 meters squared template area.

For fine substrates such as sand and silt, Ekman or Petite Ponar grab samplers may be used. Screens on the samplers must be 500 microns.

Qualitative Sampling

Discrete samples are collected from each habitat type in the sampling reach and are referred to as Qualitative Multi Habitat (QMH) samples, which are combined into a single composite qualitative sample for the reach.

D-frame kick net with 500-micron mesh netting

Handpicking selected large substrates, *e.g.*, large cobble, leaf packs, and root wads.

Grab samplers such as Ekman or Petite Ponar with 500-micron screens for fine substrates.

1 hour of sampling time is allocated to QMH at the reach, dividing time proportionally between the different habitat types within the reach.

Index Period

No guidelines presented.

Field Preservation

10% Formalin solution

DATA ANALYSIS AND INTERPRETATION

Dependent upon study objectives.

FISH COMMUNITY ASSESSMENT

FIELD METHODS

Electrofishing may be conducted with a boat-mounted, towed, or backpack electrofishing unit depending on stream size and depth. Two passes are made through the sample reach. Data for each pass is recorded separately.

Block nets may be used, but are not required for NAWQA studies.

Seining is conducted in all habitat types, with all types of seines having ¼ inch mesh. Common sense seines, collection, and bag seines of different lengths and heights may be used depending on the situation in the reach. 3 seine hauls per habitat. Three hauls from a habitat type are combined as a composite sample for that habitat type.

Field Preservation

10% Formalin solution

DATA ANALYSIS AND INTERPRETATION

Dependent upon study objectives.

ALGAL COMMUNITY ASSESSMENT

QUANTITATIVE

Select a sampling method that allows for the total area sampled from the substrate to be calculated.

Richest Target Habitat (RTH) - 3 subsamples

1) chlorophyll *a*
2) ash-free dry mass (AFDM)
3) taxa richness, cell density, relative abundance, biovolume of algal species

Depositional Targeted Habitat (DTH) - 3 subsamples

1) chlorophyll *a*
2) ash-free dry mass (AFDM)
3) taxa richness, cell density, relative abundance, biovolume of algal species

Phytoplankton (PHY) - 3 subsamples

1) chlorophyll *a*
2) particulate organic carbon (POC
3) taxa richness, cell density, relative abundance, biovolume of algal species

QUALITATIVE

Qualitative Multi Habitat (QMH)

Scraping, brushing, and siphoning periphyton from submerged substrates in five different habitats. The five samples are composited.

MACROALGAE

Qualitative samples of large filamentous algae are collected from each habitat present.

DATA ANALYSIS AND INTERPRETATION

Dependent upon study objectives.

PHYSICAL HABITAT ASSESSMENT

NAWQA protocols integrate physical habitat data taken at basin, segment, reach, and microhabitat scales. Basin and segment scale data are collected using GIS databases or calculated manually from 7.5 minute topographic maps. Reach and microhabitat scale data are collected using field-based surveys on site utilizing a transect system. The reach is divided into ten evenly spaced segments between eleven transect lines.

BASIN SCALE DATA

Total Drainage Area
Average Annual Runoff
Average Annual Air Temperature
Average Annual Precipitation
Average Annual Class A Pan Evaporation
Basin Length
Drainage Density
Entire Stream Gradient
Land Use/Land Cover
Geology
Ecoregions
Land-resource Areas
Minimum Elevation in Basin
Maximum Elevation in Basin
Basin Relief Ratio
Drainage Shape
Stream Length
Cumulative Perennial Stream Length
Drainage Texture
Estimated Flow Characteristics
Soils
Physiography
Potential Natural Vegetation
Wetlands

SEGMENT SCALE DATA

Location of Segment Boundaries
Curvilinear Channel Length and Distance to Reference Location
Segment Gradient
Stream Order
Segment Length
Upstream and Downstream Elevation
Sinuosity
Water Management Feature
Valley Sidelsope Gradient

REACH SCALE DATA

Discharge
Curvilinear Reach Length
Geomorphic Channel Units
Channel Modifications
Surface Water Gradient

TRANSECT BASED INFORMATION

Wetted Channel Width
Channel Features (bars, islands, shelves, etc.)
Bankfull Channel Width
Canopy Angles

Riparian Canopy Closure	Dominant Riparian Land Use/Land Cover
Bank Angle	Bank Height
Bank Substrate	Bank Vegetative Cover
Bank Erosion	Habitat Cover Features (presence/absence)
Thalweg Depth	Depth
Velocity	Dominant Bed Substrate
Embeddedness	Silt Presence/Absence

DATA ANALYSIS AND INTERPRETATION

Table 27. USGS Bank Stability Index an indicator of overall bank condition.

Bank Characteristic	**Measurement**	**Score**
Bank Angle (degrees)	0-30	1
	31-60	2
	>60	3
Vegetative Cover (%)	>80	1
	50-80	2
	20- <50	3
	<20	4
Bank Height (meters)	0-1	1
	1.1-2	2
	2.1-3	3
	3.1-4	4
	>4	5
Substrate (category)	bedrock, artificial	1
	boulder, cobble	2
	silt	5
	sand	8
	gravel/sand	10
	Total Score	**Interpretation**
	4-7	Stable
	8-10	At risk
	11-15	Unstable
	16-22	Very unstable

RECOMMENDED STATISTICAL TECHNIQUES

Habitat data analysis in NAWQA studies is determined by the goal of the study. Below are some of the techniques recommended.

- Graphical plotting or correlation analyses of habitat variables with respect to response variables.
- Parametric: correlation analyses, analysis of variance (ANOVA)
- Non-parametric: Spearman rank correlations, Kruskal-Wallis test, Tukey standardized range test, and Wilcoxin sign-ranks
- Principal Component Analysis (PCA)
- Indirect Gradient Analysis: Detrended Correspondence Analysis (DCA)
- Direct Gradient Analysis: Canonicial Correspondence Analysis (CCA)
- Analysis of Variance (ANOVA)

REFERENCE SITE SELECTION

No specific guidance given. However, NAWQA references Harrelson et al., who recommend the establishment of reference sites based on representative watershed types, historical conditions, and best

professional judgment (Harrelson, Rawlins et al. 1994).

IV. UNITED STATES FOREST SERVICE PROTOCOLS.

The protocols presented by USFS (Plats, Megahan et al. 1983) have the primary purpose of evaluating conditions related to salmon fish production. Habitat, invertebrate, and fish sampling and data interpretation are all aimed at assessing a stream reach's ability to promote and sustain salmonid growth and recruitment.

SAMPLING REACH CONSIDERATIONS

Transect systems are recommended for use in physical habitat assessment. The objectives of the study determine what type of transect system is used. USFS recommends the transect cluster system using one of three approaches.

1. Multiple Transect – Determine the number of transects required to detect statistical differences, then randomly select transects from the stream reach under study.

2. Multiple Station – Randomly select sample points in the reach under study, then group the required number of transects to obtain statistical significance around these points.

3. Stratified Random – The required assumption is that a good deal of information on the reach already exists (*i.e.* knowledge of the available habitats and their distribution). The stratified random design allows the investigator to target and intensively sample more complex habitats while reducing sampling in less complex and biologically important habitats.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

Sample Frequency and Size

USFS recommends a minimum of one sample per season and three replicates per habitat type. However, it is recommended that the minimum sample size needed to detect statistically significant differences should be calculated from preliminary field data for each site.

Semi-Quantitative Sampling Equipment

Hester-Dendy
Modified Hess
Surber

Field Preservation

70% ethanol or 5% formalin solution

Level of Taxonomic Identification

Dependent on project resources and objectives, but generally genus or species level.

Data Interpretation and Analysis

Species Abundance
Taxa Richness
Shannon-Wiener Diversity
Biotic Condition Index
Chandler Biotic Score

FISH COMMUNITY ASSESSMENT

The fish community assessment methods presented by USFS are designed to provide population estimates of salmonids in streams, but may be used to estimate populations of any fish species of interest. Depletion electrofishing is recommended for determining standing stocks of fish species per area of stream. Sodium cyanide, rotenone, direct underwater counting, and explosives are also presented as sampling alternatives. Several quantitative methods for determining population size are recommended. Two basic examples calculated from seine haul or electrofishing data are presented below.

Depletion sampling and estimating population size:

Two-Step Method – Block nets are used to isolate the sampling reach. Two electrofishing passes are made with numbers per species recorded for each pass individually. Population size (N) for the sample reach for each species or all species is estimated with the following formula.

$$N\ (est) = \frac{(N\ first\ pass)^2}{(N\ first\ pass - N\ second\ pass)}$$

Four-Step Zippin Method – Block nets are used to isolate the sample reach. Four electrofishing or seine haul passes are made. More or less than four passes may be used.

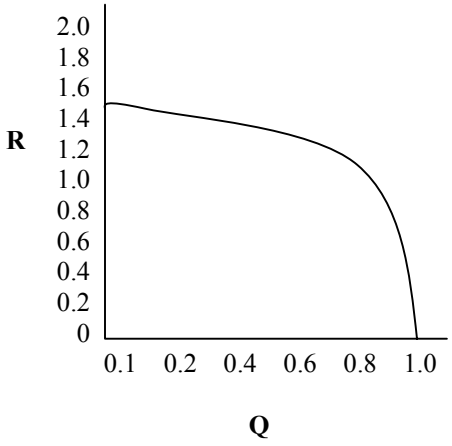
The Zippin method is a maximum likelihood model using probabilities to determine fish population size (N) within a sample reach. USFS recommends four electrofishing passes for use with the Zippin method. Zippin population size is estimated as follows.

$$N\ (est.) = T/Q$$

Where: T = total number or fish

Q = sampling fraction (determined graphically)

Graphical Determination of Q:



$$\text{Where } R = \frac{\sum_{i=1}^{k}(i-1)U_i}{T}$$

K = number of removals
U_i = number of fish collected in ith removal

PHYSICAL HABITAT ASSESSEMENT

WATER COLUMN MEASUREMENTS

Wetted Stream Width	Average Depth
Stream Shore Water Depth (esp. undercut banks)	Pool-riffle ratio
Pool, riffle, run widths and lengths	Pool quality - index
Pool Feature (feature forming the pool)	Channel bank angle

STREAM BOTTOM MEASUREMENTS

Channel elevation	Channel gradient
Channel sinuosity	Stream channel substrate
Sedimentation	Erosion and deposition
Channel debris and sediment storage	Stream order

RIPARIAN ZONE MEASUREMENTS

Streamside cover	Vegetation use by animals
Herbage production and utilization – electronic capacitance meter reading	Habitat type – stream bank
	Vegetation overhang

REFERENCE SITE SELECTION

No guidance given.

SUMMARY OF RELEVANT NATIONAL PARK SERVICE ASSESSMENT METHODS

I. GREAT PLAINS PRAIRIE CLUSTER LONG-TERM ECOLOGICAL MONITORING PROGRAM (PETERSON ET AL. 1999)

The Great Plains Prairie Cluster Long-Term Ecological Monitoring Program (Prairie Cluster) of the US National Park Service (NPS) uses macroinvertebrate biomonitoring. Macroinvertebrate communities are evaluated in accordance with the recommendations made by Peterson and coworkers (Peterson, Rizzo et al. 1999). Peterson's methodology was developed to be robust enough to be applicable to all six of the Prairie Cluster unit's streams despite some inherent stream system differences. Site conditions are compared temporally (annual changes only) and within year (i.e. sample periods) and spatial differences are not formally analyzed and potential changes are always measured against the "benchmark" conditions associated with the sampling results of 1989.

SAMPLING REACH CONSIDERATIONS

Original sampling sites and methodology followed those of (Harris, Kondratieff et al. 1991). No selection criteria were given, but the sampling sites chosen tend to reflect either entry or exit points from the park boundary, or up and downstream conditions for tributaries or point sources within park boundaries. Sites are permanently marked, visited annually, and included in subsequent monitoring designs to maintain long-term data continuity.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Statistical analyses of local data indicated that the seasonal variability was much greater than the intra-seasonal variability (i.e. from year to year). Therefore, Peterson recommended a sampling scheme of "5 replicate samples at each site on each of 3 dates, 30 days apart, during the summer season" to minimize cost and maximize usable information (Peterson et al.1999). The summer season is determined, in part, by the number of growing degree days as measured by the normal average daily temperatures for the nearest National Weather Service station. Sampling periods and dates are identified and scheduled at the beginning of the year. Initial sampling should occur within 10 days of the beginning of the sampling window, or as soon as possible thereafter if high or low flow conditions prevent sampling on the scheduled date.

Riffles –

Surber samplers (0.0929 m^2) in waters at least 25 cm deep. 200-micron mesh is recommended to avoid loss of small macroinvertebrates. 5 replicates at each site.

Runs and Pools –

Hester-Dendy samplers made of nine 57.76 cm^2 square plates separated by 3 mm spacers, providing 0.0929 m^2 of surface area for colonization. Five samplers placed at least 1 m apart at each site. Samplers are allowed to colonize for 30 days prior to retrieval.

Specimens preserved in the field with 80% ETOH.

LABORATORY METHODS

Subsampling

Specific methods as described by (Plafkin, Barbour et al. 1989). Samples are uniformly distributed over a grid of 10 equal units. Grid cells are randomly selected and processed until 100 organisms ± 20% of the total sample have been collected. Percentage of total sample used for processing is recorded.

Level of Taxonomic Identification

All macroinvertebrates are identified to genus, except as follows: Nematoda to phylum; Annelida and Acarina to class; Collembola to order; Diptera, Decapoda, Gastropoda and Pelecypoda to family. Chironomids should be identified to blood group level (red-blooded versus all others), since the two groups have different pollution tolerance levels.

DATA ANALYSIS AND INTERPRETATION

Table 28 describes the five metrics that were used to characterize macroinvertebrate community structure and condition. These metrics were selected because they differed in their sensitivity to various environmental changes (e.g. water quality, instream habitat). Total density of macroinvertebrates was only used as a metric for the Hester-Dendy samples as they were the only samples that are based on areal estimates.

Table 28. Metrics used by NPS Prairie Cluster for the interpretation of macroinvertebrate community data.

Metric	**Method of Calculation**	**Expected metric response to increasing perturbation**
Total Density	Total number of individuals across all taxa per unit area Hester-Dendy samples only	Decrease
Family Biotic Index (FBI)	$FBI = (\Sigma\ n_i a_i / N) - 0.18$ where: N = total number of individuals in sample n_i = number of individuals in *i*th family a_i = tolerance value of *i*th family	Increase
EPT ratio (R)	R = EPT / (EPT + C) where: EPT = total number of individuals of EPT taxa C = total number of individuals of Chironomidae	Decrease
Taxa Richness	Surber samples: Number of families Hester-Dendy samples: Number of genera	Decrease
Shannon-Weiner Index	$H' = - \Sigma\ (n_i / N) \ln (n_i / N)$ where: N = total number of individuals in sample n_i = number of individuals in *i*th group Surber samples: *i*th family Hester-Dendy samples: *i*th genus	Decrease

Annual changes in macroinvertebrate assemblages are evaluated using analysis of variance, and significant differences ($p \leq 0.05$) must be observed in at least two indices to conclude a significant change. If a

significant change is observed, additional research (e.g. physical habitat and stream flow data analysis) may be necessary to determine the cause.

FISH COMMUNITY ASSESSMENT

No related material.

PHYSICAL HABITAT ASSESSMENT

Physical habitat assessment consists of several quantitative measurements. Measurements are made *prior* to Hester-Dendy sampling and *immediately following* Surber sampling. An additional set of measurements is made for Hester-Dendy samplers at the final retrieval time. Stream gauge height to the nearest 0.1m and stream water temperature to the nearest 0.5 °C are recorded. With a meter stick, three depths to the nearest 0.5 cm are measured at random within a 0.5 m radius of the sampling apparatus, then recorded, and averaged. To estimate current velocity the displacement of water (to the nearest 0.5 cm) is measured at the upstream edge of the meter stick from the stream level. The current velocity is then estimated using the following equation: $C = 0.304 \ln(V) + 0.405$, where C is the current velocity in m/s and ln(V) is the natural log of the average vertical water displacement in mm. In addition, both the type of substrate and percent of surface area coverage (for each type observed) within a 0.5 m radius of the sampling apparatus are recorded.

REFERENCE SITE SELECTION

No related material.

BIOLOGICAL CRITERIA

Regional biological criteria are currently being developed for states in which most of the Prairie Cluster parks are located.

II. OZARK NATIONAL SCENIC RIVERWAYS BIOLOGICAL MONITORING PROGRAM (DOISY AND RABENI 1999)

A tiered-resources, multimetric biological monitoring program was developed by Doisy and Rabeni (Doisy and Rabeni 1999) for the Ozark National Scenic Riverways (OZAR) park service unit of the United States National Park Service (NPS). This program has two tiers of assessment, designed to give managers the ability to select a level of sampling that provides sufficient information, but still fits with their available resources. Both primary and secondary levels recommend biomonitoring of macroinvertebrates. The protocols were based on statistical analyses of data collected from OZAR, and were shown to be applicable for the whole of OZAR. However, data from different seasons could not be combined, due to significant seasonal variation in some metrics.

SAMPLING REACH CONSIDERATIONS

The habitat types for sampling were divided into 11 identifiable hydraulic habitat units (Rabeni and Jacobson 1993; Doisy and Rabeni 1999). Statistical analyses of hydraulic habitat data showed that the lowest variability of macroinvertebrate communities were in high gradient riffle (HGR) and coarse run (COR) habitats. Therefore, these habitats were considered for primary sampling and when additional resources are available, fine run (FIR) depositional habitats should also be sampled (Table 29).

Table 29. Hydraulic habitats identified in low-gradient streams of the Ozarks and used in this protocol(after Rabeni and Jacobson in Doisy and Rabeni 1999).

Habitat Type	Description
High Gradient Riffle (HGR)	Flat-bottomed channel, depth 15 - 60 cm, velocity 50 - 130 cm/s, substrate dominated by coarse gravel, cobble and boulder.
Coarse Run (COR)	Asymmetrical channel with thalweg depth 20 - 85 cm, velocity 30 - 100 cm/s, substrate dominated by well-sorted cobble and gravel.
Fine Run (FIR)	Symmetric u-shaped channel, depth 30 -90 cm, velocity 10 - 50 cm/s, substrate dominated by poorly sorted sand to gravel.

Sites representative of the HGR, COR, and FIR habitat types should be determined by personnel familiar with the hydraulic habitat units described above.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Macroinvertebrate sampling should occur annually during conditions of stable flow. The periods of suitable stable flow for Arkansas are roughly July through September and February through March. In Missouri, the corresponding periods are March 15 to April 15 and September 15 to October 15. To further reduce the inter-annual variation in data sampling efforts should occur within the same range of accumulated growing degree-days as in prior monitoring studies. Growing degree-days are calculated from the first of the year, totaling only positive differences, according to the following formula:

Degree Days = ((Tx + Tn) / 2) - Tb
where Tx = maximum daily temperature
Tn = minimum daily temperature
Tb = base temperature (50 ° Fahrenheit for this protocol)

Typically the number of degree days for spring kick net sampling is 8-12 while spring sampling with a Brown sampler is usually scheduled for days 900 through 1143. Brown sampling in the fall is done during the 3290 to 3376 degree day period. These degree-day periods are target time periods and other factors may enter into the final selection of sampling dates.

Primary Assessment (high gradient riffle or coarse run) –

D-frame kick net with 800 x 900-micron mesh. Sample approximately 0.5 m^2 and kick to a depth of 15 cm if possible. Collect six samples per location. For greater precision, it is preferred for one person to be responsible for all sampling within a given season. A periodic review of statistical analyses of metrics is used to determine the sample size required to achieve statistical significance. Table 30 clearly indicates that even to detect rather large site differences in some metrics, as many as 45 samples may be required. This may, in part, be due to the relatively small actual differences that exist between sites in the 1993 study.

Table 30. The number of samples required to detect minimum percent differences for five common metrics based on kick net data in the HGR and COR habitats of the Current River Hills ecoregion (Doisy and Rabeni 1999).

	Simpson Index	**Taxa richness**	**Total abundance**	**EPT richness**	**Biotic Index**
Spring 1993					
30%	4	5	17	6	3
20%	10	12	37	14	6
10%	40	48	150	58	24
Fall 1993					
30%	45	14	18	27	1
20%	101	31	41	60	3
10%	404	123	162	241	10

Secondary Assessment –

Brown vacuum sampler (Brown, Scham et al. 1987) with 425-micron mesh bag. For 1 minute, disturb substrate to approximately 10 cm depth. Replace mesh bag. Sample remaining materials for an additional 3 minutes. Both samples are placed on ice in a labeled whirlpak bag. Collect 12 samples per location. Sampling at locations deeper than the Brown sampler standpipe can be performed by attaching a canvas cap and elastic sleeve to the sampler, then sampling with scuba gear.

For greater precision, it is preferred for one person to be responsible for all sampling within a given season. A periodic review of statistical analyses of metrics is used to determine the sample size required to achieve statistical significance. Original data suggest the minimal detectable difference of this protocol is 30 percent and that even at this level as many as 24 samples may be required to identify diversity differences between sites (Table 31).

Table 31. The number of samples required to detect minimum percent differences for five common metrics based on Brown Sampler data in the HGR and FIR habitats at the Ratcliff Ford and Burnt Cabin locations (Doisy and Rabeni 1999).

	Simpson Index	Taxa richness	Total abundance	EPT richness	Biotic Index
Spring 1993					
HGR					
30%	24	10	135	10	1
20%	54	22	304	22	1
10%	217	88	1215	87	5
Fall 1993					
HGR					
30%	6	12	81	12	1
20%	13	27	182	26	2
10%	52	109	729	104	8
FIR					
Fall 1992 30%	11	8		13	4
Spring 1993 30%	53	8		15	2

For both assessment levels, specimens are preserved in the field with 80% ETOH or 10% formalin.

LABORATORY METHODS

Macroinvertebrate samples should be sorted using a stack of USGS sieves, with a 10,000-micron sieve on top of a 300-micron sieve. The material from the lower sieve is scooped and rinsed into a plastic storage container, using as little water as possible. The material in the upper sieve is washed into the lower sieve, then scooped and rinsed into a second plastic storage container. The contents of both containers are sorted using a 30x dissection scope. Whole specimens and body parts should be placed in double-labeled jars with 80% ETOH for later identification.

Subsampling

Allowable at primary assessment level to reduce laboratory time and allow more extensive field sampling. Not allowable at secondary assessment level. Check before subsampling that it is actually necessary; preliminary data indicate that <100 individuals may be present in some HGR samples.

Specific methods modified by Caton (Caton 1991) from that of Plafkin (Plafkin, Barbour et al. 1989). Samples are uniformly distributed over a grid of 70 2"x2" units. Three grid cells are randomly selected and processed. Once a square is started, the whole square must be completed. The number of squares required to reach 100 specimens is recorded. If at least 100 organisms are present in these three squares, subsampling is complete. Otherwise, continue until at least 100 specimens have been counted. If 100 specimens are not present in the entire sample, then the relative abundance of specimens present should be calculated and used with a notation of this procedure.

Level of Taxonomic Identification

Macroinvertebrates –

All insect specimens are identified to genus using Merrit and Cummins (Merrit and Cummins eds. 1996). Larval chironomids will be excluded from identification at the primary level of assessment, but included at family for the secondary level of assessment. Non-insect taxa will be identified to class level using Thorp and Covich (Thorp and Covich 1991).

Quality Assurance

Random checks on 5% of samples being processed, with discarded materials checked by a different person than the sorter. For subsampling, one sample from each sampling period will be divided in half and subsampled, with the alternate halves compared for replicability. Voucher specimens for all taxa will be retained in a reference collection to be identified by a second taxonomist.

DATA ANALYSIS AND INTERPRETATION

Table 32. Metrics used by OZAR for the interpretation of macroinvertebrate community data.

Metric	**Method of Calculation**	**Expected metric response to increasing perturbation**
Taxa Richness	Number of genera	Decrease
EPT Richness	Total number of genera of EPT taxa	Decrease
Simpson Index	$1 / D = 1 / \Sigma (n_i (n_i - 1) / N(N-1))$ where: N = total number of individuals in sample n_i = number of individuals in *i*th genus	Decrease
Biotic Index (Hilsenhoff)	$BI = (\Sigma n_i t_i / N) - 0.18$ where: N = total number of individuals in sample n_i = number of individuals in *i*th group	Increase
Missouri Stream Condition Index (MSCI)	$MSCI = \Sigma$ score(m_i) where: m_i = normalized value of *i*th metric for metrics that DECREASE with perturbation (i.e. Taxa Richness, EPT Richness, and Simpson Index): If $m_i >$ 25% quartile of reference, then score = 5 If 1% quartile $< m_i \leq$ 25% quartile of reference, then score = 3 If $m_i \leq$ 1% quartile of reference, then score = 1 for metrics that INCREASE with perturbation (i.e. Biotic Index): If $m_i <$ 75% quartile of reference, then score = 5 If 75% quartile $\leq m_i <$ 99% quartile of reference, then score = 3 If $m_i \geq$ 99% quartile of reference, then score = 1	Decrease

Primary Assessment –

Three types of analysis:

1. Compare calculated metrics to reference values (Table 5). Qualitative data (presence/absence) may be used if only taxa richness and EPT richness are calculated.

2. Compare replicate scores from each location for significant differences using a nonparametric Kruskal-Wallis test.
3. Calculate multi-metric Stream Condition Index (SCI) (after Rabeni, Sarver et al. 1997; Sarver, Harlan et al. 2002).

Secondary Assessment –

Simpson Index within HGR habitats is most sensitive to habitat degradation within OZAR. Using three types of analysis on all specimens in each HGR and FIR sample:

1. Quantitative similarity analyses on each pairing of samples to produce a similarity matrix. These are compared with previous years' data with either paired t-tests or Mean Similarity Analysis.
2. Normalize raw invertebrate data using the log (X + 1) transformation, then perform a Detrended Correspondence Analysis (DCA). Equal distances in ordination correspond to equal differences in species composition. Also, a Spearman rank correlation between rankings of sample scores (i.e. water quality data and physical habitat assessments) and rankings of environmental variables can show environmental gradients.
3. Compare replicate values for significant differences using a nonparametric Kruskal-Wallis test.

FISH COMMUNITY ASSESSMENT

No related material. While the introduction to this publication indicates that fish assessment methods would be identified for use in the OZARK unit, there was insufficient documentation to allow a review of these methods.

PHYSICAL HABITAT ASSESSMENT

Physical habitat assessment consists of combined qualitative and quantitative measurements. Fish cover, embeddedness, substrate, pool variability, discharge, canopy cover, channel alteration, bottom scouring and deposition, sinuosity, bank channel capacity, bank stability, riparian vegetation, and streamside cover are scored visually, and the scores are combined into an index value. Stream width and depth (m) and stream flow (m/s) are measured. Water chemistry measurements (minimally temperature in deg. C, dissolved oxygen in ppm, pH) are taken. Additional qualitative observations (water color, weather, odors, etc.) are recorded and/or documented photographically.

REFERENCE SITE SELECTION

Reference sites should be established by NPS recommendation, with a minimal number of representative sites from the upper and lower Jacks Fork and Current rivers, and several of the most threatened springs, including Alley and Blue springs. Additional sites should be located immediately up and downstream of specific areas of concern. Pilot studies should be performed on any newly selected areas to provide a baseline for comparative analysis with previous data. Reference sites should be sampled at least once per year.

Initial metric values were calculated for data previously collected in OZAR. These values may be adjusted to accommodate normal temporal variations as the monitoring plan progresses.

Table 33. Values for selected metrics using data collected in 1992 and 1993 from both kick net and Brown samplers.*

	Spring 1993 Mean Values	95% confidence levels	Fall Mean Values**	95% confidence levels
Kick Net HGR				
Taxa Richness	16.8	13.5 - 20.0	17.5	12.1 - 22.9
EPT Richness	10.0	7.9 - 12.1	8.8	4.9 - 12.5
Biotic Index	2.95	2.55 - 3.34	3.21	2.92 - 3.50
Simpson	10.7	8.8 - 12.6	6.1	2.69 - 9.54
Brown HGR				
Taxa Richness	18.9	17.2 - 20.7	21.3	18.8 - 23.8
EPT Richness	11.5	10.4 - 12.5	11.9	10.6 - 13.3
Biotic Index	4.91	4.79 - 5.03	4.73	4.57 - 4.88
Simpson	7.6	6.5 - 8.7	8.6	8.0 - 9.3

* Data used for Kick Net HGR/COR values were collected in the Current River Hills ecoregion; data used for values for Brown HGR were from Ratcliff Ford and Burnt Cabin.
** Fall values for Kick Net HGR are from 1993; fall values for Brown HGR are from 1992

BIOLOGICAL CRITERIA

The following numerical criteria for Missouri Stream Condition Index scores (MSCI) of wadeable/perennial streams of Missouri have been developed (Sarver, Harlan et al. 2002):

MSCI Score	Rating
16-20	Fully Biologically Supporting (FBS)
10-14	Partially Biologically Supporting (PBS)
4-8	Non-Biologically Supporting (NBS)

Water bodies designated as PBS and NBS are considered impaired; that is they do not meet the beneficial use of Protection of Aquatic Life as stated in the Missouri Water Quality Standards (Sarver et al. 2002).

III. DEVELOPMENT OF A MULTIMETRIC SYSTEM OF BIOLOGICAL WATER QUALITY MONITORING FOR THE BUFFALO NATIONAL RIVER (MATHIS 2001)

In 2001 Mathis developed a biological monitoring program using on a multimetric approach for the Buffalo National River park service unit (BUFF) of the NPS (Mathis 2001). The program is based on biological monitoring of macroinvertebrate communities and was developed independently of the Arkansas Department of Environmental Quality (ARDEQ) protocols, in order to promote the use of quantitative methods for subtle habitat changes over the rapid bioassessment methodologies and compliance monitoring based protocols that ARDEQ currently employs. Determination of sampling location, frequency and methods, as well as metric calculation were based on the analysis of empirical data collected both within and around BUFF. The resultant Index of Community Integrity is similar to that used by Ohio EPA and other state agencies (after Mathis 2001).

SAMPLING REACH CONSIDERATIONS

Macroinvertebrate samples should be collected at the same locations as existing NPS water quality sampling locations with the following additional considerations:

1. macroinvertebrate sampling sites should be in permanently flowing riffles, upstream from major tributaries or sufficiently downstream (~ 1km) to minimize their impact on biota;
2. macroinvertebrate sampling sites should be located where local influences (e.g. fords, bridges, bluffs, springs, groundwater inputs, bank instabilities, etc.) are minimal;
3. macroinvertebrate sampling sites on tributaries should be located sufficiently upstream from the Buffalo river (i.e. above the floodplain) whenever possible, to minimize river influences on the tributary;
4. replicated sampling of three adjacent riffles at two sites (preferably with high and low water quality, respectively) should be performed during each sampling period for quality assurance/quality control (QA/QC); QA/QC sites need not stay the same from year to year.

MACROINVERTEBRATE COMMUNITY ASSESSMENT

FIELD METHODS AND INDEX PERIOD

Samples are collected annually between early December and late March from permanently flowing riffles at each site. Samples should be collected within a three weeks window if possible. Otherwise, all samples from the main channel should be collected during a consolidated time period and all samples from the tributaries should be collected within another time period. Additionally, samples should be collected only during base flow conditions, and not before a minimum two-week period after any major flood event.

Four random samples are collected at each riffle location with a Hess sampler (0.363 mm mesh). The location of each sample within the riffle location is randomly determined by visually dividing the riffle into an equally spaced 6x12 grid and rolling dice to determine the appropriate row (i.e. one die for longitudinal) and column (one die for perpendicular) positions within the matrix. Sample each of four locations as close as possible to the determined positions as substrate and depth will allow. Cobble-size and larger substrate particles should be individually scrubbed into the collection bag and visually inspected for further specimens before discarding. The smaller substrate should be disturbed with a hand rake 200 times to a depth of approximately 10 cm. If several people are collecting samples for the monitoring program, multiple collections at a reference site should be performed to determine variation between personnel. Differences greater than 30% may be attributable to human error and attempts made to minimize this error.

Samples are preserved in the field with Kahle's solution (11 parts concentrated formalin, 28 parts 100% ethanol, 2 parts glacial acetic acid, 59 parts distilled water).

LABORATORY METHODS

One week after sampling, specimens should be transferred to 75% ETOH for storage. Samples should be sorted, and debris material should be reexamined by another person to prevent loss of specimens. If a significant number of specimens are repeatedly missed by a particular investigator, his/her technique should be altered or he/she should no longer participate in sorting.

Specimens are retained in 75% ETOH for further identification if necessary. A voucher collection should be prepared and verified by regional taxonomic specialists.

Subsampling

Not allowed.

Level of Taxonomic Identification

All macroinvertebrates are identified to family, except the following organisms that have been previously identified to higher levels:

Phylum Annelida, Class Hirudinea
Phylum Arthropoda, Class Arachnoidea, Order Hydracarina
Phylum Arthropoda, Class Crustacea, Order Ostracoda
Phylum Nermetea

Lower level taxonomic identifications may be done later if necessary.

DATA ANALYSIS AND INTERPRETATION

An Index of Community Integrity (ICI) was developed for the Buffalo River. Based on the biological metrics listed in Table 34, this ICI uses two differing percentile ranges (Scenario A and B, Table 35); producing either four or five scoring categories for metrics. Using the five category scoring scenario, theoretical ICI values could range from 0 to 100, with 0 being most impaired and 100 being a perfect reference condition (Table 35). Mathis did not elaborate on his comparisons of the two differing scoring approaches but it appears that he favored Scenario B as presented in Table 35.

Table 34. Metrics used for the Index of Community Integrity (ICI) for the macroinvertebrate communities of the Buffalo River.

Metric	**Method of Calculation**	**Expected metric response to increasing perturbation**
Margalef's Index of Taxa Richness	$d = s - 1 / \ln (N)$ where: s = total number of taxa present N = total number of individuals in sample	Decrease
Shannon's Taxa Diversity Index	$H' = ((N * \ln N) - (\Sigma n_i * \ln n_i)) / N$ where: N = total number of individuals in sample n_i = number of individuals in *i*th family	Decrease

% Dominant Three Taxa	%Dominant 3 Taxa = 100 *(Σ D_1 + D_2 +D_3) / N where: N = total number of individuals in sample D_1 = number of individuals in most abundant family D_2 = number of individuals in 2nd most abundant family D_3 = number of individuals in 3rd most abundant family	Increase
% Chironomidae	% of Total Abundance from Chironomids	Increase
% Plecoptera	% of Total Abundance from Plecopterans	Decrease
% Trichoptera	% of Total Abundance from Trichopterans	Decrease
% Elmidae	% of Total Abundance from Elmidans	Decrease
% Corbicula	% of Total Abundance from Corbiculans	Increase
% Intolerant Organisms	% of Total Abundance from organisms with tolerance values of 0 or 1	Decrease
% Collector - Filterers	% of Total Abundance from organisms that gather particles of organic matter less than 1mm in diameter	Decrease
Index of Communtiy Integrity (ICI)	$ICI_A = \Sigma$ ICI score(m_i) where: ICI_A = combined ICI for site A m_i = normalized value of *i*th metric of site A score(m_i) = ICI score of *i*th metric based on percentile rank value (see table below)	Decrease

Table 35. Index of Community Integrity (ICI) scores for metrics associated with the macroinvertebrate communities of the Buffalo River.

Metric Score for calculation of ICI	**Percentile Rank of Metric Value**			
	Decreasing[1]		**Increasing[2]**	
	Scenario A	**Scenario B**	**Scenario A**	**Scenario B**
2		< 15		> 85
4	< 25	15 to 40	>75	61 to 85
6	25 to 50	41 to 65	51 to 75	36 to 60
8	51 to 75	66 to 90	25 to 50	10 to 35
10	> 75	> 90	< 25	< 10

[1] Decreasing Metrics are expected to decrease with increasing habitat perturbation. These metrics include: Margalef Diversity Index, Shannon Index, % Plecoptera, % Trichoptera, % Elmidae, % Collector-Filterers, and % Intolerant Taxa

[2] Increasing Metrics are expected to increase with increasing habitat perturbation. These metrics include: % Chironomidae, % Corbicula, and % Dominant Taxa.

ICI scores should be calculated annually for all NPS sampling locations and plotted as bar graphs. Sites with the highest ICI values should be considered reference sites. The condition of remaining sites may be determined by comparison. Sites with ICI values of 70 - 50% of the reference ICI are considered slightly

to moderately impacted. Sites with ICI values that are less than 50% the reference ICI are considered severely impacted.

Metrics and ICI are determined by standardized spreadsheets, which transform raw data entered manually.

FISH COMMUNITY ASSESSMENT

No related material.

PHYSICAL HABITAT ASSESSMENT

Physical habitat assessment following USEPA or USGS standard methods, as determined by NPS personnel.

REFERENCE SITE SELECTION

Reference sites are designated by high ICI scores. Observed ICI and metrics values for various stations, seasons, and percentile distributions along the main channel of the Buffalo River were calculated by Mathis (Mathis 2001).

BIOLOGICAL CRITERIA

Region-specific biological criteria are currently being developed for states containing the Buffalo River watershed.

COMPOSITE SUMMARY OF PROGRAMMATIC ELEMENTS OF STATE, NATIONAL, AND PARK SERVICE RELATED ENTITIES

I. SUMMARY OF BIOLOGICAL MONITORING COMPONENTS, SAMPLING PERIODS, AND REFERENCE CONDITIONS.

We have restricted our review of biological monitoring programs and methods to those geo-political entities that most likely manage similar ecosystems and have similar management goals. Most methods included in this review are associated with state programs and are often based on methods developed by and used in many federal environmental programs. We believe that a close examination of existing programs and their methods can be used to select or modify biological monitoring design components and methods most suited to the long-term monitoring of aquatic resources located on and influencing the environmental quality of Heartland Network facilities. The following compiled information and summary statements suggest that certain "core" approaches and methods are common to many environmental monitoring programs. The commonality of many methods and monitoring components indicates these elements are robust, have broad applicability and are easily adopted (or modified) for use by NPS. These monitoring methods have been developed and time tested by managers of similar ecosystems with similar goals. Using these methods would have two major benefits for NPS: 1) it would facilitate outside comparisons by reducing differences in information based on varying methods, and 2) it would expand NPS's ability to assess the condition of its aquatic resources through time and space by comparison with regional data from similar resource populations (e.g. reference lakes and streams, healthy stream segments).

Fourteen state, national, and national park service biological monitoring programs were examined. All programs included habitat and macroinvertebrate monitoring components that were monitored during normal or base flow conditions, most often occurring in the growing season. Generally, state and federal programs try to sample macroinvertebrates communities between spring and late fall. Only Mathis (Mathis 1999) in his work in the Buffalo River recommended that macroinvertebrate samples (quantitative) be taken in the winter months. Nearly all programs used a reference condition/site approach to evaluate relative conditions associated with monitoring sites. Most programs used ranking systems based on objective data to classify biological conditions (e.g. excellent, moderately impaired, etc.). The following specific commonalities were observed in our evaluations of the reviewed programs:
14 state, national, and national park service biological monitoring protocols were examined, and the following generalities were observed:

1. All (14 of 14) reviewed protocols recommend physical habitat assessments and monitor macroinvertebrate communities. Most (10 of 14) recommend monitoring of fish communities, and few (2 of 14) recommend monitoring of algal communities (Table 36).

2. All (14 of 14) specify sampling at base flow conditions, and most (11 of 14) sampling periods fall within the growing season for macroinvertebrates (Table 37).

3. Most (10 of 14) protocols designate some method for determination of reference sites or conditions. The two most common methods of determination for reference sites are best professional judgment (5 of 10) and least disturbance (4 of 10) (Table 38).

Table 36. Primary programmatic elements of biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Invertebrates	Fish	Algae	Habitat
Arkansas	X	X		X
Indiana	X	X		X
Iowa	X	X		X
Kansas	X	X		X
Minnesota	X	X		X
Missouri	X			X
Nebraska	X	X		X
Ohio	X	X		X
USEPA	X	X	X	X
USGS	X	X	X	X
USFS	X	X		X
Prairie Cluster (NPS)	X			X
OZAR (NPS)	X			X
BUFF (NPS)	X			X

The programmatic elements compiled in Table A are primarily elements related to the monitoring and assessment of running water environments. Few state and federal programs have established monitoring guidelines for wetlands. However, the monitoring and assessment of lakes and reservoirs is common and relies mainly on water chemistry data and measurements of planktonic chlorophyll (an indicator of algal biomass) and the areal extent vascular plant communities. Detailed habitat evaluations for lakes and reservoirs are seldom part of programmatic efforts, but general morphology data (e.g. lake volume, mean depth, water residence time) is determined for many of the larger lakes and reservoirs within environmental programs. Occasionally, fish and invertebrate monitoring is designed into monitoring programs, but the collection and evaluation of such data tends to be sporadic within state and federal programs in the mid-continent area.

Table 37**.** Index periods and sampling regimes of biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Index Period(s)	Sampling Regime	
		Visits/ Site/Year	Samples/Site
Arkansas	Apr - Jun Sep - Oct	1	1 composite
Indiana	Jul - Oct	No more than 1 every other yr	No guideline
Iowa	Jul 15 - Oct 15	1	1 or 3
Kansas[1]	Spring, Summer, Fall	1 in rotating seasons	2
Minnesota[2]	Jun - Jul	1	1 composite
Missouri	Sep 15 - Oct 15	1	1 composite
Nebraska[3]	May - Sep#	1	1
Ohio	Jun 15 - Sep 30	1	3 - 5
USEPA	No guideline	1	1 composite
USGS[3]	No guideline	1	1
USFS	Year round	4 (1 per season)	3
Prairie Cluster (NPS)	Summer	3	5
OZAR (NPS)	Mar 15 - Apr 15 Sep 15 - Oct 15	1	6 or 12
BUFF (NPS)	Mar - Dec	1	4

[1] Spring one year, Summer the second year, Fall the third, then repeat.
[2] Earlier sampling allowed based on temperature.
[3] No specific guideline; determined from associated data.

Table 38. Approaches used to define and identify reference conditions and sites employed in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Determination of Reference Sites						
	Historical Conditions	**Paired Watersheds**	**Best Professional Judgment**	**Objective Data**	**Least Disturbed**	**Statistical Distribution**	**No Guidance/ Under Development**
Arkansas							X
Indiana			X				
Iowa						X	
Kansas					X		
Minnesota							X
Missouri					X		
Nebraska					X		
Ohio	X		X		X		
USEPA		X	X				
USGS	X		X				
USFS							X
Prairie Cluster (NPS)							X
OZAR (NPS)			X				
BUFF (NPS)						X	

Several categories listed in Table C represent general concepts that incorporate the use of diverse variables and factors, depending upon the protocol. In addition, while different groups use similar factors or variables, they may or may not be measured quantitatively. Nearly all approaches can involve subjective evaluations of factors and their "weighting value" in selecting reference sites. "Historical conditions" refers to the use of historic data in determining typically pre-settlement or un-disturbed conditions. However, if major land use changes have occurred (e.g. urban growth areas), historic data can be data that is only several decades old. Often, historic data suffers from a lack of data quality information and may not have the level of resolution necessary to address regional or local issues. "Paired watersheds" refers to the use of watersheds of similar type and size that have known differences in potential stressors. This approach works well if the study objectives are limited in spatial area and address issues that are more " site specific" in nature. "Best professional judgment (BPJ)" represents an approach that relies upon the subjective knowledge and experience of involved personnel to determine whether or not an aquatic resource is free enough from impairments to be considered a reference system or site. Often the type and amount of quantitative or qualitative information varies considerably among people and organizations; thus, BPJ suffers from information inconsistencies and inherent evaluation biases related to individual and collective educational and work experiences. It is suggested that BPJ is often a beginning point in the selection process, and that BPJ results be further evaluated as a more formal method is adopted and data needs are

addressed. The “Least disturbed” approach to identifying reference conditions and sites is based on the identification and evaluation of stressor and receptors in aquatic ecosystems of interest and their watersheds. Typically, factors relating to water quality, in-stream and near-habitat conditions, and watershed or landscape variables and potential stressors are used to evaluate reference sites, and biological variables are used to validate and test these evaluations. Minimally impacted areas are considered reference sites. When dealing with large populations of similarly classified aquatic systems (e.g. wadeable streams) a “Statistical distribution” approach is used when reference conditions and sites are either unknown or absent. The statistical distribution of factors or condition values is then used to determine a relative ranking of conditions and/or sites, based on some assessed statistical property(s) such as an upper or lower quartile. The final category (No guidance/under development) was used to identify entities that have not explicitly described the reference site determination process.

II. SUMMARY OF MACROINVERTEBRATE COMMUNTIY MONITORING PROTOCOLS.

Further evaluation of the macroinvertebrate programs and protocols examined in this review revealed a number of common factors. Most groups recommend use of at least semi-quantitative collection methods, often sampling with either a D-framed net over multiple habitats, or artificial substrate samplers for a prescribed time period. Monitoring groups working in stream systems with well-defined and reoccurring rifle areas in stream segments of interest have recommended the use of a quantitative riffle sampler such as a Hess or Surber sampler. Additional non-riffle areas required the use of different sampling gear.

The majority of approaches used replicated samples (typically 3 to 5 samples) in assessing site and system conditions. Within the monitoring programs evaluated, macroinvertebrate samples were most often systematically subsampled to reduce data processing time and expenses. The raw data was then used to generate a number of evaluation metrics. All but one of the monitoring programs used these metrics to create a multimetric index and index scoring scenario that was used in interpreting temporal and spatial change in the macroinvertebrate community within and among sites (and streams). Eighty different metrics were used in at least one or more of the 14 programs surveyed, while only 16 different metrics were noted to be included in three or more of the programs. Only 10 metrics were found to be common to four or more of the programs using macroinvertebrates for monitoring purposes. By far the most frequently used metrics were taxa richness, number of EPT taxa, percent dominant taxa and scores from the Hilsenhoff Biotic Index. The following generalities were observed among the surveyed entities that used macroinvertebrates in a biological monitoring program:

1. Most protocols (11 of 14) require semi-qualitative, multi-habitat sampling, some (4 of 14) require semi-qualitative, single habitat sampling, and some (4 of 14) require qualitative, single habitat sampling (Table 39).

2. Two types of sampling equipment were used in more than 25% of protocols: D-frame kick nets (6 of 14) and Artificial Substrate samplers (5 of 14) (Table 40, Table 41).

3. A majority of protocols recommend replicate samples (9 of 14), subsampling (9 of 14), and multimetric indices (13 of 14) (Table 42).

4. Ten primary metrics were used in at least 25% (i.e. 4 or more) of the protocols:

% Chironomidae	Total Number of Taxa
% Ephemeroptera	Number of Dipteran Taxa
% Trichoptera	Number of Ephemeropteran Taxa
% Dominant taxon	Number of Trichopteran Taxa
Hilsenhoff Biotic Index	Number of EPT Taxa

Table 39. Comparison of qualitative and semi-quantitative macroinvertebrate protocols used in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Qualitative		Semi-quantitative	
	Single Habitat	Multi-habitat	Single Habitat	Multi-habitat
Arkansas			X	X
Indiana				X
Iowa		X		X
Kansas				X
Minnesota	X			
Missouri				X
Nebraska		X		X
Ohio		X	X	
USEPA				X
USGS		X		X
USFS				X
Prairie Cluster (NPS)				X
OZAR (NPS)	X		X	X
BUFF (NPS)			X	

Table 40. Invertebrate sampling equipment used in qualitative sampling efforts in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Sampling Equipment							
	D-net	Eckman	Ponar	Peterson	Surber	Artificial Substrate	Hand Picking	Modified Hess
Arkansas								
Indiana								
Iowa						X	X	
Kansas	X							
Minnesota	X							
Missouri								
Nebraska	X						X	
Ohio	X	X	X	X				
USEPA								
USGS	X	X	X				X	
USFS								
Prairie Cluster (NPS)								
OZAR (NPS)	X							
BUFF (NPS)								

Table 41. Invertebrate sampling equipment used in quantitative sampling efforts in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Sampling Equipment								
	D-net	Eckman	Ponar	Peterson	Surber	Artificial Substrate	Hand Picking	Modified Hess	Brown
Arkansas	X								
Indiana									
Iowa						X		X	
Kansas	X								
Minnesota									
Missouri	X						X		
Nebraska	X		X	X	X	X			
Ohio		X	X	X		X			
USEPA	X								
USGS	X	X	X						
USFS					X	X		X	
Prairie Cluster (NPS)					X	X			
OZAR (NPS)	X								X
BUFF (NPS)								X	

Table 42. Methods used for data analysis and interpretation by state and national entities for the evaluation of macroinvertebrate data.

Monitoring Entity	Replicate Samples	Sub-sampling	Multi-metric Indices	Multi-variate Indices	Hypothesis Testing	Level of Taxonomic Identification
Arkansas		X	X			lowest practical
Indiana		X	X			family
Iowa	X	X	X			lowest practical
Kansas	X		X			lowest practical
Minnesota	X		X			genus
Missouri	X	X	X			lowest practical
Nebraska		X	X			lowest practical
Ohio	X	X	X			lowest practical
USEPA		X	X	X		lowest practical
USGS						lowest practical
USFS	X		X			genus or species
Prairie Cluster (NPS)	X	X	X		X	genus
OZAR (NPS)	X	X	X	X		genus
BUFF (NPS)	X		X			family

III. SUMMARY OF FISH COMMUNITY MONITORING PROTOCOLS .

Ten of the 14 evaluated biological monitoring programs recommended the biological monitoring of fish communities, but only 9 programs provided the specific protocols used in their fish monitoring efforts. Most programs identified the minimum length of the stream reach to be sampled, as well as the types of habitats included in their sampling effort. For those programs that listed specific collection methods, some form of seining and electrofishing were the primary sampling methods. Most programs developed and used a number of fish metrics, and those metrics were typically combined into a multimetric index for purposes of evaluation. The following generalities were observed among the fish protocols evaluated:

1. More than half (7 of 10) of the protocols recommended a defined sampling reach length; of those protocols that recommended defined reach lengths, most (5 of 7) recommended lengths with upper and lower limits. Half of the protocols recommended the use of block nets to restrict immigration and emigration of fish from the sampling reach. More than half of the programs recommended multi-habitat sampling for fish (Table 43).

2. The two most common types of equipment recommended for fish sampling were backpack electrofishing equipment (9 of 10) and seines (6 of 10) (Table 44).

3. Multimetric indices were recommended in 70% of protocols that monitor fish (7 of 10) (Table 45).

Table 43. Elements of field fish sampling protocols used in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Sampling Reach Length	Block Nets Used	Single Habitat Sampling	Multi-Habitat Sampling	Avoid Areas of Human Impact
Arkansas					
Indiana					
Iowa	150-500 m				
Kansas	150-300 m	X		X	X
Minnesota	35 times the mean width			X	
Missouri	20 times the mean width				
Nebraska		X		X	
Ohio	500 m			X	
USEPA	150-300 m	X		X	X
USGS	150-300 m	X		X	
USFS		X			
Prairie Cluster (NPS)					
OZAR (NPS)					
BUFF (NPS)					

Table 44. Fish sampling equipment used in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Seine	Backpack Electrofishing	Tote Barge Electrofishing	Boat Mounted Electrofishing	Passive Sampling Gear
Arkansas	X	X		X	X
Indiana					
Iowa		X	X		
Kansas	X	X			
Minnesota		X	X		
Missouri					
Nebraska	X	X	X	X	X
Ohio		X		X	X
USEPA	X	X			
USGS	X	X		X	
USFS	X	X			
Prairie Cluster (NPS)					
OZAR (NPS)					
BUFF (NPS)					

Table 45. Methods used for data analysis and interpretation by state and national entities for the evaluation of fish data.

Monitoring Entity	Replicate Samples	Sub-sampling	Multimetric Indices	Multivariate Indices	Hypothesis Testing
Arkansas					
Indiana			X		
Iowa			X		
Kansas			X		
Minnesota			X		
Missouri					
Nebraska			X		
Ohio			X		
USEPA			X	X	
USGS					
USFS					
Prairie Cluster(NPS)					
OZAR (NPS)					
BUFF (NPS)					

IV. SUMMARY OF ALGAL COMMUNITY MONITORING PROTOCOLS.

No states having Heartland Network facilities and none of the evaluated NPS programs used algae in a biological monitoring program. Two national biological monitoring protocols recommended biological monitoring of algal communities. Often, chlorophyll concentrations were identified as a measurement variable. These two programs recommended periphyton (i.e. attached algae) monitoring, using both qualitative and quantitative methods (Table 46). Subsampling was also used in both programs to generate community level variables (Table 47).

Table 46. Algal communities sampled and protocol type used in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Periphyton	Phytoplankton	Filamentous Algal Mats	Qualitative	Quantitative
Arkansas					
Indiana					
Iowa					
Kansas					
Minnesota					
Missouri					
Nebraska					
Ohio					
USEPA	X			X	X
USGS	X	X	X	X	X
USFS					
Prairie Cluster (NPS)					
OZAR (NPS)					
BUFF (NPS)					

Table 47. Methods used for data analysis and interpretation by state and national entities for the evaluation of algae data.

Monitoring Entity	Replicate Samples	Sub-sampling	Multi-metric Indices	Multi-variate Indices	Hypothesis Testing	Level of Taxonomic Identification
Arkansas						
Indiana						
Iowa						
Kansas						
Minnesota						
Missouri						
Nebraska						
Ohio						
USEPA		X	X	X		lowest practical
USGS		X				species
USFS						
Prairie Cluster (NPS)						
OZAR (NPS)						
BUFF (NPS)						

V. SUMMARY OF PHYSICAL HABITAT ASSESSMENT PROTOCOLS.

All 14 of the evaluated state, national, and NPS biological monitoring protocols recommended physical habitat assessment in conjunction with biological monitoring. Among these, the following generalities were observed:

All (14 of 14) recommended visual based assessment methods. Most (12 of 14) additionally recommended quantitative methods, and many (10 of 14) also recommended habitat development indices (Table 48).

Table 48. Habitat assessment methods used in biological assessment programs of state and national regulatory and management entities.

Monitoring Entity	Visual Based	Quantitative	Habitat Development Indices
Arkansas	X		X
Indiana	X	X	X
Iowa	X		
Kansas	X	X	X
Minnesota	X	X	
Missouri	X	X	X
Nebraska	X	X	X
Ohio	X	X	X
USEPA	X	X	X
USGS	X	X	X
USFS	X	X	
Prairie Cluster (NPS)	X	X	
OZAR (NPS)	X	X	X
BUFF (NPS)	X	X	X

APPENDIX I. REFERENCES

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APPENDIX II. GLOSSARY

Acclimation - response by an animal that enables it to tolerate a change in a single factor (e.g. temperature) in its environment.
Adaptation - adjustments made by animals in respect of their environments. The adjustments may occur by natural selection, as individuals with favorable genetically acquired traits breed more prolifically than those lacking these traits (genotypic adaptation), or they may involve non-genetic changes in individuals, such as physiological modification (e.g. acclimatization) or behavioral changes (phenotypic adaptation).
Aquatic Assemblage - an organism group of interacting populations in a given waterbody, for example, fish assemblage or a benthic macroinvertebrate assemblage.
Aquatic Biota - collective term describing the organisms living in or depending on the aquatic environment.
Aquatic Community - association of interacting assemblages in a given waterbody, the biotic component of an ecosystem (see also aquatic assemblage).
Aquatic Life Use - a beneficial use designation in which the waterbody provides suitable habitat for survival and reproduction of desirable fish, shellfish, and other aquatic organisms.
Attribute - a measurable component of a biological system.
Benthic marcroinvertebrates - see benthos.
Benthos - animals without backbones, living in or on the sediments, a size large enough to be seen by the unaided eye, and which can be retained by a U.S. Standard No. 30 sieve (28 openings/inch, 0.595-mm openings). Also referred to as bentic macroinvertebrates, infauna, or macrobenthos.
Bioavailability - degree to which chemicals can be taken up by organisms.
Biodiversity - Refers to the variety and variability among living organisms and the ecological complexes in which they occur. Diversity can be defined as the number of different items and their relative frequencies. For biological diversity, these items are organized at many levels, ranging from complete ecosystems to the biochemical structures that are the molecular basis of heredity. Thus, the term encompasses different ecosystems, species, and genes.
Biological Assessments or Bioassessments - evaluation of the biological condition of a waterbody using biological surveys and other direct measurements of resident biota in surface waters.
Biological Criteria or Biocriteria - narrative or numeric expressions that describe the biological condition (structure and function) of aquatic communities inhabiting waters of a designated aquatic life use. Biocriteria are based on the numbers and kinds of organisms present and are regulatory-based biological measurements.
Biological Integrity - the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region.
Biological Monitoring or Biomonitoring - use of a biological entity as a detector and its response as a measure to determine environmental conditions. Toxicity tests and ambient biological surveys are common biological monitoring methods.
Biological Survey or Biosurvey - collecting, processing, and analyzing a representative portion of the resident aquatic community to determine its structural and/or functional characteristics.
Bioregion - any geographical region characterized by a distinctive flora and fauna (see also ecoregion).
Clean Water Act (CWA) - An act passed by the U.S. Congress to control water pollution (formerly referred to as the Federal Water Pollution Control Act of 1972). Public Law 92-500, as amended. 33 U.S.C. 1251 et seq.
Clean Water Act Section 303(d) - annual report to Congress from EPA that identifies those waters for which existing controls are not sufficiently stringent to achieve applicable water quality standards.
Clean Water Act Section 305(b) - biennial reporting requires description of the quality of the Nation's surface waters, evaluation of progress made in maintaining and restoring water quality, and description of the extent of remaining problems by using biological data to make aquatic life use support decisions.
Community - all the groups of organisms living together in the same area, usually interacting or depending on each other for existence.

Criteria - statements of the conditions presumed to support or protect the designated use or uses of a waterbody. Criteria may be narrative or numeric.
Density-Dependence - regulation of the size of a population by mechanisms that are themselves controlled by the size of that population (e.g. the availability of resources) and whose effectiveness increases as population size increases.
Designated Use - classification specified in water quality standards for each waterbody or segment describing the level of protection from perturbation afforded by the regulatory programs. The designated aquatic life uses established by the state or authorized tribes set forth the goals for restoration and/or baseline conditions for maintenance and prevention from future degradation of the aquatic life in specific waterbodies.
Diatom - microscopic algae with cell walls made of silicon and have two separating halves.
Ecological Integrity - the condition of an unimpaired ecosystem as measured by combined chemical, physical (including physical habitat), and biological attributes.
Ecoregions - a relatively homogeneous ecological area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variables (see also bioregions).
Habitat - a place where the physical and biological elements of ecosystems provide a suitable environment including the food, cover, and space resources needed for plant and animal livelihood.
Historical Data - data sets from previous studies, which can range from handwritten field notes to published journal articles.
Impact - change in the chemical, physical (including habitat) or biological quality or condition of a waterbody caused by external sources.
Impairment - detrimental effect on the biological integrity of a waterbody caused by an impact that prevents attainment of the designated use.
Index of Biological Integrity (IBI) - an integrative expression of site condition across multiple metrics. An index of biological integrity is often composed of at least seven metrics. The plural form is either indices or indexes.
Macroinvertebrates - animals without backbones of a size large enough to be seen by the unaided eye and which can be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings).
Metric - A calculated term or enumeration representing some aspect of biological assemblage, function, or other measurable aspect and is a characteristic of the biota that changes in some predictable way with increased human influence. A multimetric approach involves combinations of metrics to provide an integrative assessment of the status of aquatic resources.
Microinvertebrates - animals without backbones that are not large enough to be seen by the unaided eye; they will not be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings).
Minimally Impaired - sites or conditions with slight anthropogenic perturbation relative to the overall region of the study.
Multimetric - analysis techniques using several measurable characteristics of a biological assemblage.
Multivariate Community Analysis - statistical methods (e.g. ordination or discriminant analysis) for analyzing physical and biological community data using multiple variables.
Narrative Biological Criteria - general statements of attainable or attained conditions of biological integrity and water quality for a given designated aquatic life use.
Non-Point Source Pollution - pollution that occurs when rainfall, snowmelt, or irrigation runs over land or through the ground, picks up pollutants, and deposits them into rivers, lakes, and coastal waters or introduces them into ground water.
NPDES - National Pollutant Discharge Elimination System.
Numeric Biocriteria - numerical indices that describe expected attainable community attributes for different designated aquatic life uses.
Point Source - origin of a pollutant discharge from a discrete conveyance typically thought of as an effluent from the end of a pipe.
Population - aggregate of individuals of a biological species that are geographically isolated from other members of the species and are actually or potentially interbreeding.
Reference Condition - set of selected measurements or conditions of unimpaired or minimally impaired waterbodies characteristic of a waterbody type in a region.
Reference Site - specific locality on a waterbody which is unimpaired or minimally impaired and is representative of the expected biological integrity of other localities on the same waterbody or nearby waterbodies.

Regionalization or Ecoregionalization - procedure for subdividing a geographic area into regions of relative homogeneity in ecological systems or in relationship between organisms and their environment.
Stressors - physical and biological factors that adversely affect aquatic organisms.
Taxa - a grouping of organisms given a formal taxonomic name such as species, genus, family, etc.
Total Maximum Daily Loads (TMDLs) - calculation of the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards and an allocation of that amount to the pollutant's source.
Use Attainability Analysis (UAA) - analysis that describes factors limiting designated use of waterbodies.